PTO/SB/28 (07-09)
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PETITION TO MAKE SPECIAL UNDER ACCELERATED EXAMINATION PROGRAM

Attorney Docket Number	I001-0002USC4	First Named Inventor	James S. Baldassarre
Application Number (if Known)			
Title of Invention	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension		

APPLICANT HEREBY PETITIONS TO MAKE THE ABOVE-IDENTIFIED APPLICATION SPECIAL UNDER THE REVISED ACCELERATED EXAMINATION PROGRAM. See Instruction sheet on page 3.

1. **Claims of the application:**
   a. The application must contain three (3) or fewer independent claims and twenty (20) or fewer total claims. The application may not contain any multiple dependent claims.

   b. **Applicant hereby agrees not to separately argue the patentability of any dependent claim during any appeal** in the application. Specifically, the applicant agrees that the dependent claims will be grouped together with and not argued separately from the independent claim from which they depend in any appeal brief filed in the application (37 CFR 41.37(c)(1)(vii)).

   c. The claims must be directed to **a single invention**.

2. **Interviews:**
   Applicant hereby agrees to have (if requested by examiner):
   a. An interview (including an interview before a first Office action) to discuss the prior art and any potential rejections or objections with the intention of clarifying and possibly resolving all issues with respect to patentability at that time, and

   b. A telephonic interview to make an election without traverse if the Office determines that the claims are not obviously directed to a single invention.

3. **Preexamination Search Statement and Accelerated Examination Support Document:**
   With this petition, applicant is providing: a **preexamination search statement**, in compliance with the requirements set forth in item 8 of the instruction sheet, and an **"accelerated examination support document"** that includes:
   a. An **information disclosure statement** in compliance with 37 CFR 1.98 citing each reference deemed most closely related to the subject matter of each of the claims;

   b. For each reference cited, **an identification of all the limitations of the claims** that are disclosed by the reference specifying where the limitation is disclosed in the cited reference;

   c. A **detailed explanation of how each of the claims are patentable** over the references cited with the particularity required by 37 CFR 1.111(b) and (c);

   d. A concise **statement of the utility** of the invention as defined in each of the independent claims (unless the application is a design application);

   e. An identification of any cited references that may be disqualified as prior art under 35 U.S.C. 103(c) as amended by the CREATE act; and

   f. **A showing of where each limitation of the claims finds support under the first paragraph of 35 U.S.C. 112** in the written description of the specification. If applicable, the showing must also identify: (1) each means- (or step-) plus-function claim element that invokes consideration under 35 U.S.C. 112, ¶6; and (2) the structure, material, or acts that correspond to any means- (or step-) plus-function claim element that invokes consideration under 35 U.S.C. 112, ¶6. If the application claims the benefit of one or more applications under title 35, United St ates Code, the showing must also include where each limitation of the claims finds support under the first paragraph of 35 U.S.C. 112 in each such application in which such support exists.

PETITION TO MAKE SPECIAL UNDER ACCELERATED EXAMINATION PROGRAM (Continued)

Attorney Docket Number	I001-0002USC4	First Named Inventor	James S. Baldassarre

Attachments:

a.	X	Accelerated Examination Support Document (see item 3 above).
b.		A statement, in compliance with the requirements set forth in item 8 of the instruction sheet, detailing the preexamination search which was conducted.
c.	X	Information Disclosure Statement.
d.	☐	Other (*e.g.*, a statement that the claimed subject matter is directed to environmental quality, energy, or countering terrorism (37 CFR 1.102(c)(2)).

Fees: The following fees must be filed electronically via EFS or EFS-Web:

a.	The basic filing fee, search fee, examination fee, and application size fee (if required) under 37 CFR 1.16.
b.	Petition fee under 37 CFR 1.17(h) - unless the petition is filed with a showing under 37 CFR 1.102(c)(2).

Signature:

Click Remove if you wish to remove this signatory Remove

Signature	[signature]	Date	21 June 2010
Name (Print/Typed)	Christopher P. Rogers	Registration Number	36334

Click Add if you wish to add additional signatory Add

***Note:** Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Application Serial Number	TBD
Confirmation Number	TBD
Filing Date	Herein
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	TBD
Examiner	TBD
Attorney Docket Number	I001-0002USC4

Pre-Examination Search Document

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This pre-examination search statement is provided in support of the Petition for Accelerated Examination filed herewith.

A pre-examination search was conducted involving U.S. patents and patent application publications, foreign patent documents and non-patent literature as indicated below. The results of the search are provided on an Information Disclosure Statement filed concurrently herewith.

The search primarily includes the following aspects:

- The method of reducing adverse events in patients in need of treating with nitric oxide - excluding patients with pre-existing left ventricular dysfunction.
- The patients have a pulmonary capillary wedge pressure greater than 20mm Hg.
- Patients with left ventricular dysfunction have conditions like systolic or diastolic dysfunction, hypertensive, viral, iodopathic cardiomyopathy, autoimmune disease related cariomyopathy, structural heart disease, idiopathic pulmonary arterial hypertension, pulmonary hypertension cardiomyopathy.

- The patient's population are children and adults.
- Adverse events are pulmonary edema, hypotension, cardiac arrest, ECG changes, hypoxemia, hypoxia and bradycardia.
- The patient in need of nitric oxide inhalation has PCWP<=15mg, PVRI>3micro.sq.meters.
- Left ventricular afterload is minimized by administering a pharmaceutical dosage form comprising nitroglycerin and calcium channel blocker to the patient, using an inter-aortic balloon pump.

8 (A) Pre-examination Search

Details of US Patent Classification Codes used

http://www.uspto.gov/go/classification/

128-Surgery

128/200.14 – Liquid Medicament Atomizer or Sprayer

128/200-24 – Respiratory Method or Device

128/203.15 – Particular treating agent carried by breathes gas

128/203.12 – Means for mixing treating agent with respiratory gas

558- Organic Compounds

558/486 – Glyceryl trinitrate per se (i.e., trinitroglycerin)

423 – Chemistry or Inorganic Compounds

423/405 – Nitric Oxide (NO)

600 – Surgery

600/481 – Cardiovascular

600/513 – Detecting heartbeat electric signal and diverse cardiovascular characteristic

Details of IPC-8 Codes used

http://www.wipo.int/classifications/ipc/ipc8/?lang=en

A61K – Preparations for Medical, Dental, or Toilet Purposes

A61K 33/00 – Medicinal preparations containing inorganic active ingredients

A61K 33/08 – Oxides; Hydroxides

A61P – Specific Therapeutic Activity of Chemical Compounds or Medicinal Preparations

A61P 9/00 – Drugs for disorders of the cardiovascular system

A61P 9/04 – Inotropic agents, i.e. stimulants of cardiac contraction; drugs for heart failure

A61P 9/08 – Vasodilators for multiple indications

A61P 43/00 – Drugs for specific purposes

C01B – Non-Metallic Elements; Compounds Thereof

C01B 21/24 – Nitric oxide (NO)

Dates Conducted: May 10, 2010 and May 17, 2010

Database Searches

Database Service: Legal Advantage

Data Searched: All patents and Non-patent literature

Database Used: MicroPatent, USPTO, European Patent Office/Espacenet, WIPRO, JPO, Google, Springerlink, Wiley Interscience, ScienceDirect, Scirus, Journal of Medicinal Chemistry, ACS Publications, and, Journal of American Academy of Pediatrics.

Search Logic

Search No.	Concept	Keywords
1	Nitric oxide	Nitric oxide, nitrogen monoxide, nitrogen oxide, iNO, NO
2	Inhale	Inhale, breath, gasp
3	Reduce	Reduce, minimize, prevent, avoid, exclude, reject, except, omit
4	Adverse event	Adverse/undesirable/unfavorable/unfavorable event/effect/consequence/indication, side effect, toxicity, toxin
5	Identify	Identify, select, choose, opt, pick, screen, find, segregate, separate, distinguish, take out
6	Left ventricular dysfunction	Left ventricular dysfunction, LVD, diastolic/systolic dysfunction, cardiomyopathy, heart disease
7	Pulmonary Capillary wedge pressure	Pulmonary Capillary wedge pressure, PCWP
8	Respiratory failure	Respiratory failure, Pulmonary edema, hypotension or cardiac arrest, heart failure, heart attack, electrocardiogram/ECG change, hypoxia, hypoxemia, bradycardia

8(B) Search Directed to the Invention

The pre-examination search was directed to the claimed invention, encompassing all the features of the claims and giving the claims their broadest reasonable interpretation.

8(C) Search Directed to the Disclosure

No disclosed features that are unclaimed at this time are currently seen as features that may be claimed later.

8(D) Search Report from a Foreign Patent Office

Search reports from Australia, Japan, and the EPO are attached herewith

8(E) Statement of Good Faith

All statements above in support of the petition to make special are based on a good faith belief that the search was conducted in compliance with the requirements of this rule.

Respectfully Submitted,

Christopher P. Rogers, Reg. No. 36,334
___[signature]___
Date: 21 June 2010

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15 March 2010

RECEIVED 17 MAR 2010

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Your Ref: 28686IKA/AMM:Is

Examiner's first report on patent application no. 2009202685
by Ikaria Holdings, Inc.

Last proposed amendment no.

Dear Madam/Sir,

I am replying to the request for normal examination. I have examined the application and I believe that there are lawful grounds of objection to the application. These grounds of objection are:

1. The invention defined in claims 1-30 does not involve an inventive step when compared to the disclosure of each of the following prior art documents*:

   D1: LOH, E. *et al.* "Cardiovascular Effects of Inhaled Nitric Oxide in Patients with Left Ventricular Dysfunction". CIRCULATION, 1994, vol.90: 2780-2785.
   D2: CUJEC, B. *et al.* "Inhaled Nitric Oxide Reduction in Systolic Pulmonary Artery Pressure is Less in Patients with Decreased Left Ventricular Ejection Fraction". CANADIAN JOURNAL OF CARDIOLOGY, 1997, vol.13(9): 816-824.
   D3: ROSALES, A *et al.* "Adverse Hemodynamic Effects Observed with Inhaled Nitric Oxide After Surgical Repair of Total Anomalous Pulmonary Venous Return". PEDIATRIC CARDIOLOGY, 1999, vol.20: 224-226.
   D4: BOCCHI, E. *et al.* "Inhaled Nitric Oxide Leading to Pulmonary Edema in Stable Severe Heart Failure". THE AMERICAN JOURNAL OF CARDIOLOGY, 1994, vol.74: 70-71.
   D5: ARGENZIANO, M. *et al.* "Inhaled Nitric Oxide is not a Myocardial Depressant in a Porcine Model of Heart Failure". THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY, 1998, vol.115: 700-704.

   The problem addressed by the current application is reducing adverse events or serious adverse events associated with inhaled nitric oxide in patients who have pre-existing left ventricular dysfunction.

   The cited art is directed to a problem similar to the applicant's problem, and in searching the problem a person skilled in the art could reasonably be expected to have found, and to have ascertained, understood, and regarded, this prior art as relevant.

   D1 investigated the use of inhalation of the pulmonary vasodilator, nitric oxide (NO), in patients with heart failure due to left ventricular dysfunction (LVD). The cause of heart failure in half the patients was ischemic cardiomyopathy and in the other half it was caused by idiopathic dilated cardiomyopathy (see abstract and Methods: Study Population). Following administration of NO via a face masks patients showed an increase in the mean pulmonary artery wedge pressure associated with decreases in cardiac index and stroke volume index (see Results). It is suggested that selective pulmonary vasodilation is not desirable in patients with left ventricular failure (see page 2784, last paragraph).

D2 discloses that there have been reports that a decrease in pulmonary vascular resistance following iNO inhalation occurs in patients with LVD as a result of an increase in pulmonary capillary wedge pressure. D2 further investigated the effects of iNO in a group of patients with a broad range of left ventricular function in a randomized manner (see page 817, left col.). Some of the patients received oxygen in addition to NO (see page 818, Study protocol). Three patients with depressed left ventricular ejection fraction (LVEF) presented with pulmonary oedema after administration of nitric oxide (see page 821, left col. 1st paragraph and page 822, right col., lines 4-6). Other adverse events to occur in patiens with depressed LVEF were an increase in pulmonary wedge pressure and decreased pulmonary vascular resistance (the latter patients were also cardiomyopathy patients) (see page 821, right col.). There is a clear suggestion that the use of nitric oxide is limited in patients with pre-existing LVD (see CONCLUSIONS).

D3 discloses a case report of a one month old patient who underwent corrective surgery with pulmonary vein confluence to left atrial anastomosis (see abstract). The patient was treated with NO therapy following development of sudden onset systemic-level pulmonary pressure with concomitant systemic hypotension. However, favourable changes were followed by "rebound" pulmonary hypertension that occurred with concomitant systemic hypotension and central venous pressure. Therapy with NO was discontinued based on the rationale that this episode of pulmonary hypertension may have been caused by left atrial hypertension secondary to a sudden increase in pulmonary blood flow into a noncompliant left atrium and ventricle (see page 225, 4th and 5th paragraphs). As a result, D3 states that NO therapy can be detrimental in patients with LVD and/or cardiomyopathy as these patients may develop pulmonary oedema (see abstract and page 226, left col., last paragraph).

D4 pertains to a study in which patients with refractory heart failure and severe pulmonary hypertension having impaired LVEF and severe and diffuse systolic dysfunction were administered NO via inhalation. Following NO therapy patients presented with an increase in pulmonary wedge pressure and developed pulmonary oedema (see whole document).

D5 discloses that there have been reports of increases in left ventricular end-diastolic pressure and episodes of pulmonary oedema during the clinical use of inhaled nitric oxide (iNO) in patients with pre-existing LVD (see abstract and the introduction).

Each of D1-D5 differs from the instant specification in that they do not specifically disclose excluding patients with LVD from iNO treatment nor the steps of informing a medical provider that excluding patients with LVD from iNO treatment reduces adverse events. However, each of D1-D5 discloses that adverse events occur in patients with pre-existing LVD following administration of iNO and they clearly suggest that precautions should be taken when administering iNO.

Therefore the person skilled in the art would directly and without difficulty, by routine steps, arrive at a solution which is the same as the claimed solution, and therefore the claimed invention lacks an inventive step.

* As found during a national phase search

NOTE: There is a current postponement of acceptance in place. If you overcome all other objections before the expiration of that postponement, the Commissioner will only accept the application at that time if you have filed a clear and unambiguous statement requesting the withdrawal of that postponement. Otherwise, a further adverse report will be issued.

You have 21 months from the date of this report to overcome all my objection(s) otherwise your application will lapse.

You will need to pay a monthly fee for any response you file after 12 months from the date of the first report.

You will also need to pay any annual continuation fees that apply. These will normally be first due five years from the filing date. Please note however that earlier commencement dates apply for divisional applications.

Information about fees may be obtained by phoning 1300 651 010.

Yours faithfully,

EDWINA VANDINE
Patent Examination A
A1 - PBR Plants & Biotechnology
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REC'D 10 MAY 2010
Action by ....

Date
10.05.10

Reference	Application No./Patent No.
N.108660-TJD	09251949.5 - 2123
Applicant/Proprietor	
Ikaria Holdings, Inc.	

Communication

The extended European search report is enclosed.

The extended European search report includes, pursuant to Rule 62 EPC, the European search report (R. 61 EPC) or the partial European search report/ declaration of no search (R. 63 EPC) and the European search opinion.

Copies of documents cited in the European search report are attached.

☑ 1 additional set(s) of copies of such documents is (are) enclosed as well.

The following have been approved:

☑ Abstract ☑ Title

☐ The Abstract was modified and the definitive text is attached to this communication.

The following figure(s) will be published together with the abstract:

Refund of the search fee

If applicable under Article 9 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

Europäisches Patentamt · European Patent Office · Office européen des brevets

Europäisches Patentamt
European Patent Office
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EUROPEAN SEARCH REPORT

Application Number
EP 09 25 1949

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	LOH EVAN ET AL: "Cardiovascular Effects of Inhaled Nitric Oxide in patients With Left Ventricular Dysfunction" CIRCULATION, vol. 90, no. 6, 1994, pages 2780-2785, XP002577161 ISSN: 0009-7322 * the whole document * -----	1-9	INV. A61K33/00 A61P9/08 A61P9/12
X,D	SEMIGRAN MARC J ET AL: "Hemodynamic effects of inhaled nitric oxide in heart failure" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 24, no. 4, 1994, pages 982-988, XP009131903 ISSN: 0735-1097 * the whole document * -----	1-9	
X,D	HAYWARD C S ET AL: "Inhaled nitric oxide in cardiac failure: Vascular versus ventricular effects" JOURNAL OF CARDIOVASCULAR PHARMACOLOGY, vol. 27, no. 1, 1996, pages 80-85, XP009131904 ISSN: 0160-2446 * the whole document * -----	1-9	TECHNICAL FIELDS SEARCHED (IPC) A61K
X	OVODOV ET AL: "Nitric oxide: Clinical applications" SEMINARS IN ANESTHESIA, SAUNDERS, CO, NEW YORK, NY, US LNKD- DOI:10.1053/SA.2000.6785, vol. 19, no. 2, 1 June 2000 (2000-06-01), pages 88-97, XP005426335 ISSN: 0277-0326 * page 90, column 1 * * page 93, column 2 - page 94 * ----- -/--	1-9	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	13 April 2010	Albrecht, Silke

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

2

EPO FORM 1503 03.82 (P04C01)

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 09 25 1949

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	HENRICHSEN ET AL: "Inhaled nitric oxide can cause severe systemic hypotension" JOURNAL OF PEDIATRICS, MOSBY-YEAR BOOK, ST. LOUIS, MO, US LNKD- DOI:10.1016/S0022-3476(96)70230-5, vol. 129, no. 1, 1 July 1996 (1996-07-01), page 183, XP022199226 ISSN: 0022-3476 * the whole document *	1-9	
X	ADATIA ET AL: "Inhaled nitric oxide and hemodynamic evaluation of patients with pulmonary hypertension before transplantation" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, NEW YORK, NY, US LNKD- DOI:10.1016/0735-1097(95)00048-9, vol. 25, no. 7, 1 June 1995 (1995-06-01), pages 1656-1664, XP005857183 ISSN: 0735-1097 * page 1663, column 1 *	1-9	TECHNICAL FIELDS SEARCHED (IPC)
X	CUJEC BIBIANA ET AL: "Inhaled nitric oxide reduction in systolic pulmonary artery pressure is less in patients with decreased left ventricular ejection fraction" CANADIAN JOURNAL OF CARDIOLOGY, vol. 13, no. 9, 1997, pages 816-824, XP002577162 ISSN: 0828-282X * the whole document *	1-9	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	13 April 2010	Albrecht, Silke

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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L : document cited for other reasons

& : member of the same patent family, corresponding document

2

EPO FORM 1503 03.82 (P04C01)

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 09 25 1949

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	FINDLAY G P: "Paradoxical haemodynamic response to inhaled nitric oxide" INTERNATIONAL JOURNAL OF INTENSIVE CARE 1998 GB, vol. 5, no. 4, 1998, pages 134-139, XP001536771 ISSN: 1350-2794 * the whole document * -----	1-9	
X,D	BOCCHI E A ET AL: "Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure" AMERICAN JOURNAL OF CARDIOLOGY, CAHNERS PUBLISHING CO., NEWTON, MA, US LNKD- DOI:10.1016/0002-9149(94)90496-0, vol. 74, no. 1, 1 July 1994 (1994-07-01), pages 70-72, XP023278686 ISSN: 0002-9149 [retrieved on 1994-07-01] * the whole document * -----	1-9	

TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	13 April 2010	Albrecht, Silke

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

2

EPO FORM 1503 03.82 (P04C01)

(Translation of Official Action)

NOTIFICATION OF REASON FOR REJECTION

Mailed: February 23, 2010

Japanese Patent Application No. 2009-157623

Applicant: IKARIA HOLDINGS, INC.

The present application should be rejected for the following reason(s). If the applicant has any argument against the reason(s), an Argument must be filed within three months of the mailing date of this Official Action.

REASON 1
The present invention as claimed in the following claim(s) is unpatentable under Article 29, paragraph 1, sub-paragraph 3 of the Japanese Patent Law as being anticipated by the following publication(s) distributed in Japan or elsewhere or as being identical with an invention made available to the public through electric telecommunications prior to the filing of the present application.

REASON 2
The present invention as claimed in the following claim(s) is unpatentable under Article 29, paragraph 2 of the Japanese Patent Law since the invention could have been easily made by those skilled in the art to which it pertains on the basis of the invention(s) described in the following publication(s) distributed in Japan or elsewhere or an invention/inventions made available to the public through electric telecommunications prior to the filing of the present application.

NOTE:

Citation 1: Inglessis, I. *et al.*, Journal of the American College of Cardiology, 2004, Vol. 44, No. 4, pp. 793-798

Citation 2: Loh, E. *et al.*, Circulation, 1994, 90, pp. 2780-2785

Citation 3: Steinhorn, R.H. *et al.*, Pulmonary Hypertension, Persistent-Newborn, emedicine, updated Apr. 19, 2007 [http://emedicine.medscape.com/article/898437-overview]

Citation 4: BOCCHI, E.A. *et al.*, The American Journal of Cardiology, 1994, Vol. 74, pp. 70-72

A.

Reasons 1 and 2/ Claims 1 to 14/ Citation 1

Citation 1 discloses that inhaled nitric oxide is known as a selective pulmonary vasodilator (Abstract), and that inhaled nitric oxide, when administered to patients with right ventricular myocardial infarction and cardiogenic shock, reduced the pulmonary arterial pressure (Abstract). Citation 1 also discloses that the inhalation of nitric oxide is known to decrease pulmonary vascular tone in adults and children with pulmonary hypertension (page 793, right column, lines 11 to 6 from the bottom), and that nitric oxide is delivered by means of a ventilator or is mixed with oxygen (page 795, left column, "NO administration"). Especially, Table 2 presents hemodynamic parameters of target patients at the time of study enrollment, indicating that most of the patients have a pulmonary capillary wedge pressure (PCWP) of less than 20 mmHg.

In light of the present specification (paragraph [0013]), the patients of Citation 1 having a PCWP of less than 20 mmHg are not deemed to have pre-existing left ventricular dysfunction (LVD).

Thus, the present invention as claimed in claims 1 to 14 is indistinguishable from the invention disclosed in Citation 1.

(The present invention and the invention disclosed in Citation 1 are identical in active ingredient and target patients, and thus are deemed to necessarily provide the same functions/effects.)

B.

Reason 2/ Claims 1 to 14/ Citations 1 to 4

Inhaled nitric oxide is well known as a selective pulmonary vasodilator, as disclosed in Citation 1.

On the other hand, Citation 2 (for example, Abstract) discloses that inhaled nitric oxide, when administered to patients with left ventricular dysfunction, may cause a decrease in pulmonary vascular resistance associated with an increase in left ventricular filling pressure, leading to the risk of the occurrence of adverse events.

Citation 3 (for example, see Abstract and "Treatment with iNO") discloses that, although inhaled nitric oxide is used for the treatment of pulmonary hypertension of newborns, patients suffering from congenital cardiac disease characterized by left ventricular outflow tract obstruction and severe left ventricular dysfunction have a contraindication to the treatment with inhaled nitric oxide.

Citation 4 (page 71, left column, lines 13 to 15) discloses that inhaled nitric oxide, when administered to patients with severe heart disease, may cause pulmonary edema.

In view of the above, it would have been obvious to those skilled in the art to exclude patients with pre-existing left ventricular dysfunction from patients to be treated with a selective pulmonary vasodilator, in order to avoid the occurrence of adverse events, based on Citations 1 to 4.

Further, the present invention as claimed in claims 1 to 14 is not deemed to provide particularly remarkable advantages, in view of Citations 1 to 4.

REASON 3
The present application should be rejected on the grounds that the recitation of the claim(s) fails to meet the requirement of Article 36, paragraph 6, sub-paragraph 2 of the Japanese Patent Law in the following respect(s).

NOTES:

(1) The abbreviations "PAPm," "PCWP" and "PVRI" are unclear in meaning.

(2) The term "near" renders the scope of the claimed invention unclear, and thus is inappropriate as an expression for use in the claims.

Background Art Information*

Field of Search: IPC A61K33/00

*The information provided herein constitutes no reason for rejection.

拒絶理由通知書

特許出願の番号	特願２００９－１５７６２３
起案日	平成２２年　２月　９日
特許庁審査官	辰己　雅夫　　　　　　　４４９８　４Ｃ００
特許出願人代理人	吉武　賢次（外　３名）　様
適用条文	第２９条第１項、第２９条第２項、第３６条

この出願は、次の理由によって拒絶をすべきものです。これについて意見がありましたら、この通知書の発送の日から３か月以内に意見書を提出してください。

理　由

１．この出願の下記の請求項に係る発明は、その出願前に日本国内又は外国において、頒布された下記の刊行物に記載された発明又は電気通信回線を通じて公衆に利用可能となった発明であるから、特許法第２９条第１項第３号に該当し、特許を受けることができない。

２．この出願の下記の請求項に係る発明は、その出願前に日本国内又は外国において頒布された下記の刊行物に記載された発明又は電気通信回線を通じて公衆に利用可能となった発明に基いて、その出願前にその発明の属する技術の分野における通常の知識を有する者が容易に発明をすることができたものであるから、特許法第２９条第２項の規定により特許を受けることができない。

３．この出願は、特許請求の範囲の記載が下記の点で、特許法第３６条第６項第２号に規定する要件を満たしていない。

記　　　（引用文献等については引用文献等一覧参照）

Ａ．
・理由　１，２
・請求項　１－１４
・引用文献等　１
・備考：

引用文献１には、吸入用一酸化窒素は選択的肺血管拡張剤として知られていること（Abstract）、右心室心筋梗塞および心臓ショックを有する患者に吸入用一

酸化窒素を投与したところ、肺動脈圧が減少したこと（Abstract）が記載されている。同文献にはまた、一酸化窒素の吸入は、成人や小児の肺高血圧患者の肺血管緊張を減少させることが知られていること（p.793 右欄下から11行-下から6行）、ベンチレーターを使用して送達することや酸素と混合すること（p.795 左欄"NO administration"）についても記載されており、特に、Table2には、対照患者の試験登録時の血行動態パラメーターが記載され、多くの患者の肺毛細血管楔入圧（ＰＣＷＰ）が２０ｍｍＨｇ未満であることが示されている。

ここで、本願明細書【００１３】の記載からみて、引用文献１のＰＣＷＰが２０ｍｍＨｇ未満の患者は、先在性左心室機能障害（ＬＶＤ）を有していないものと認められる。

してみると、請求項１－１４に係る発明は引用文献１に記載された発明と区別することができない。

（本願発明と引用文献１記載の発明は、有効成分と対象患者が同一であるから、当然に同様の作用効果を奏するものといえる。）

Ｂ．
・理由　２
・請求項　１－１４
・引用文献等　１－４

上記の引用文献１に記載されるように、吸入用一酸化窒素は選択的肺血管拡張剤として周知のものである。

一方、引用文献２（Abstract等）には、左心室機能不全の患者に吸入用一酸化窒素を投与すると、左心充満圧の上昇に伴う肺血管抵抗の低下を引き起こし、有害事象が生ずる可能性があることが記載されている。

引用文献３（Abstract, "Treatment with iNO"等）には、新生児肺高血圧の治療に吸入用一酸化窒素が用いられるものの、左心室流路障害で特徴づけられる先天性心疾患や、重篤な左心室機能不全の患者に対しては、吸入用一酸化窒素による治療は禁忌であると記載されている。

引用文献４（p.71 左欄第13-15行）には、重篤な心疾患の患者に吸入用一酸化窒素を投与すると、肺水腫を引き起こす可能性があることが記載されている。

してみると、引用文献１－４の記載に基づき、有害事象の発生を避けるべく、選択的肺血管拡張剤の対象患者から、先在性左心室機能障害を有する患者を除外することは当業者が容易に想到し得たことである。

そして、請求項１－１４に係る発明が引用文献１－４の記載からみて格別顕著な効果を奏するとも認められない。

Ｂ．
・理由　３
（１）

・請求項　７

「ＰＡＰｍ」、「ＰＣＷＰ」、「ＰＶＲＩ」は略語であり、その意味が不明である。

（２）

・請求項　１０

「ほぼ」なる記載は発明の範囲を不明確とするものであって、特許請求の範囲の記載として適切でない。

引　用　文　献　等　一　覧

1．Inglessis, I. et al., Journal of the American College of Cardiology, ２００４年, Vol.44, No.4, p.793-798

2．Loh, E. et al., Circulation, １９９４年, 90, p.2780-2785

3．Steinhorn, R.H. et al., Pulmonary Hypertension, Persistent-Newborn, emedicine, Updated Apr 19, 2007 [http://emedicine.medscape.com/article/898437-overview]

4．BOCCHI, E.A. et al., The American Journal of Cardiology, １９９４年, V01.74, p.70-72

（注）法律又は契約等の制限により、提示した非特許文献の一部又は全てが送付されない場合があります。

先行技術文献調査結果の記録

・調査した分野　　ＩＰＣ　Ａ６１Ｋ３３／００

この拒絶理由通知の内容に関するお問い合わせ、または面接のご希望がございましたら下記までご連絡下さい。

特許審査第三部医療　高橋樹理

ＴＥＬ．０３（３５８１）１１０１　内線３４５０

ＦＡＸ．０３（３５８１）１３４２

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Application Serial Number	TBD
Confirmation Number	1376
Filing Date	Herein
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	1614
Examiner	TBD
Attorney Docket Number	I001-0002USC4

ACCELERATED EXAMINATION SUPPORT DOCUMENT

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This Accelerated Examination Support Document (AESD) is submitted in support of the Petition for Accelerated Examination filed herewith.

Claims 1-20 are currently pending in the continuation application. A listing of the claims starts on page 2 herein.

The remaining sections of the AESD begin on page 6. Consideration and grant of the Petition to Accelerate Examination is respectfully requested.

CLAIMS

1. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, said method comprising:

a. providing pharmaceutically acceptable nitric oxide gas to a medical provider; and,

b. informing the medical provider that excluding said patients who have pre-existing left ventricular dysfunction from said treatment reduces the risk or prevents the occurrence of the adverse event or serious adverse event associated with said medical treatment.

2. The method of claim 1, wherein the adverse event or serious adverse event is one or more of pulmonary edema, hypotension, cardiac arrest, electrocardiogram changes, hypoxemia, hypoxia and bradycardia, or, associations thereof.

3. The method of claim 1, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

4. The method of claim 3, wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient.

5. The method of claim 3, wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

6. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, said method comprising:

a. providing pharmaceutically acceptable nitric oxide gas to a medical provider; and,

b. informing the medical provider that such patients that have pre-existing left ventricular dysfunction experience an increased rate of adverse events or serious adverse events associated with said medical treatment.

7. The method of claim 6, further comprising informing the medical provider of a risk of an adverse event or a serious adverse event in such patients who have a pulmonary capillary wedge pressure greater than 20 mm Hg.

8. The method of claim 6, further comprising informing the medical provider that there is a risk associated with using inhaled nitric oxides in such patients who have pre-existing or clinically significant left ventricular dysfunction and that such risk should be evaluated on a case by case basis.

9. The method of claim 6, further comprising informing the medical provider that there is a risk associated with using inhaled nitric oxide in such patients who have left ventricular dysfunction.

10. The method of claim 6, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

11. The method of claim 10, wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient.

12. The method of claim 10, wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

13. A method of reducing one or more adverse events or serious adverse events in an intended patient population comprising children in need of being treated with inhaled nitric oxide comprising:

a. identifying a patient eligible for inhaled nitric oxide treatment;

b. evaluating and screening the patient to identify if the patient has pre-existing left ventricular dysfunction; and

c. excluding from inhaled nitric oxide treatment any patient having pre-existing left ventricular dysfunction.

14. The method of claim 13, wherein the patient having pre-existing left ventricular dysfunction also exhibits a pulmonary capillary wedge pressure greater than 20 mm Hg.

15. The method of claim 13, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

16. The method of claim 15,

wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient, or,

wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

17. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, the method comprising:

a. identifying said patient in need of receiving inhalation of nitric oxide treatment;

b. evaluating and screening the patient to identify if the patient has pre-existing left ventricular dysfunction; and

c. administering the inhalation of nitric oxide if the patient has not been diagnosed as having pre-existing left ventricular dysfunction, thereby reducing the risk or preventing the occurrence of the adverse event or significant adverse event associated with the inhalation of nitric oxide treatment.

18. The method of claim 17, wherein the patient diagnosed as having pre-existing left ventricular dysfunction also exhibits a pulmonary capillary wedge pressure greater than 20 mm Hg.

19. The method of claim 17, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

20. The method of claim 19,

wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient, or,

wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

9(A) References Deemed Most Closely Related

An Information Disclosure Statement in compliance with 37 CFR 1.98 has been filed herewith citing each of the following references deemed most closely related to the subject matter of the claims. The references listed in the IDS submitted herewith but not listed in this Petition are not closely related to the claimed invention particularly as compared to the references listed and discussed herein.

List of Most Closely Related References

Use of Nitric Oxide, American Academy of Pediatrics, Pediatrics, Vol. 106, No. 2, August 2000, pp. 344-345. ("AAP").

Lipshultz, SE, Ventricular dysfunction clinical research in infants, children and adolescents, Progress in Pediatric Cardiology, 12 (2000):1-28. ("Lipshultz").

The Neonatal Inhaled Nitric Oxide Study Group, Inhaled Nitric Oxide In Full-Term and Nearly Full-Term Infants With Hypoxic Respiratory Failure, N Engl J Med, 1997, Vol. 336, No. 9, pp. 597-604. Correction at N Engl J Med 1997;337:434. ("NINOS").

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Loh, E., et al., Cardiovascular Effects of Inhaled Nitric Oxide in Patients with Left Ventricular Function, Circulation, 1994, Vol. 90:2780-2785. ("Loh").

Inglessis I et al., Hemodynamic effects of inhaled nitric oxide in right ventricular myocardial infarction and cardiogenic shock, JACC, Vol. 44, No. 4, August 18, 2004:793-8. ("Inglessis 2004").

Inglessis I et al., Hemodynamic effects of inhaled nitric oxide in right ventricular myocardial infarction and cardiogenic shock, Reply, JACC, Vol. 45, No. 6, March 15, 2005:962-7. ("Inglessis 2005").

Bocchi EA et al., Inhaled Nitric Oxide Leading to Pulmonary Edema in Stable Severe Heart Failure, The American Journal of Cardiology, Vol. 74, July 1, 1994. ("Bocchi").

Cujec, B., et al., Inhaled Nitric Oxide Reduction in Systolic Pulmonary Artery Pressure is Less in Patients with Decreased Left Ventricular Ejection Fraction, Canadian Journal of Cardiology, 1997, vol. 13(9):816-824. ("Cujec").

Rosales, A, et al., Adverse Hemodynamic Effects Observed with Inhaled Nitric Oxide After Surgical Repair of Total Anomalous Pulmonary Venous Return, Pediatric Cardiology, 1999, vol. 20:224-226. ("Rosales").

Argenziano, M, et al., Inhaled Nitric Oxide is not a Myocardial Depressant in a Porcine Model of Heart Failure, The Journal of Thoracic and Cardiovascular Surgery, 1998, vol. 115:700-704. ("Argenziano").

Steinhorn RH et al., Inhaled nitric oxide enhances oxygenation but not survival in infants with alveolar capillary dysplasia, J Pediatr, March 1997;130(3):417-22 (3rd). ("Steinhorn 1997").

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Krasuski RA et al., Inhaled Nitric Oxide Selectivity Dilates Pulmonary Vasculature in Adult Patients With Pulmonary Hypertension, Irrespective of Etiology, JACC, Vol. 36, No. 7, December 2000:2204-11. ("Krasuski").

Semigran MJ et al., Hemodynamic Effects of Inhaled Nitric Oxide in Heart Failure, JACC, Vol. 24, No. 4, October 1994:982-8. ("Semigran").

Dickstein ML et al., A Theoretic Analysis of the Effect of Pulmonary Vasodilation on Pulmonary Venous Pressure: Implications for Inhaled Nitric Oxide Therapy, J Heart Lung Transplant, 1996;15:715-21. ("Dickstein").

Henrichsen T et al., Inhaled nitric oxide can cause severe systemic hypotension, The Journal of Pediatrics, Vol. 129, No. 1, p. 183, 1July1996. ("Henrichsen").

Ovodov KJ et al., Nitric Oxide: Clinical Applications, Seminars in Anesthesia, Perioperative Medicine and Pain, Vol. 19, No. 2, June 2000, pp. 88-97. ("Ovodov")

Adatia I et al., Inhaled Nitric Oxide and Hemodynamic Evaluation of Patients With Pulmonary Hypertension Before Transplantation, JADD, Vol. 25, No. 7, June 1995, pp. 1656-64. ("Adatia").

Findlay GP et al., Paradoxical haemodynamic response to inhaled nitric oxide, International Journal of Intensive Care, Vol. 5, No. 4, 1998, pp. 134-139. ("Findlay").

9(B) Identification of Limitations Disclosed by References

AAP:

In August 2000, the Committee on Fetus and Newborn of the American Academy of Pediatrics issued a report on the use of iNO in infants. A relevant portion states:

> iNO should be administered using FDA-approved devices that are capable of administering iNO in constant concentration ranges in parts per million or less throughout the respiratory cycle. Infants who receive iNO therapy should be monitored according to institutionally derived protocols designed to avoid the potential toxic effects associated with iNO administration. These effects include methemoglobinemia (secondary to excess nitric oxide concentrations), direct pulmonary injury (attributable to excess levels of nitrogen dioxide), and ambient air contamination.

(P. 344, 2nd col.). AAP also lists seven RECOMMENDATIONS. (Pp. 344-345). However, AAP is completely silent respecting excluding from iNO treatment any child patient diagnosed with pre-existing left ventricular dysfunction.

Lipshultz:

Lipshultz teaches that data or information gleaned from iNO studies in adults does not correlate or is otherwise probative of iNO studies in children. In other words, children with ventricular dysfunction must be diagnosed, understood, and treated differently than adult patients diagnosed with ventricular dysfunction. Relevant statements are found in the abstract:

> Many changing developmental properties of the pediatric myocardium and differences in the etiologies of ventricular dysfunction in children compared with adults [exist] ... invalidating the concept that children can safely be considered small adults for the purpose of understanding heart failure pathophysiology and treatment.

At page 2, the author states:

> The disease processes resulting in ventricular dysfunction are often different in children than adults. Many pediatric conditions have no close analogies in the adult ... [hence] the effects of intervention may be unlike those seen in adults.

And, at page 5, the author states:

> when trying to understand the proper therapy for children with ventricular dysfunction it is usually important not to view the child as a small adult and extrapolate the effects of ventricular dysfunction therapy for adult ischemia or post-infarction patients to the child where a multitude of non-ischemic, non post-infarction etiologies exist.

NINOS:

At page 597 under "Conclusions" it states:

> Nitric oxide therapy reduced the use of extracorporeal membrane oxygenation, but had no apparent effect of mortality, in critically ill infants with hypoxic respiratory failure.

As set forth in the "Results" section on page 597, the study included 121 infants in the control group and 114 infants in the nitric oxide group. Left ventricular dysfunction was not mentioned.

As to patient eligibility, NINOS states:

> Infants born at 34 or more weeks of gestation who required assisted ventilation for hypoxic respiratory failure and had an oxygenation index of at

> least 25 on two measurements made at least 15 minutes apart were eligible for the trial.
> Infants were considered ineligible for the study if they were more than 14 days old, had a congenital heart disease, or if it had been decided not to provide full treatment.

(P. 598 under "Study Patients").

Hayward 1996:

The ten patients (19 to 59 years old) in this study had severe LV dysfunction and secondary pulmonary hypertension. (See p. 81 under "Methods" and Results" headings). iNO was administered in 10, 20 and 40 ppm doses. (Id. at 2nd col.). The study concludes stating:

> Our results confirm the safety and utility of INO in short-term assessment of pulmonary hypertension in patients with severe cardiac impairment. The possibility of worsening cardiac function in some patients is worrisome, however, and suggests that INO should be used cautiously in such patients and only in combination with other treatments that have been shown to improve LV function. Safety guidelines for the use of INO were recently formulated. We recommend that these guidelines be expanded to include caution regarding the use of INO in patients with severe LV dysfunction. Further study of the haemodynamic effects of INO on the left ventricle is needed.

(P. 84).

Hayward 1997:

This study was conducted in eleven adults being 51-69 years old with normal LV function. (P. 49, under "Methods" heading). The objective of the study was to determine the effects of iNO on load-independent indexes of normal human LV function. (Id. under "Objectives" heading). The results were that iNO had no effect on steady state LV pressure, volume, contractility duration, active relaxation, diastolic compliance or PVR. (Id. under "Results" heading). Thus, it was concluded that 20 ppm of iNO does not significantly affect normal LV function. (Id. under "Conclusions" heading).

Roberts:

The study included 30 newborn infants having "severe hypoxemia even though they were receiving mechanical ventilation at an FiO_2 of 1.0" (p. 606 under "Criteria for Eligibility") to determine whether iNO decreases severe hypoxemia in infants with persistent pulmonary hypertension. (See Abstract and Results, p. 605). The study concluded that "[i]nhaled nitric oxide improves systemic oxygenation in infants with persistent pulmonary hypertension and may reduce the need for more invasive treatments." (See Conclusions, p. 605).

Roberts further states under the "Criteria for Eligibility" heading:

> Infants were excluded from the study if they had any of the following: previous treatment with extracorporeal membrane oxygenation or high-frequency oscillatory or jet ventilation, a congenital diaphragmatic hernia or suspected lung hypolasia, structural cardiac lesions (other than a patent ductus arteriosus), uncorrected hypotension (a mean aortic pressure below 40 mm Hg) or polycythemia (an arterial hematocrit of at least 70 percent), an unevacuated pneumothorax, or a phenotype consistent with a lethal chromosomal abnormality. Since infants who have received exogenous surfactant without sustain increases in systemic oxygenation have responses to inhaled nitric oxide similar to those of infants not previously treated with surfactant, they were not excluded from the study.

Loh:

This is a study of 19 patients with an average age of 52 +/- 3 years. (See p. 2780 under "Study Population" heading). These adult patients suffered from ischemic cardiomyapathy (heart failure due to coronary artery disease and resultant partial cardiac muscle death) and idiopathic dilated cardiomyopathy. (Id.). Fourteen of the patients were diagnosed with left ventricular dysfunction. (See p. 2780 under "Methods and Results" heading).

Loh discloses:

> The most prominent hemodynamic effect of NO inhalation was the increase in pulmonary artery wedge pressure (median increase 26%).
> Thus, more severe LV dysfunction (as evidenced by higher left heart filling pressures, lower stroke volume, and larger LV cavity size) was present in the

patients who had the largest increases in pulmonary artery wedge pressure with inhaled NO.

(P. 2782 under "Hemodynamic Determinants of an Increase in Pulmonary Artery Wedge Pressure With Inhaled NO" heading).

Loh further discloses:

The major finding of this study is that in patients with reactive pulmonary arterial hypertension secondary to LV failure, inhalation of NO causes reciprocal changes in the PVR (decrease) and LV filling pressure (increase). In contrast, in patients with LV failure, we found that inhalation of NO is associated not with a decrease in pulmonary artery pressure, but rather, with an increase in LV filling pressure that accounts for the decrease in PVR.

(P. 2783 under "Discussion" heading).

Inglessis 2004:

This is a study of 13 patients with an average age of 65 +/- 3 years. (See p. 793 under "Methods" heading). The objective of the study was to see if iNO improved "cardiac performance in patients with RVMI and CS." (See p. 794).

Under the "Methods" heading at p. 794, the reference discloses:

Patients were then included for further study if their right atrial (RA) pressure was >10 mm Hg, their PCWP was no >5 mm Hg higher than the RA pressure, and their CI was <2.5 $l/min/m^2$. Patients were excluded from the study if they had severe pulmonary edema (PCWP >25 mm Hg; n=4), mechanical complications of MI requiring urgent surgical correction (N=0), severe mitral or aortic valvular disease (n=1), persistent hemodynamically significant tachyarrhythmias (n=1), or a history of clinically significant pulmonary disease (n=0).

The reference further discloses:

In this study, PCWP did not change during NO inhalation by RVMI patients, as has been previously observed during administration to patients with severe LV systolic dysfunction. In patients with severe LV systolic dysfunction, which is usually accompanied by poor diastolic ventricular compliance, breathing NO is thought to increase pulmonary venous return, resulting in an increase in LV filling pressure. The RVMI patients in this study had primarily RV systolic and diastolic function, and the degree of LV dysfunction was not as severe as in

> those patients in whom the PCWP has been reported to increase during NO inhalation.

(P. 797, 2nd col.).

Inglessis 2005:

In a reply, the author states "[p]atients with severe LV systolic function should be monitored carefully during chronic NO inhalation because of the possibility of their developing pulmonary venous hypertension." (P. 965, 2nd col.).

Bocchi:

This study included 3 patients ages 40, 41, and 52 years old suffering from either ischemic or idiopathic cardiomyapathy. (P. 70, 1st col.). All three adults had severe pulmonary HTN and refractory heart failure and were candidates for cardiac transplantation. (Id.) All three patients were treated with iNO.

The reference discloses:

> Results of this investigation demonstrate that acute inhaled nitric oxide produces rapid pulmonary vasodilation in the absence of hypoxia in patients with severe heart failure. However, nitric oxide inhalation was associated with an increment in pulmonary pressure, mainly pulmonary wedge pressure, and an improvement in cardiac output. In addition, inhaled nitric oxide may lead to pulmonary edema in patients with severe heart failure.

(P. 71, 1st col.).

Cujec:

This is a case study involving 33 adults with a mean age of 69 +/- 11 years, most of whom had significant valvular disease and dysfunctional LV characterized by a reduced ejection fraction. (P. 816 under "Patients" heading, and p. 819 under "Results" heading).

Cujec concludes at page 823 stating:

> We found in a randomized and blinded trial that the reduction in pulmonary artery systolic pressure following nitric oxide inhalation depends on the pre-existing LVEF. Our results in patients with a broader mix of cardiac pathology confirm previous case series. These observations suggest further limitations for the clinical role of inhaled nitric oxide. We postulate that in patients with the

least cardiac reserve, decreasing venous but not arterial pulmonary vascular resistance may cause an increase in regional pulmonary edema. Through reflex mechanisms, this could further impair cardiopulmonary function resulting in cardiac decompensation, worsening pulmonary hypertension and generalized pulmonary edema. This study cautions against the ubiquitous use of inhaled nitric oxide in the treatment of all critically ill patients. Nitric oxide is not just a pulmonary vasodilator but has profound effects on many other systems. The adverse effects of nitric oxide may become most evident in patients with the least cardiac reserve.

Rosales:

This is a case report of a one-month old neonate that developed rebound pulmonary hypertension after receiving iNO. (See Abstract at p. 224). The infant patient was diagnosed with total anomalous pulmonary venous return (three pulmonary veins draining into the portal system below the diaphragm and the remaining upper left pulmonary vein draining into the innominate vein). (Id.).

This infant underwent surgical correction and in the post operative period received iNO. (See p. 225, 1st col.). iNO was discontinued based on the rationale that the episode of pulmonary HTN may have been caused by left atrial hypertension secondary to a sudden increase in pulmonary blood flow into a non-compliant left atrium and ventricle due in part to the redirection of blood flow from the surgical correction. (See p. 225, 2nd col.).

Argenziano:

This study in pigs resulted in the following conclusion:

In conclusion, we have reproduced, in a porcine model of heart failure and pulmonary hypertension, the constellation of clinically observed hemodynamic responses to inhaled NO therapy, including dose-dependent decreases in pulmonary arterial pressure and PVR and increases in LVEDP. Furthermore, determination of the ESPVR, PRSW, EDPVR, and T in these animals has demonstrated no effect of inhaled NO on myocardial contractility or relaxation. An alternative explanation that has been proposed on theoretical grounds is that volume shifts caused by pulmonary vasodilation are responsible for clinically observed elevations in left atrial pressure and may also explain why patients with preexisting ventricular dysfunction are at greatest risk for these pressure elevations. Although clinical validation of our findings in humans is necessary and is the subject of current investigations, an understanding of this mechanism may lead to strategies allowing the safe use of inhaled NO in heart failure, perhaps by adjunctive vasodilator therapy.

(P. 707).

Steinhorn 2007:

This is a review article of persistent pulmonary HTN. It is a general discussion and review, not a clinical study. No data is provided. It points out that iNO is contraindicated in congenital heart disease (e.g., interrupted AO arch, critical AO stenosis, and hypolplastic LV) and severe LV dysfunction.

Under the heading "Treatment with iNO," it states:

> Treatment with iNO for newborns with an OI>25. Nitric oxide (NO) is an endothelial-derived gas signaling molecule that relaxes vascular smooth muscle and that can be delivered to the lung by means of an inhalation device (INOVent; Datex-Ohmeda Ind, Madison, WI).
> In 2 large randomized trials, NO reduced the need for ECMO support by approximately 40%.
> Contraindications to iNO include congenital heart disease characterized by left ventricular outflow tract obstruction (eg, interrupted aortic arch, critical aortic stenosis, hypoplastic left heart syndrome) and severe left ventricular dysfunction.

Krasuski:

This reference reports the results of a clinical study in forty-two adult patients (26 to 77 years old) having pulmonary hypertension during cardiac catheterization and receiving iNO. (See Abstract, p. 2204). The reference concludes that

> Nitric oxide is a safe and effective screening agent for pulmonary vasoreactivity. Regardless of etiology of pulmonary hypertension, pulmonary vasoreactivity is frequently demonstrated with the use of NO. Right ventricular diastolic dysfunction may predict a poor vasodilator response.

(Id. under "Conclusions" heading).

Semigran:

This study included 16 adults (13 men and 3 women) having a mean age of 51 ± 2 years each having class III or IV heart failure and being considered for heart transplantation. (See p. 983, 1st col.). No patient had a history of primary pulmonary disease, and pulmonary function testing was consistent with chronic left heart failure. (Id.). The patients were treated with digoxin, diuretic drugs, vasodilators and amiodarone. (Id.) iNO was administered at 20, 40 and 80 ppm. (Id. at 2nd col.).

The reference concludes stating:

> Inhaled nitric oxide is a selective pulmonary vasodilator in patients with severe chronic heart failure. The selectivity of inhaled nitric oxide for the pulmonary circulation offers a potential advantage over nonselective vasodilators such as nitroprusside in the identification of reversible pulmonary vasoconstriction in potential heart transplant recipients. Nitric oxide increases left ventricular filling pressure in patients with severe heart failure by an unknown mechanism.

(P. 982 under "Conclusions" heading).

Dickstein:

The reference teaches mathematical (see Appendix at p. 720) and electric circuit (see Figure 1 at p. 717) models of a cardiovascular system as "time varying elastances: the pulmonary and systemic vascular systems were each modeled as a series of resistive and compliance elements." (P. 715 under "Methods" heading).

The reference concludes stating:

> Pulmonary vasodilation by itself can lead to an increase in pulmonary venous pressure that is mediated by shifts of blood between arterial and venous compartments of the pulmonary bed. Furthermore, impairment in ventricular contractile state by itself has relatively little effect on pulmonary venous pressure. The magnitude of the increase in pulmonary venous pressure is largely determined by the volume status and the initial value of pulmonary vascular resistance.

(P. 715 under "Conclusions" heading).

Dickstein further discloses:

> The present analysis suggests that it is not necessary for this agent [i.e., nitric oxide] to work as a negative inotrope to cause pulmonary venous pressure to rise: its pulmonary vasodilating actions alone are sufficient to explain why patients with preexisting heart failure are at greatest risk for pulmonary edema.

(P. 719, 2nd col.).

Henrichsen:

This reference is a letter to the editor of journal reporting iNO treatment of a baby born at 38 weeks of gestation diagnosed with persistent pulmonary hypertension of the newborn (PPHN) and severe left ventricular dysfunction. The baby was treated with 20 ppm iNO which "resulted in an immediate fall in the mean systemic arterial blood pressure from 48 to 35 mm Hg, which reversed when the NO therapy was discontinued." In other words, the iNO caused systemic hypotension.

As second iNO treatment thirty hours later "resulted in a marked improvement in oxygenation, from an arterial oxygen tension to 16 to 420 mm Hg without a change in the systemic arterial blood pressure."

Ovodov:

The review article discusses various clinical studies of PPHN using iNO. (P. 95, 2nd col.). In particular, the reference cites the NINOS trial. (Id.) It concludes that "[s]afety of low-dose inhaled nitric oxide in newborns has been suggested by several studies" and that "there are no reports of any related adverse clinical manifestations." (P. 96, 1st col.).

Adatia:

This reference reports the results of a study involving 11 patients ranging in age from 0.7 to 27 years with a median of 13 years diagnosed with pulmonary hypertension. (P. 1656, 2nd col.). Some of the patients were diagnosed with "severe left ventricular failure despite optimal medical management with digoxin, diuretic drugs and, when appropriate, maximal afterload reduction therapy." (P. 1657, 1st col.).

The reference concludes stating:

> These preliminary observations suggest that nitric oxide is a potent pulmonary vasodilator with minimal systemic effects. It may be useful in discriminating patients needing combined heart and lung transplantation from those requiring exchange of the heart alone.

(P. 1656 under Conclusions heading).

Findlay:

This reference is a case report concerning a 22-year old man treated with iNO where the patient had a "paradoxical response to inhaled nitric oxide, where a rise in mean pulmonary artery and pulmonary artery occlusion pressure and a fall in cardiac output and stroke volume occurred, in a young man with meningococcaemia." (P. 134, 1st col.).

Henrichsen is a report of a single near-term neonate having PPHN and LVD that experienced systemic hypotension when treated with iNO, which is contrary to the accepted understanding that is a selective vasodilator, i.e., non-systemic. Moreover, the subsequent iNO treatment had a positive therapeutic outcome. Henrichsen fails to teach LVD as exclusionary criteria in the claimed patient population, and it teaches away from the invention by merely cautioning iNO treatment.

The instant claims are patentable over Ovodov, Adatia and Findlay at least because each reference fails to teach or suggest excluding the claimed patient population having LVD from being treated with iNO.

9(C) Detailed Explanation of Patentability

None of the references disclose excluding from iNO treatment any patient in the patient population (comprising children) that have been diagnosed as having pre-existing left ventricular dysfunction (LVD) in order to avoid adverse events or serious adverse events. (See independent claims 1, 6, 13 and 17). Thus, independent claims 1, 6, 13 and 17 are patentably novel and nonobvious over the listed most relevant references as well as the other references of record. Moreover, dependent claims 2-5, 7-12, 14-16 and 18-20 are patentably novel and nonobvious for at least the same reasons set forth herein respecting independent claims 1, 6, 13 and 17.

The AAP reference is highly relevant due to the prominence of the Pediatric Committee. The fact that it is silent respecting excluding from iNO treatment any child patient diagnosed with pre-existing left ventricular function speaks louder than words.

Lipshultz teaches that data and information gleaned from iNO studies in adults do not correlate or are otherwise probative of iNO studies in children. Thus, the Hayward 1996 & 1997, Loh, Inglessis 2004 & 2005, Bocchi, Cujec, Krasuski, Findlay and Semigran references are not probative of the instantly claimed invention.

Pre-existing LVD is not mentioned in the NINOS reference involving infants. While the Roberts involves neonate patients, it fails to teach excluding such patients if they have been diagnosed with pre-existing LVD.

Rosales involves a one-month old neonate patient undergoing surgical correction and post operative iNO treatment. Rosales also fails to teach or suggest pre-existing LVD as exclusionary criteria for iNO treatment.

Argenziano is a pig study that also fails to teach or suggest pre-existing LVD as exclusionary criteria for iNO treatment.

Steinhorn 2007 is a general discussion and review. No data is provided. Therefore, Steinhorn 2007 is a non-enabling reference.

Dickstein is a "purely theoretic analysis of the impact of NO therapy on pulmonary venous pressure." (P. 719, 2nd col.). The reference fails to disclose any data to support this unpredictable science which is also not well understood, therefore, Dickstein is non-enabling prior art. The reference also teaches away from excluding a patient from being treated with iNO where the patient has been diagnosed with pre-existing LVD. In contrast, the reference theorizes that increased volume causes the risk of adverse events stating:

> results of the present analysis would suggest that patients with heart failure are at increased risk for development of pulmonary edema during NO therapy because of the high effective volume status.

(P. 719, 2nd col.).

Henrichsen is a report of a single near-term neonate having PPHN and LVD that experienced **systemic** hypotension when treated with iNO, which is contrary to the accepted understanding that nitirc oxide is a selective vasodilator, i.e., non-systemic. Moreover, the subsequent iNO treatment had a positive therapeutic outcome. Henrichsen fails to teach LVD as exclusionary criteria in the claimed patient population, and it teaches away from the invention by merely cautioning iNO treatment.

The instant claims are patentable over Ovodov, Adatia and Findlay at least because each reference fails to teach or suggest excluding the claimed patient population having LVD from being treated with iNO.

9(D) Concise Statement of Utility

The instantly claimed invention is eligible subject matter under 35 USC 101 for patentable utility in that the claims are generally directed to a method of excluding patients in need of being treated with inhaled nitric oxide. The purpose of such mandatory exclusion is to reduce the incidence of adverse events or serious adverse events. Patients in an intended patient population are excluded from such treatment (even though the inhaled nitric oxide treatment would be potentially beneficial to the patient) if the patient has pre-existing left ventricular dysfunction.

9(E) Showing of Support under 35 USC 112, First Paragraph

Support and antecedent basis for the claimed invention is found at least in the SUMMARY OF THE INVENTION as originally filed at pages 2-4 and ¶¶[0005]-[0020]. Enablement of the claimed invention is found at least in the DETAILED DESCRIPTION OF THE EXEMPLARY EMBODIMENTS at pages 4-13 and ¶¶[0021]-[0050] as well as in EXAMPLE1: INOT22 STUDY at pages 13-22 and ¶¶[0051]-[0069].

9(F) Identification of References Disqualified as Prior Art under 35 USC 103(c)

None of the cited references are disqualified as prior art under 35 USC 103(c).

Respectfully Submitted,

Christopher P. Rogers, Reg. No. 36,334

Date: 21 June 2010

Lee & Hayes, PLLC
601 W. Riverside Avenue, Suite 1400
Spokane, WA 99201

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Application Serial Number	TBD
Confirmation Number	TBD
Filing Date	Herein
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	TBD
Examiner	TBD
Attorney Docket Number	I001-0002USC4

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

From: Christopher P. Rogers (Tel.; Fax 509-323-8979)
Customer Number: 49584
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601 W. Riverside Avenue, Suite 1400
Spokane, WA 99201

Fees will be paid by credit card through the EFS Web; however the Commissioner is hereby authorized to charge any deficiency of fees and credit any overpayments to Deposit Account Number 12-0769.

Respectfully Submitted,

Dated: 21 June 2010

By: [signature]
Christopher P. Rogers
Reg. No. 36,334

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Priority Application Serial No	12/494,598
Priority Filing Date	06/30/2006
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Priority Group Art Unit	1614
Priority Examiner	TBD
Attorney Docket Number	I001-0002USC4

INFORMATION DISCLOSURE STATEMENT

The citations listed are submitted in compliance with the duty of disclosure defined in 37 CFR §1.56. Copies of the cited references were cited or submitted with the priority application and are therefore not submitted herewith.

The Examiner is requested to make these citations of official record in this application.

Respectfully Submitted,

Date: 21 June 2010

By: [signature]
Christopher P. Rogers
Reg. No. 36,334

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Examiner Name	
	Attorney Docket Number	I001-0002USC4

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	4	WO2010019540;(A1)			2010-02-18	NOVARTIS AG [CH]; PASC		☐

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
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CERTIFICATION STATEMENT

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☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement.

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(A1) The present invention relates to a method for the detection of predisposition to high altitude pulmonary edema (HAPE). It particularly relates to an allelic variants of iNOS (inducible nitric oxide synthase) gene, which has been found to be related with the prevalence of HAPE.

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(54) **Title:** METHOD OF DETECTING PREDISPOSITION TO HIGH ALTITUDE PULMONARY EDEMA

(57) **Abstract:** The present invention relates to a method for the detection of predisposition to high altitude pulmonary edema (HAPE). It particularly relates to an allelic variants of iNOS (inducible nitric oxide synthase) gene, which has been found to be related with the prevalence of HAPE.

METHOD OF DETECTING PREDISPOSITION TO HIGH ALTITUDE PULMONARY EDEMA

TECHNICAL FIELD

The present invention relates to a method for the detection of predisposition to high altitude pulmonary edema (HAPE). It particularly relates with the allelic variants of iNOS (inducible nitric oxide synthase) gene, which has been found to be related with the prevalence of HAPE.

BACKGROUND AND PRIOR ART

High altitude pulmonary edema (HAPE) is a form of noncardiogenic pulmonary edema that develops in approximately 10% of randomly selected mountaineers within 24h after rapid ascent to altitude above 4,000 m. A similar phenomenon is observed in the lowlander inductees to a height above 3000 m for various business reasons. An even higher incidence rate of about 60% has been demonstrated in subjects who are susceptible to HAPE as documented by previous occurrence of the disease (Houston CS et al 1960, Bartsch P et al 1997, 1990). HAPE can be effectively prevented by prophylactic use of vasodilators or slow ascent. Nevertheless, it remains the most common cause of death related to high altitude exposure during trekking or mountaineering (Hackett PH et al 1990). The morbidity rate in Himalayan mountaineers was estimated to be 50% if immediate treatment with supplemental oxygen or rapid descent is impossible (Lobenhoffer HP et al 1982). Observed differences in clinical presentations and severity of the disease between racial and ethnic groups together with familial clustering favor a significant hereditary predisposition to the disease.

Although knowledge of the factors influencing the development of HAPE is still incomplete, there is experimental evidence that an exaggerated hypoxic pulmonary vasoconstriction (HPV) plays an important role (Scherrer U et al 1996). An excessive rise in pulmonary artery pressure has been demonstrated by invasive and noninvasive measurements at high altitude in individuals with HAPE. The uneven vasoconstriction in the capillaries sometimes results in "capillary leakage" followed by edema formation (Bartsch P et al 1991). Human subjects who are susceptible to the disease demonstrate an increased pulmonary vascular response even during a brief exposure of high altitude. The underlying pathophysiological mechanism for this exaggerated HPV is still unknown. There is, however, evidence that the endogenous vasodilator nitric oxide (NO) modulates vascular reactivity (Palmer RMJ et al 1987). Regulation of vascular tone by NO is attributed to the intermediates of cGMP pathway (Bellamy TC et al 2002).

CONFIRMATION COPY

The following studies emphasize the involvement of NO in HAPE:
NO exerts its effect mainly via improvement of ventilation/perfusion ratio and lowering of alveolar to arterial oxygen tension difference by increasing arterial oxygen saturation (Scherrer U et al 1996). However, in the healthy volunteers, administration of the NO synthesis antagonist N^G-monomethyl-L-arginine (L-NMMA) during hypoxia increases pulmonary artery pressure and vascular resistance which is similar to that observed in HAPE. Due to this NO has been used as an inhalation therapy for the treatment of HAPE in the affected individuals (Anand IS et al 1998).
Phosphodiesterase 5 is the key enzyme responsible for cGMP hydrolysis in the lungs. The inhibitors of Phosphodiesterase 5 have been found to inhibit hypoxia induced pulmonary hypertension (Goldstein I et al 1998). Hypoxia decreases exhaled NO in mountaineers susceptible to HAPE indicating decreased NO production in such cases (Busch et al 2001). Thus defective NO synthesizing machinery imparting lower NO level may be envisaged to be responsible for the pathogenesis of HAPE. NO is synthesized by three isozymes nNOS (neuronal nitric oxide synthase, NOS1), iNOS (inducible nitric oxide synthase, NOS2) and eNOS (endothelial nitric oxide synthase, NOS3) (Michel T et al 1997). NOS1 and NOS3 are constitutively expressed while NOS2 is expressed upon induction. Among these the best candidate which is supposed to be defective in HAPE is eNOS (endothelial nitric oxide synthase) while induction of iNOS (inducible nitric oxide synthase) seems to be inevitable for the immediate recovery of the total NO reserve (Xia Y et al 1998). Moreover, robust cell signaling mechanisms generally favor the recruitment of inducible genes for immediate early physiological responses. It can be speculated that a defect in iNOS which doesnot permit its activation may not recover the reduced NO level in individuals exposed to hypoxia resulting in HAPE.
The defect in iNOS may occur at genetic level in HAPE patients. In numerous cases, the expression of the genes has been found to get altered by the polymorphisms in the gene sequence (Qadar Pasha MA et al 2001). Hence, it is always possible that polymorphism in iNOS gene may alter its
expression and associates with the disease.
Current status of the treatment of HAPE:
1. NO therapy: NO is being used as an inhalation therapy for the treatment of HAPE. It exerts its effect mainly via improvement of ventilation/perfusion ratio and lowering of alveolar to arterial oxygen tension difference by increasing arterial oxygen saturation.
NO induced improvement in arterial oxygenation in subjects with HAPE was accompanied

by a shift in blood flow in the lung away from edematous segments and toward nonedematous segments results in evening/homogeneity of the vasoconstriction throughout the capillaries (Scherrer U et al 1996, Anand IS et al 1998).

2. Rapid descent: Rapid descent of HAPE patients not only prevents the worsening but even improves the pathogenesis of the disease (Hackett PH et al 2001).

3. Portable Air Chambers (PACs): PACs in the form of small cylinders filled with oxygen is often used as inhalation therapy for HAPE (Hackett PH et al 2001).

4. Genetic predisposition: The only study in this context suggests that genetic variation in endothelial nitric oxide synthase gene (eNOS) and angiotensin converting enzyme gene (ACE) may predispose individuals to HAPE (Droma Y et al 2002). The results are as follows:

	Controls	Patients
Glu298Asp (eNOS)	9.8%	25.6%
B/A (eNOS)	6.9%	32.2%
I/D (ACE)	4%	22%

Limitations of the available therapies for HAPE:

1. HAPE patients do not found to have homogenous response to NO inhalation. Moreover, concentration of required NO varies with the severity of the disease. Sometimes inadequate inhalation results in hypotension or even septic shock to the patients.
2. Immediate descent of the HAPE patients often remains impossible due to severe weather and rugged terrain (Anand IS et al 1998, Hackett PH et al 2001).
3. Carriage of PACs sometimes appears to be not feasible due to overloading problem. Improved conditions of the disease are often temporary as removal of chambers renders the patient worse (Hackett PH et al 2001).
4. The reported polymorphisms associated with HAPE are not specific but have also been shown to be associated with the disorders like diabetes, coronary artery disease, hypertension and myocardial infarction where elevated blood pressure is observed

(Monti LD et al 2003, Via M et al 2003). The allelic frequency difference mentioned appears to be the same with other diseases. Hence the possibility of allelic contribution to the disease may be due to other related pathophysiologies like hypertension, which involves the exacerbations of HAPE. Moreover, the study does not include HA natives (high landers), a population residing blissfully in the same environment where the disease occurs.

Novelty of the invention is in providing a novel method for the detection of predisposition to HAPE.

Still another novelty is for providing a novel marker region in iNOS gene.

Still another novelty is for providing a novel SNP in iNOS gene.

Still another novelty is to demonstrate association of the allelic variants of iNOS gene with HAPE.

Another novelty is to provide novel primers and probes for amplification, which contains the novel SNP.

OBJECTS OF THE INVENTION:

Main object of the present invention is to provide a method for the detection of predisposition to HAPE, which obviates the limitations listed above.

Still another object is providing a novel SNP in iNOS gene.

Another object is to provide novel primers and probes for amplification, which contains the novel SNP.

Another object is to perform association analysis for the allelic variants between low landers and HAPE patients so that the relation with the disease could be scored.

SUMMARY OF THE INVENTION:

The present invention relates to the method of detection of predisposition to HAPE. It particularly relates with the allelic variants of iNOS gene, which has been related to the prevalence of HAPE. Defective Nitric Oxide (NO) synthesizing machinery imparting lower NO level has been envisaged to be responsible for the pathogenesis of HAPE. iNOS gene has been shown to be responsible for NO production as the inhibitors of NO production increased the severity of HAPE. Present invention provides a method for detection of predisposition to HAPE as the novel allelic variants of iNOS gene in the disclosed marker region was shown to be negatively associated with the prevalence of HAPE in a population.

BRIEF DESCRIPTION OF ACCOMPANYING FIGURES/DRAWINGS

Figure 1 Schematic representation of the gene of inducible Nitric Oxide Synthase (iNOS) localization: 17 cenq[11.2]. The vertical bars showing the exonic regions (From Gene bank Nucelotide Sequence ID No. NT_010799).

Figure 2 shows sequence file of the individual with AA homozygote.

Figure 3 shows sequence file of the individual with GG homozygote.

Figure 4 shows sequence file of the individual with AG heterozygote.

Figure 5 shows sequence file of the individual with TC heterozygote.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the preferred embodiments of the invention given for the purpose of disclosure. Alternative embodiments of the invention can be envisaged by those skilled in the art. All such alternative embodiments are intended to lie within the scope of this invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the method of detection of predisposition to HAPE. It particularly relates with the allelic variants of iNOS gene, which has been found to be related to the prevalence of HAPE.

I. Identification of the marker region on the iNOS gene:

Taking in consideration the important functions of NO at high altitude, iNOS, the inducible nitric oxide synthase gene was selected as the candidate gene for the study.

II. Selection of the study subjects:

Clinical severity of HAPE was assessed by Lake Louise acute mountain sickness (AMS) scoring system. Briefly, the patients were assessed for the presence of five symptoms: headache, gastrointestinal upset, fatigue, weakness, or both, dizziness, lightheadedness, or both, and difficulty in sleeping. Change in mental status, ataxia and peripheral edema were also assessed. Each of these symptoms were rated between 0 and 3. A score of 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. HAPE score is the sum of all 8 symptoms and patients were characterized by HAPE score>6 (Anand IS et al 1998). Lowlanders (LLs) were subjects who even after induction to high altitudes atleast thrice never found to have any of the above mentioned symptoms. High altitude (HA) natives were the permanent residents of HA from ancient times.

III. Extraction of genomic DNA from leukocytes:

Genomic DNA was extracted from blood using salting out method. Lysis of red blood cells in presence of high salt was followed by treatment with Nucleus lysis buffer (NLB). Proteins were precipitated and extraction of DNA was obtained in ethanol (Miller SA et al 1988).

IV. Identification of the allelic variants of the iNOS gene:

Novel polymorphism of the invention:

As a first step to the present invention, the applicants carried out the PCR amplification of marker region of the iNOS gene using self designed oligonucleotide primers. The primers were designed in accordance with the human iNOS gene sequence (Gene Bank Accession Number NT_010799). The sequencing of the purified PCR product revealed a novel single nucleotide polymorphism in Intron 7 of the human iNOS gene. It was apparent, therefore that there is a hitherto unrecognized allele or subtype of the human iNOS gene.

The present invention provides a sequence for the allelic variants of human iNOS gene comprising the following novel single nucleotide polymorphism compared with the human iNOS gene sequence in the database.

For example, the nucleotide sequence of the allelic variant of human iNOS gene (SEQ ID NO: 1) having the polymorphic site listed in Table 1 may be-

5'CAGCGGAGTGATGGCAAGCACGACTTCCGGGTGTGGAATGCTCAGCT
CATCCGCTATGCTGGCTACCAGATGCCAGATGGCAGCATCAGAGGGGA
CCCTGCCAACGTGGAATTCACTCAGGTACCCGGCCCAGCCTCAGCC
A*/GCCGGCCATTGGGGCGGGGAGCCCCGTGGTGAGCGAGTGACAGAGT
GGAGCCCAGAGGAGACACGCAGCCCGGGCTTACAGACTCACAGGGCCC
GTCTTGTTCCCCAGCTGTGCATC3'

In the above sequence the SNP* is shown in bold.

Table 1

Site of change	Base change	Mutation type
19480	A/G	Transition

V. Association Analysis with the disease

Analysis of the SNP in 42 HA natives, 39 HAPE controls and 18 HAPE patients revealed three genotypes, namely AA, AG and GG. The distribution of alleles is summarized in Table 2.

Table 2

Study subjects	A	G
HAPE controls (n=39)	0.35	0.65
HAPE patients (n=18)	0.58	0.42
HA natives (n=42)	0.18	0.82

The frequency of the G allele was found to be in the order of HA natives>HAPE controls>HAPE subjects. The biostatistical analysis showed a significant association of G allele with HA adaptation and A allele with the disease as mentioned in Table 3.
Herein the odds ratio (OR) and 95% confidence of interval was used as a measure of the strength of the association between genotypic combination and the disease. P value of <0.05 was considered statistically significant.

Table 3

Association type	χ^2 value	p value	Odds ratio	95% CI	Relative risk
HAPE patients & HAPE controls	10.63	0.001	2.56	1.45-4.54	1.66 (1.21-2.27)
HAPE patients & HA natives	33.96	<0.001	6.29	3.30-12.01	3.22 (2.05-5.06)
HAPE controls & HA natives	7.42	0.006	-	-	-

Nitric oxide synthase for its reaction to synthesize nitric oxide, requires oxygen which acts as a cofactor in the reaction. Oxygen binds to the oxygenase domain in iNOS and contributes to the synthesis of NO. In hypoxic condition scarcity of oxygen may lead to lower NO production, however any modification in the oxygenase domain, which modify the activity of the enzyme in such a way that it requires no oxygen or less oxygen may contribute to normal NO production. NO improves oxygenation of hemoglobin and normal NO production may involve the mechanisms acting in acclimatization, hence any alteration in oxygenase domain may be favorable for the production of NO. In the present investigation the novel SNP found in intron 7 is present near to the oxygenase domain of NOS2 gene which spans exon 7 to exon 16. It is quite possible that the SNP found is in linkage disequilibrium to a nearby SNP, which is contributing to the final impact on NO production by NOS2 gene.

VI. Diagnostic kits

The invention further provides diagnostic kit comprising at least one or more allele specific oligonucleotides as described in SEQ ID 2 and 3. Often, the kits contain one or more pairs of allele-specific oligonucleotides hybridizing to different forms of a polymorphism. In some kits, the allele-specific oligonucleotides are provided immobilized to a substrate. For example, the same substrate can comprise allele-specific oligonucleotide probes for detecting at least the polymorphism shown in Table1. Optional additional components of the kit include, for example, restriction enzymes, reverse transcriptase or polymerase, the substrate nucleoside triphosphates, means used to label (for example, an avidin enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for reverse transcription, PCR, or hybridization reactions. Usually, the kit also contains instructions for carrying out the methods.

VII. Nucleic acid vectors

Variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer, which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage promoters, glycolytic enzyme promoters and tRNA promoters, depends on the host selected. Commercially available expression vectors can also be used. Suitable host cells include bacteria such as E.coli, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell lines and derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide.

The invention further provides transgenic non-human animals capable of expressing an exogenous variant gene and/or having achieved by operably linking the gene to a promoter and optionally an enhancer, and microinjecting the construct into a zygote. Inactivation of endogenous variant genes can be achieved by forming a transgene in which a cloned variant gene is inactivated by insertion of a positive selection marker. The transgene is then introduced in to an embryonic stem cell, where it undergoes homologous recombination with an endogenous variant gene. Mice and other rodents are preferred animals. Such animals provide useful drug screening systems.

Accordingly, the main embodiment of the present invention relates to a method for detecting predisposition to high altitude pulmonary edema (HAPE), said method comprising the steps of:

(a) selecting study subjects by monitoring high altitude pulmonary edema associated symptoms,
(b) extracting genomic DNA from leukocytes by conventional methods from the study subjects,
(c) amplifying Intron 7 of the human iNOS gene of SEQ ID No.1 by designing and synthesizing Forward and Reverse oligonucleotide primers of SEQ ID No. 2 and SEQ ID No. 3, respectively,
(d) identifying computationally novel Single Nucleotide Polymorphism (SNP) by comparing with the already existing sequence of human iNOS gene,
(e) screening the high altitude native population (HA natives), low lander natives (HAPE controls) and low lander HAPE patients for the novel single nucleotide polymorphism, using above said primers of SEQ ID No. 2 (Forward Primer) and SEQ ID 3 (Reverse Primer),
(f) computing the frequencies of AA, AG and GG genotypes in the populations of step (e) for establishing the association of the genotypes with high altitude pulmonary edema, and
(g) predicting and statistically analyzing the differences in the distribution of the allelic variants (AA, AG and GG genotypes) in the populations wherein GG genotype at 19480 position are at low risk to high altitude pulmonary edema and AA genotype at 19480 position are at high risk of the disease.

Another embodiment of the present invention relates to the oligonucleotide primers capable for amplification of Intron 7 of human iNOS gene are selected from group comprising of

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

Yet another embodiment of the present invention relates to the oligonucleotide primers contain one or more polymorphic sites selected group comprising of

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer.

Still another embodiment of the present invention relates to the allelic variants wherein the allelic variants of the of iNOS gene have AA, AG and GG genotypes

A diagnostic kit for the detection of SNP genotypes having predisposition to high altitude pulmonary edema (HAPE) said kit comprising of primers and probes:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

One more embodiment of the present invention relates to the Primers suitable for amplification of iNOS gene region containing one or more polymorphic sites, said primers include:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer

(b) SEQ ID 3: 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

In another embodiment of the present invention relates to the nucleic acid vectors containing the allelic variants of the iNOS gene.

The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of the present invention.

EXAMPLES

EXAMPLE 1

Identification of the marker gene:

Taking in consideration the important functions of NO at HA, iNOS, the inducible nitric oxide synthase was selected as the candidate gene for the study.

EXAMPLE 2

Selection of the study subjects:

Clinical severity of HAPE was assessed by Lake Louise acute mountain sickness (AMS) scoring system. Briefly, the patients were assessed for the presence of five symptoms: headache, gastrointestinal upset, fatigue, weakness, or both, dizziness, lightheadedness, or both, and difficulty in sleeping. Change in mental status, ataxia and peripheral edema were also assessed. Each of these symptoms were rated between 0 and 3. A score of 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. HAPE score is the sum of all 8 symptoms and patients were characterized by HAPE score>6 (Anand IS et al 1998). LLs were subjects who even after induction to high altitudes atleast thrice never found to have any of the above mentioned symptoms. HA natives were the permanent residents of HA from ancient times.

EXAMPLE 3

Extraction of genomic DNA from leukocytes:

Genomic DNA was extracted from blood using salting out method. Lysis of red blood cells in presence of high salt was followed by treatment with Nucleus lysis buffer (NLB). Proteins were precipitated and DNA was extracted from peripheral blood leukocytes using a modification of the salting out procedure. The concentration of the DNA was determined by measuring the optical density of the sample, at a wavelength of 260 nm. (Miller SA et al 1988).

EXAMPLE 4

Identification of the allelic variants of the iNOS gene:

This example describes the identification of allelic variants of iNOS gene by PCR and sequencing using certain oligonucleotide primers according to the invention. The DNA was then amplified by polymerase chain reaction by using the oligonucleotide primers:

1. 5'CAG CGG AGT GAT GGC AAG CAC GAC 3'(as listed in SEQ ID NO:2) and
2. 5' GAT GCA CAG CTG GGG AAC AAG ACG 3'(as listed in SEQ ID NO:3).

Polymerase chain reaction was carried out using the following conditions:

Step 1 94^0C for 4 min
Step 2 94^0C for 30 sec
Step 3 62.5 ^{0}C for 30 sec
Step 4 72 ^{0}C for 45 sec
Step 5 34 times to Step 2
Step 6 72^0C for 10 min

PCR was performed in a Perkin Elmer GeneAmp PCR System 9600. This reaction produced a DNA fragment of 258bp when analyzed by 2% agarose gel electrophoresis. The PCR product was purified from band cut out of agarose gel using a Amersham Pharmacia gel extraction kit (Amersham) and both the strands of the PCR product were directly sequenced using dye terminator chemistry on an ABI Prism 377 automated DNA sequencer. The PCR product was identical to the human iNOS gene sequence except of the novel single base pair change mentioned in Table1.

EXAMPLE 5

Nucleotide sequence of the Allelic Variant of the iNOS gene:

The nucleotide sequence of the allelic variant of iNOS gene derived using the method as described in example 1 -

5'CAG CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT GCC AAC GTG GAA TTC ACT CAG GTA CCC GGC CCA GCC TCA GCC **A*/G**CC GGC CAT TGG GGC GGG GAG CCC CGT GGT GAG CGA GTG ACA GAG TGG AGC CCA GAG GAG ACA CGC AGC CCG GGC TTA CAG ACT CAC AGG GCC CGT CTT GTT CCC CAG CTG TGC ATC 3'

In the above sequence the SNP* is shown in bold.

EXAMPLE 6

G allele is related with adaptation and A allele associates with the disease:

A method as described in example 4 is applied to a series of DNA samples extracted from HA natives, HAPE controls and HAPE patients. A highly significant association of G allele with the HA adaptation and A allele with the disease has been observed. The results are summarized in the table below:

Association type	χ^2 value	p value	Odds ratio	95% CI	Relative risk
HAPE patients & HAPE controls	10.63	0.001	2.56	1.45-4.54	1.66 (1.21-2.27)
HAPE patients & HA natives	33.96	<0.001	6.29	3.30-12.01	3.22 (2.05-5.06)
HAPE controls & HA natives	7.42	0.006	-	-	-

Hence, individuals with GG genotype being at low risk and those with AA genotype being at high risk for HAPE, can be expected to hold true for other populations also.

EXAMPLE 7

Nucleic acid vectors containing the iNOS variant sequences:

Vectors and host cells transformed with the allelic variants of the iNOS gene containing one or more polymorphic sites as listed in table 1, can be prepared, for example, as detailed below.

Variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer, which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage, glycolytic enzyme and tRNA, depends on the host selected. Commercially available expression vectors can also be used. Suitable host cells include bacteria such as E.coli, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell lines and derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide.

Advantages of the present invention:

The present invention adds following points to the treatment of HAPE.

1. Inducible nitric oxide synthase gene as a novel marker for HAPE studies.
2. Novel primer sequences responsible for the amplification of PCR product containing novel SNP.
3. Novel SNP (19480 A/G) that can be used for further association studies.
4. A significant association of wild type allele (A) to the disease (Table 2 and 3).
5. A significant association of mutant allele (G) to adaptation (Table 2 and 3).
6. A significant difference between the frequency of alleles with respect to HA native and HAPE controls (Table 2 and 3).
7. The presence of G allele predisposes an individual to less chances of getting diseased.
8. It may help individuals to decide visiting high altitude for various reasons.

Provided below is the sequence listing information for SEQ ID Nos. 1, 2 and 3

SEQUENCE LISTING

<u>GENERAL INFORMATION</u>

APPLICANT: CSIR

TITLE OF INVENTION: Method for the detection of predisposition to high altitude pulmonary edema (HAPE).

NUMBER OF SEQUENCES: 03

CORRESPONDING ADDRESS: Institute of genomics and integrative biology, CSIR, Delhi University Campus, Mall Road-110007, India.

Telephone: +91-11-27666156 Fax: +91-11-27667471

<u>INFORMATION FOR SEQUENCE ID NO: 1</u>

1. SEQUENCE CHARACTERISTICS:

   1. LENGTH: 258 bp

   2. TYPE: <u>DNA</u>

5'CAG CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT GCC AAC GTG GAA TTC ACT CAG GTA CCC GGC CCA GCC TCA GCC **A*/G**CC GGC CAT TGG GGC GGG GAG CCC CGT GGT GAG CGA GTG ACA GAG TGG AGC CCA GAG GAG ACA CGC AGC CCG GGC TTA CAG ACT CAC AGG GCC CGT CTT GTT CCC CAG CTG TGC ATC 3'

   3. ORGANISM: *Homo sapiens* (Humans)

   4. IMMEDIATE SOURCE: PCR

   5. NAME/KEY: Marker Region

   6. SEQUENCE ID # 1

<u>INFORMATION FOR SEQUENCE ID NO: 2</u>

1. SEQUENCE CHARACTERISTICS:

LENGTH: 24 bp

TYPE: DNA

5'CAG CGG AGT GAT GGC AAG CAC GAC 3'

ORGANISM: Artificial sequence

IMMEDIATE SOURCE: Synthetic

NAME/KEY: Synthetic Oligonucleotide

SEQUENCE ID # 2

<u>INFORMATION FOR SEQUENCE ID NO: 3</u>

1. SEQUENCE CHARACTERISTICS

LENGTH: 24 bp

TYPE: DNA

5' GAT GCA CAG CTG GGG AAC AAG ACG 3'

ORGANISM: Artificial sequence

IMMEDIATE SOURCE: Synthetic

NAME/KEY: Synthetic Oligonucleotide

SEQUENCE ID # 3

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Synthase Gene Polymorphisms With High-Altitude Pulmonary Edema.Circulation 2002;106: 826-830.

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We claim:

1. A method for detecting predisposition to high altitude pulmonary edema (HAPE), said method comprising the steps of:

   (a) selecting study subjects by monitoring high altitude pulmonary edema associated symptoms,
   (b) extracting genomic DNA from leukocytes by conventional methods from the study subjects,
   (c) amplifying Intron 7 of the human iNOS gene of SEQ ID No.1 by designing and synthesizing Forward and Reverse oligonucleotide primers of SEQ ID No. 2 and SEQ ID No. 3, respectively,
   (d) identifying computationally the Novel Single Nucleotide Polymorphism (SNP) by comparing with the already existing sequence of human iNOS gene,
   (e) screening the high altitude native population (HA natives), low lander natives (HAPE controls) and low lander HAPE patients for the novel single nucleotide polymorphism, using above said primers of SEQ ID No. 2 (Forward Primer) and SEQ ID 3 (Reverse Primer),
   (f) computing the frequencies of AA, AG and GG genotypes in the populations of step (d) for establishing the association of the genotypes with high altitude pulmonary edema, and
   (g) predicting and statistically analyzing differences in the distribution of the allelic variants (AA, AG and GG genotypes) in the populations and wherein GG genotype at 19480 position are at low risk to high altitude pulmonary edema and AA genotype at 19480 position are at high risk to of the high altitude pulmonary edema.

2. A method as claimed in claim 1 wherein, the oligonucleotide primers capable for amplification of Intron 7 of human iNOS gene are selected from group

   (a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and
   (b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

3. A method as claimed in claim 3 wherein, the oligonucleotide primers contain one

or more polymorphic sites selected group comprising of:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer.

4. A method as claimed in claim 1 wherein, the allelic variants of iNOS gene have AA, AG and GG genotypes.

5. A diagnostic kit for the detection of SNP genotypes having predisposition to high altitude pulmonary edema (HAPE) said kit comprising of primers and probes:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

6. A pair of primers suitable for amplification of iNOS gene region containing one or more polymorphic sites, said primers include:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer

(b) SEQ ID 3: 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

7. The nucleic acid vectors containing the allelic variants of the iNOS gene.

AMENDED CLAIMS

[received by the International Bureau on 31 August 2004 (31.08.2004); original claims 1-7 replaced by new claims 1-6 (2 pages)]

1. A method for detecting predisposition to high altitude pulmonary edema (HAPE), said method comprising the steps of:

   (a) selecting study subjects by monitoring high altitude pulmonary edema associated symptoms,
   (b) extracting genomic DNA from leukocytes by conventional methods from the study subjects,
   (c) amplifying Intron 7 of the human iNOS gene of SEQ ID No.1 by designing and synthesizing Forward and Reverse oligonucleotide primers of SEQ ID No. 2 and SEQ ID No. 3, respectively,
   (d) identifying computationally the Novel Single Nucleotide Polymorphism (SNP) by comparing with the already existing sequence of human iNOS gene,
   (e) screening the high altitude native population (HA natives), low lander natives (HAPE controls) and low lander HAPE patients for the novel single nucleotide polymorphism, using above said primers of SEQ ID No. 2 (Forward Primer) and SEQ ID 3 (Reverse Primer),
   (f) computing the frequencies of AA, AG and GG genotypes in the populations of step (d) for establishing the association of the genotypes with high altitude pulmonary edema, and
   (g) predicting and statistically analyzing differences in the distribution of the allelic variants (AA, AG and GG genotypes) in the populations and wherein GG genotype at 19480 position are at low risk to high altitude pulmonary edema and AA genotype at 19480 position are at high risk to of the high altitude pulmonary edema.

2. A method as claimed in claim 1 wherein, the oligonucleotide primers capable for amplification of Intron 7 of human iNOS gene are selected from group

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and
(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

3. A method as claimed in claim 2 wherein, the oligonucleotide primers identify one or polymorphic sites responsible for HAPE.

4. A method as claimed in claim 1 wherein, the allelic variants of iNOS gene have AA, AG and GG genotypes

5. A diagnostic kit for the detection of SNP genotypes having predisposition to high altitude pulmonary edema (HAPE) said kit comprising of primers and probes:
(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer
(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

6. A pair of primers suitable for amplification of iNOS gene region containing one or more polymorphic sites, said primers include

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer
(b) SEQ ID 3: 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

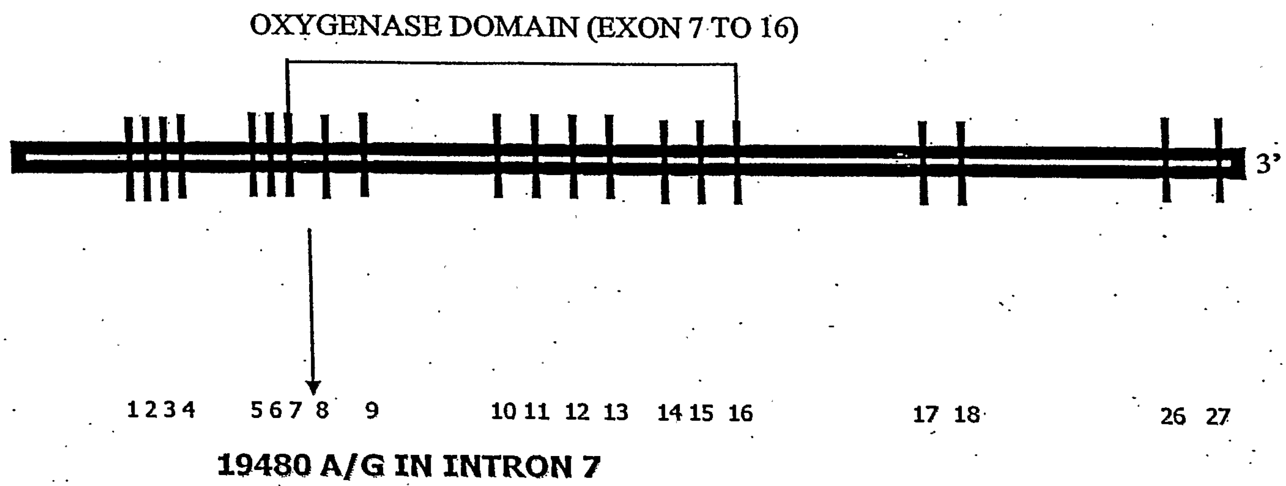
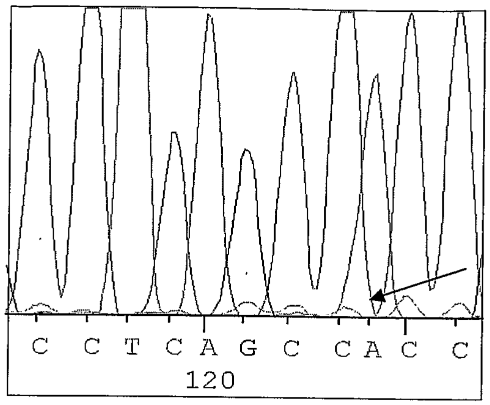
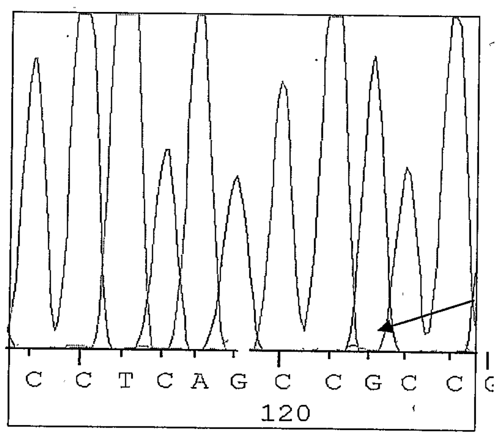

OXYGENASE DOMAIN (EXON 7 TO 16)
3'
1 2 3 4
5 6 7 8
9
10 11 12 13
14 15 16
17 18
26 27
19480 A/G IN INTRON 7

Fig. 1



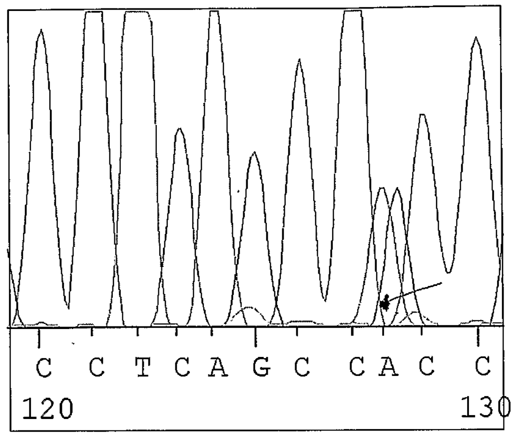
19480 AA

Figure 2



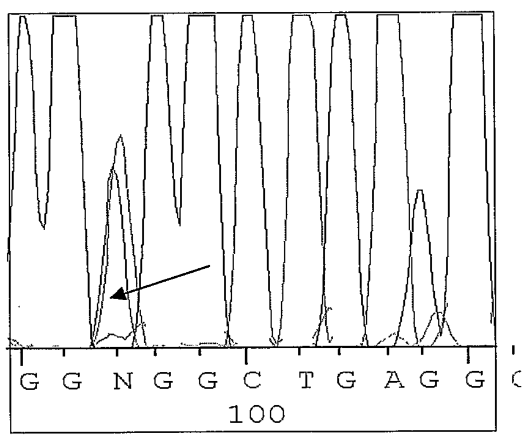
19480 GG

Figure 3



19840 AG

Figure 4



19480 TC

Figure 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/05158

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, BIOSIS, WPI Data, EMBASE, SEQUENCE SEARCH, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI; Retrieved on 04.06.2004, "Alignment display for SEQ ID NO:1" retrieved from EBI Database accession no. AC131306 XP002283507	7
A	abstract ---	1-6
A	DATABASE GENBANK 'Online! Partial sequence, 19 February 1904 (1904-02-19) "H.sapiens, chromosome 17, genomic contig" retrieved from NCBI Database accession no. NT_010799 XP002283508 abstract ---	1-7

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 2004	26/07/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bradbrook, D

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BASNYAT B ET AL: "High-altitude illness" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 361, no. 9373, 7 June 2003 (2003-06-07), pages 1967-1974, XP004429770 ISSN: 0140-6736 page 1971, column 2, paragraph 4 abstract ---	1-7
A	DROMA YUNDEN ET AL: "Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema" CIRCULATION, vol. 106, no. 7, 13 August 2002 (2002-08-13), pages 826-830, XP002283504 ISSN: 0009-7322 cited in the application abstract ---	1-7
A	WEISS JOHANNA ET AL: "Lack of evidence for association of high altitude pulmonary edema and polymorphisms of the NO pathway." HIGH ALTITUDE MEDICINE & BIOLOGY. UNITED STATES 2003 FALL, vol. 4, no. 3, October 2003 (2003-10), pages 355-366, XP001181946 ISSN: 1527-0297 abstract ---	1-7
A	XU WEIMING ET AL: "Molecular cloning and structural organization of the human inducible nitric oxide synthase gene (NOS2)" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 219, no. 3, 1996, pages 784-788, XP002283505 ISSN: 0006-291X figure 1; table 1 -& DATABASE GENBANK ‘Online! H.sapiens NOS2 gene, exons 8 and 9, 19 August 1996 (1996-08-19) retrieved from NCBI Database accession no. X85766 XP002283548 abstract ---	1-7
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/05158

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHARTRAIN NICOLE A ET AL: "Molecular cloning, structure, and chromosomal localization of the human inducible nitric oxide synthase gene" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 9, 1994, pages 6765-6772, XP002283506 ISSN: 0021-9258 figure 1; table 1 ---	1-7
A	DATABASE SNP 'Online! SNP in iNOS gene at pos. 845034 of NT_010799, 11 May 2003 (2003-05-11) retrieved from NCBI Database accession no. RS2297520 XP002283509 abstract -----	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/05158

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-4 (in part)
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-4,7 (in part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-4 encompass a surgical step carried out on the human/animal body, the search has been carried out assuming the absence of such a step.

Continuation of Box I.1

Claims Nos.: 1-4 (in part)

Rule 39.1(iv) PCT - Method of surgery on the human or animal body (Claims 1-4)

Continuation of Box I.2

Claims Nos.: 1-4,7 (in part)

Claims 1-4 are unclear contrary to the requirements of Art.6 PCT for the following reasons:

Claims 1-4 are directed to a method for detecting a predisposition to HAPE according to the genotype of an individual at a particular polymorphic site in the iNOS gene. According to the application, said polymorphism is in intron 7 of the iNOS gene, at position 19480 (cf p.6, l.10-11 and Table 1). However, said definition does not unambiguously identify the polymorphic site: no indication is given as to how the stated position relates to any disclosed nucleotide sequence for the iNOS gene. Separate reference is made to the contiguous genomic sequence with Gene Bank Accession Number NT_010799 (cf Fig.1), wherein the position of the iNOS gene is between bases 820786 and 864549. The sequence defined by SEQ ID NO.1 contains the polymorphism (cf p.6, l.16-23): a search using this sequence provided matches with GenBank database sequences AC131306, AL354047 and AC130289 (cf D1: sequence alignments), which are genomic clones from human chromosome 17, and which do not indicate the position of the iNOS gene. A 94.6% match was also found with sequence X85766, with the polymorphism located at position 300, i.e. in intron 8 of the iNOS gene (cf D1 and D6: Xu et al and X85766). This corresponds with position 845034 of NT_010799 (cf D2: NT_010799 partial sequence).

Therefore, this is taken as being the position of the polymorphism, and search was based on this polymorphic sites an SEQ ID NO.1.

Claim 3 is unclear (Art.6 PCT) in that it refers to the primers containing one or more polymorphic sites, yet these have not been defined. It is noted that neither of the primers would hybridize across the polymorphic site of interest. Therefore, claim 3 was searched only insofar as it relates to the primer sequences defined by SEQ ID NOs 2 and 3.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claim 7 is unclear (Art.6 PCT) in that it refers to "The nucleic acid vectors containing the allelic variants of the iNOS gene". It is unclear what vectors and what allelic variants are being referred to. As the only variant referred to in the application is that discussed above, claim 7 was searched with respect to any vector comprising SEQ ID NO.1 or the polymorphism defined above.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

WO2005004884

Publication Title:

TREATMENT OF SPECIFIC CARDIOVASCULAR CONDITIONS WITH NITRITE

Abstract:

Abstract of WO2005004884

It has been surprisingly discovered that administration of nitrite to subjects causes a reduction in blood pressure and an increase in blood flow to tissues. The effect is particularly beneficial, for example, to tissues in regions of low oxygen tension. This discovery provides useful treatments to regulate a subject's blood pressure and blood flow, for example, by the administration of nitrite salts. Provided herein are methods of administering a pharmaceutically-acceptable nitrite salt to a subject, for treating, preventing or ameliorating a condition selected from : (a) ischemia-reperfusion injury (e.g., hepatic or cardiac or brain ischemia-reperfusion injury); (b) pulmonary hypertension (e.g., neonatal pulmonary hypertension); or (c) cerebral artery vasospasm. Data supplied from the esp@cenet database - Worldwide c70

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 January 2005 (20.01.2005)

PCT

(10) International Publication Number
WO 2005/004884 A2

(51) International Patent Classification[7]: A61K 33/00

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(25) Filing Language: English

(26) Publication Language: English

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60/485,959 9 July 2003 (09.07.2003) US
60/511,244 14 October 2003 (14.10.2003) US

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(54) **Title:** TREATMENT OF SPECIFIC CARDIOVASCULAR CONDITIONS WITH NITRITE

(57) **Abstract:** It has been surprisingly discovered that administration of nitrite to subjects causes a reduction in blood pressure and an increase in blood flow to tissues. The effect is particularly beneficial, for example, to tissues in regions of low oxygen tension. This discovery provides useful treatments to regulate a subject's blood pressure and blood flow, for example, by the administration of nitrite salts. Provided herein are methods of administering a pharmaceutically-acceptable nitrite salt to a subject, for treating, preventing or ameliorating a condition selected from : (a) ischemia-reperfusion injury (e.g., hepatic or cardiac or brain ischemia-reperfusion injury); (b) pulmonary hypertension (e.g., neonatal pulmonary hypertension); or (c) cerebral artery vasospasm.

TREATMENT OF SPECIFIC CARDIOVASCULAR CONDITIONS WITH NITRITE

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/485,959, filed July 9, 2003, and No. 60/511,244, filed October 14, 2003, both of which are incorporated herein by reference in their entirety.

Government Interest Statement

Aspects of this invention were developed with government support under Grant Nos. HL58091 (D.B.K.-S.), and HL70146 (R.P.P.), both awarded by the National Institutes of Health. The government has certain rights in aspects of the invention. The government also may have certain rights in the invention due to at least one inventor's employment by the National Institutes of Health.

Background of the Disclosure

The last decade has seen an increase in the understanding of the critical role nitric oxide as a blood vessel dilator contributing to the regulation of blood flow and cardiovascular homeostasis. Nitric oxide may be oxidized in blood to nitrite (NO_2-), an anion considered to be an inert metabolic end product of such nitric oxide oxidation. *In vivo* plasma levels of nitrite have been reported to range from 150 to 1000 nM, and the nitrite concentration in aortic ring tissue has been reported to be in excess of 10,000 nM (Rodriguez *et al.*, *Proc Natl Acad Sci U S A, 100*, 336-41, 2003; Gladwin *et al.*, *Proc Natl Acad Sci U S A, 97*, 9943-8, 2000; and Rassaf *et al.*, *Nat Med, 9*, 481-3, 2003). This potential storage pool for NO is in excess of plasma S-nitrosothiols, which have been reported to be less than 10 nM in human plasma (Rassaf *et al.*, *Nat Med, 9*, 481-3, 2003; Rassaf *et al.*, *Free Radic Biol Med, 33*, 1590-6, 2002; Rassaf *et al.*, *J Clin Invest, 109*, 1241-8, 2002; and Schechter *et al.*, *J Clin Invest, 109*, 1149-51, 2002). Mechanisms have been proposed for the *in vivo* conversion of nitrite to NO, for example, by enzymatic reduction by xanthine oxidoreductase or by non-enzymatic disproportionation/acidic reduction (Millar *et al.*, *Biochem Soc Trans, 25*, 528S, 1997; Millar *et al.*, *FEBS Lett, 427*, 225-8, 1998; Godber *et al.*, *J Biol Chem, 275*, 7757-63, 2000; Zhang *et al.*, *Biochem Biophys Res Commun, 249*, 767-72, 1998 [published erratum appears in *Biochem Biophys Res Commun 251*, 667, 1998]; Li *et al.*, *J Biol Chem, 276*, 24482-9, 2001; Li *et al.*, *Biochemistry, 42*, 1150-9, 2003; Zweier *et al.*, *Nat Med, 1*, 804-9, 1995; Zweier *et al.*, *Biochim Biophys Acta, 1411*, 250-62, 1999; and Samouilov *et al.*, *Arch Biochem Biophys, 357*:1-7, 1998).

Arterial-to-venous gradients of nitrite across the human forearm at rest and during regional NO synthase inhibition have been observed, with increased consumption of nitrite occurring with exercise (Gladwin *et al.*, *Proc Natl Acad Sci U S A, 97*, 9943-8, 2000; Gladwin *et al.*, *Proc Natl Acad Sci USA, 97*, 11482-11487, 2000; and Cicinelli *et al.*, *Clin Physiol, 19*:440-2, 1999). Kelm and colleagues have reported that large artery-to-vein gradients of nitrite form across the human forearm during NO synthase inhibition (Lauer *et al.*, *Proc Natl Acad Sci USA, 98*, 12814-9, 2001). Unlike the more simple case of oxygen extraction across a vascular bed, nitrite may be both consumed, as evidenced by artery-to-vein gradients during NO synthase inhibition and exercise, and produced in the vascular bed by endothelial nitric oxide synthase-derived NO reactions with oxygen.

At high concentrations, nitrite has been reported to be a vasodilator *in vitro* (Ignarro *et al.*, *Biochim Biophys Acta, 631*, 221-31, 1980; Ignarro *et al.*, *J Pharmacol Exp Ther, 218*, 739-49, 1981; Moulds *et al.*, *Br J Clin Pharmacol, 11*, 57-61, 1981; Gruetter *et al.*, *J Pharmacol Exp Ther, 219*, 181-6, 1981; Matsunaga *et al.*, *J Pharmacol Exp Ther, 248*, 687-95, 1989; and Laustiola *et al.*, *Pharmacol Toxicol, 68*, 60-3, 1991). The levels of nitrite shown to vasodilate *in vitro* have always been in excess of 100,000 nM (100 μM) and usually at millimolar concentrations.

Consistent with the high concentrations of nitrite required to vasodilate *in vitro*, when Lauer and colleagues infused nitrite into the forearm circulation of human subjects, they reported no vasodilatory effects, even with concentrations of 200 μM in the forearm (Lauer *et al.*, *Proc Natl Acad Sci USA, 98*, 12814-9, 2001). Lauer *et al.* reported that a "complete lack of vasodilator activity of intraartierial infusions of nitrite clearly rules out any role for this metabolite in NO delivery" and concluded that "physiological levels of nitrite are vasodilator-inactive." Furthermore, Rassaf and colleagues also failed to find a vasodilatory effect in humans following infusion of nitrite (Rassaf *et al.*, *J Clin Invest, 109*, 1241-8, 2002). Thus, *in vivo* studies have concluded that physiological levels of nitrites do not serve as a source for NO, and that physiological levels of nitrites do not have a role in regulating blood pressure.

Historically, nitrite has been used as a treatment for cyanide poisoning. High concentrations are infused into a subject suffering cyanide poisoning in order to oxidize hemoglobin to methemoglobin, which will bind cyanide. These high concentrations of nitrite produce clinically significant methemoglobinemia, potentially decreasing oxygen delivery. While these high concentrations of nitrite have been shown to decrease blood pressure in humans, the amount of methemoglobin formed precluded a use for nitrite in the treatment of other medical conditions.

Therefore, the state of the art was that nitrite was not a significant vasodilator at concentrations below 100 μM *in vitro*, and even when infused into humans at concentrations of 200 μM in the forearm. It was also the state of the art that nitrite was not converted to nitric oxide in the human blood stream.

Summary of the Disclosure

It has been surprisingly discovered that administration of pharmaceutically-acceptable salts of nitrite is useful in the regulation of the cardiovascular system. It has also been surprisingly discovered that nitrite is reduced to nitric oxide *in vivo*, and that the nitric oxide produced thereby is an effective vasodilator. These effects surprisingly occur at doses that do not produce clinically significant methemoglobinemia. These discoveries now enable methods to prevent and treat conditions associated with the cardiovascular system, for example, high blood pressure, pulmonary hypertension, cerebral vasospasm and tissue ischemia-reperfusion injury. These discoveries also provide methods to increase blood flow to tissues, for example, to tissues in regions of low oxygen tension. It is particularly surprising that the nitrite does not need to be applied in an acidified condition in order for it to be effective in regulating the cardiovascular system, and more particularly to act as a vasodilator *in vivo*.

It has now been surprisingly discovered by the inventors that nitrite can serve as a vasodilator in humans at much lower concentrations (as low as 0.9 μM) than have been used in the past for cyanide poisoning. The mechanism is believed to involve a reaction of nitrite with deoxygenated hemoglobin and red blood cells, to produce the vasodilating gas nitric oxide. This potent biological effect is observed at doses of nitrite that do not produce clinically significant methemoglobininemia (for instance, less than 20%, more preferably less than 5% methemoglobin in the subject).

It has been discovered that nitrite is converted to nitric oxide *in vivo*, and that the nitric oxide produced thereby is an effective vasodilator. Further, it has been surprisingly discovered that administration of nitrite, for instance a pharmaceutically-acceptable salt of nitrite, to a subject causes a reduction in blood pressure and an increase in blood flow to tissues, for example, to tissues in regions of low oxygen tension. These discoveries now enable useful methods to regulate the cardiovascular system, for instance to prevent and treat malconditions associated with the cardiovascular system, for example, high blood pressure, or organs, tissues, or systems suffering a lack of or inadequate blood flow. Non-limiting examples of contemplated malconditions include stroke, heart disease, kidney disease and failure, eye damage including hypertensive retinopathy, diabetes, and migraines.

In one example embodiment, the present disclosure provides a method for decreasing a subject's blood pressure or increasing blood flow, including in a particular embodiment administering to the subject sodium nitrite at about 36 μmoles per minute into the forearm brachial artery.

The present disclosure additionally provides a method for increasing blood flow to a tissue of a subject, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite, such as a salt thereof, so as to increase blood flow to a tissue of the subject. The blood flow may be specifically increased in tissues in regions of low oxygen tension. The present disclosure also provides a method for decreasing a subject's blood pressure, comprising administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to decrease the subject's blood pressure.

The present disclosure further provides a method for treating a subject having a condition associated with elevated blood pressure, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to treat at least one vascular complication associated with the elevated blood pressure.

Also provided is a method for treating a subject having a hemolytic condition, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to treat at least one vascular complication associated with the hemolytic condition.

The disclosure further provides a method for treating a subject having a condition associated with elevated blood pressure in the lungs, *e.g.* pulmonary hypertension, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite. In some embodiments, this includes treating a subject having neonatal pulmonary hypertension. In some embodiments, this includes treating a subject having primary and/or secondary pulmonary hypertension. In some embodiments for treating subjects having a condition associated with elevated blood pressure in the lungs, the nitrite is nebulized.

Also contemplated herein are methods for treating, ameliorating, or preventing other conditions of or associated with blood flow, including vasospasm, stroke, angina, revascularization of coronary arteries and other arteries (peripheral vascular disease), transplantation (*e.g.*, of kidney, heart, lung, or liver), treatment of low blood pressure (such as that seen in shock or trauma, surgery and cardiopulmonary arrest) to prevent reperfusion injury to vital organs, cutaneous ulcers (*e.g.*, with topical, non-acidified nitrite salt), Raynauds phenomenon, treatment of hemolytic conditions (such as sickle cell, malaria, TTP, and HUS), hemolysis caused by immune incompatibility before and after birth, and other conditions listed herein.

Also provided herein are methods of administering a pharmaceutically-acceptable nitrite salt to a subject, for treating, preventing or ameliorating a condition selected from: (a) ischemia-reperfusion injury (*e.g.*, hepatic or cardiac or brain ischemia-reperfusion injury); (b) pulmonary hypertension (*e.g.*, neonatal pulmonary hypertension); or (c) cerebral artery vasospasm. Also contemplated are methods for treatment, prevention, and/or amelioration of gestational or fetal cardiovascular malconditions.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

Brief Description of the Figures

Figure 1 is a graph, depicting hemodynamic and metabolic measurements at baseline and during exercise in 18 subjects. **Figure 1A** shows effects on each of the indicated values without inhibition of NO synthesis. **Figure 1B** shows effects with inhibition of NO synthesis. *Key*: MAP – mean arterial pressure, mmHg; FBF – forearm blood flow, mL/min/100mL; O_2 saturation, %; pO_2 – venous oxyhemoglobin saturation, partial pressure of oxygen, mmHg; pH, units; * = $p<0.05$ vs. Baseline 1 or 2, respectively; ** = $p<0.01$ vs. Baseline 1 or 2, respectively; † = $p<0.05$ vs. Baseline 1; †† = $p<0.01$ vs. Initial Exercise.

Figure 2 is a graph, depicting effects of infusion of sodium nitrite in bicarbonate-buffered normal saline into the brachial arteries of 18 healthy subjects. **Figure 2A** shows effects on each of the indicated values without inhibition of NO synthesis. **Figure 2B** shows effects with inhibition of NO synthesis. *Key as for Figure 1, plus*: Nitrite – venous nitrite, μM; NO-heme – venous iron-nitrosyl-hemoglobin, μM; and MetHb – venous methemoglobin, %; + = $p<0.01$ vs. Initial Exercise.

Figure 3 is a series of graphs, illustrating the effects of infusion of low-dose sodium nitrite into the brachial arteries of 10 healthy subjects at baseline and during exercise, without and with inhibition of NO synthesis. **Figure 3A** shows forearm blood flow at baseline and following a five-minute infusion of $NaNO_2$. **Figure 3B** shows forearm blood flow with and without low-dose nitrite infusion at baseline and during L-NMMA infusion with and without exercise stress. **Figure 3C** shows venous levels of nitrite from the forearm circulation at the time of blood flow measurements. **Figure 3D** shows venous levels of S-nitroso-hemoglobin (S-NO) and iron-nitrosyl-hemoglobin (Hb-NO) at baseline and following nitrite infusion during exercise stress.

Figure 4 is a pair of graphs, showing formation of NO-hemoglobin adducts. **Figure 4A** shows formation of iron-nitrosyl-hemoglobin and S-nitroso-hemoglobin, comparing baseline, with nitrite infusion, and nitrite infusion with exercise. **Figure 4B** compares formation of NO-hemoglobin adducts with hemoglobin-oxygen saturation in the human circulation, during nitrite infusion.

Figure 5A shows NO release following nitrite injections into solutions of PBS ("PBS"), deoxygenated red blood cells ("deoxy-RBC"), and oxygenated red blood cells ("oxy-RBC"). **Figure 5B** shows the rate of NO formation from nitrite mixed with PBS (first bar in each set), and oxygenated and deoxygenated red blood cells (second and third bar in each set, respectively).

Figure 6 is a mutlipanel figure showing nitrite therapy in hepatic ischemia-reperfusion injury. **Figure 6A** illustrates the experimental protocol used for murine model of hepatic ischemia-reperfusion injury. **Figure 6B** is a graph showing serum AST levels in mice following hepatic ischemia-reperfusion. $^*p < 0.05$ vs. vehicle (0 μM) and $^{**}p < 0.01$ vs. vehicle (0 μM) **Figure 6C** is a graph showing serum ALT levels in mice following hepatic ischemia-reperfusion. $^*p < 0.05$ vs. vehicle (0 μM) and $^{**}p < 0.01$ vs. vehicle (0 μM) **Figure 6D** is a representative photomicrographs of hepatic histopathology following 45 minutes of ischemia and 24 hours of reperfusion. **Figure 6E** is a bar graph showing pathological scoring of hepatic tissue samples following 45 minutes of ischemia and 24 hours of reperfusion. **Figure 6F** is a bar graph showing hepatocellular apoptosis as measured by TUNEL staining following 45 minutes of ischemia and 24 hours of reperfusion. ** $p < 0.001$ vs. I/R alone group

Figure 7 is a multipanel figure showing nitrite therapy in myocardial ischemia-reperfusion injury. **Figure 7A** illustrates the experimental protocol used for myocardial ischemia-reperfusion studies in mice. **Figure 7B** is a representative photomicrographs of the murine hearts following 30 minutes of myocardial ischemia and reperfusion. **Figure 7C** is a bar graph comparing myocardial area-at-risk (AAR) per left ventricle (LV), infarct size (INF) per AAR, and infarct per left ventricle in mice treated with nitrate or nitrite. **Figure 7D** is a bar graph comparing myocardial ejection fraction at baseline and following 45 minutes of myocardial ischemia and 48 hours of reperfusion. **Figure 7E** is a bar graph comparing left ventricular fractional shortening at baseline and following 45 minutes of myocardial ischemia and 48 hours of reperfusion.

Figure 8 is a series of graphs, illustrating blood and liver tissue levels of nitrite, RSNO and RxNO. **Figure 8A** shows blood nitrite, RSNO, and RxNO levels (μmol/L) in animals (n=3-5 per group) subjected to sham hepatic ischemia-reperfusion (I/R) or hepatic ischemia and either 1 or 30 minutes of reperfusion. ** $p < 0.001$ vs. sham **Figure 8B** shows liver tissue nitrite levels in mice (n=3-5 per group) subjected to hepatic ischemia-reperfusion (I/R) injury. **Figure 8C** shows liver tissue RSNO levels (μmol/L) in mice (n = 3-5 per group) subjected to hepatic ischemia and varying periods of reperfusion. **Figure 8D** shows hepatic tissue RxNO levels (μmol/L) following hepatic ischemia and reperfusion in mice (n = 3-5 per group).

Figure 9 is a multipanel figure, illustrating nitrite mediated hepatoprotection and the nitric oxide and heme oxygenase-1 signaling pathways. **Figure 9A** is a graph, comparing serum aspartate aminotransferase (AST) levels in mice receiving saline vehicle, nitrite (24 μM), the nitric oxide (NO) scavenger PTIO, or nitrite (24 μM) + PTIO. **p < 0.01 vs. the vehicle group. **Figure 9B** is a graph comparing serum levels of AST in eNOS deficient (-/-) mice receiving saline vehicle or sodium nitrite (24 μM). **Figure 9C** is an image showing hepatic protein levels of heme oxygenase-1 (HO-1) determined using western blot analysis in sham operated animals and in animals subjected to hepatic ischemia (45 minutes) and reperfusion (5 hours). **Figure 9D** is a graph comparing serum AST levels in mice treated with nitrite (24 μM) or the HO-1 inhibitor zinc deuteroporphyrin bis glycol (ZnDPBG) in the setting of hepatic ischemia reperfusion injury.

Figure 10 is a series of panels, showing the effects of nitrite anion inhalation in newborn hypoxic lambs (*n*=7) (**Figure 10A**) on hemodynamic and metabolic measurements. After a hypoxic gas mixture (FiO_2 =0.12) had been started at time 0, nitrite by aerosol reduced pulmonary artery pressure (PAP) from hypoxic levels by 63 +/- 3% (P <0.01 versus hypoxic baseline) with little change in mean arterial pressure (MAP), cardiac output, or methemoglobin levels, but a marked increase in exhaled NO (P <0.01 compared to baseline). **Figure 10B** illustrates the effect of saline inhalation on pulmonary artery pressure in hypoxic lambs (*n*=7). **Figure 10C** is a multipanel graph, showing maximal effects of nitrite nebulization as compared to saline nebulization on PAP, MAP, and exhaled NO (eNO). Data are mean ± SEM.

Figure 11 illustrates effects of nitrite anion inhalation in newborn lambs during stable, normoxic (SaO_2 ~ 99 %) pulmonary hypertension induced by the infusion of an endoperoxide analog of thromboxane (U46619) (*n*=6). After infusion of U46619 was started at time 0, nitrite by aerosol reduced pulmonary artery pressure (PAP) from infusion baseline level by 23 ± 6% (P <0.05 compared to infusion baseline) with no measurable change in mean arterial pressure (MAP) and with a moderate increase in exhaled NO (P <0.01 compared to baseline).

Figure 12A compares the change in pulmonary arterial pressure (PAP), exhaled NO, and iron-nitrosyl-hemoglobin as measured by both chemiluminescence and electron paramagnetic resonance (EPR) after nitrite inhalation in animals with pulmonary hypertension induced with either hypoxia or infusion of the thromboxane analog U46199. Data for iron-nitrosyl-hemoglobin, measured by areas of output peaks after tri-iodide based reductive chemiluminescence (**Figure 12B**) and by depth of peak at 3350 Gauss in electron paramagnetic resonance (EPR) (**Figure 12C**; red line: drug induced, blue line: hypoxic) measured 20 minutes after nitrite inhalation was begun. **Figure 12D** shows change in mean pulmonary artery pressure during hypoxia after inhalation of nebulized sodium nitrite was related to blood pH, with increased vasodilation associated with decreasing pH (r = 0.57 P = 0.055). Data are mean ± SEM.

Figure 13 is a multipanel figure, showing duration of effect of NO gas inhalation (*n*=7) (**Figure 13A**) or nitrite nebulization (*n*=7) (**Figure 13B**) on hemodynamic and metabolic measurements during hypoxic-induced pulmonary hypertension. Treatment with nitrite aerosol resulted in a rapid sustained reduction in hypoxic-induced pulmonary vasoconstriction and a graded increase in exhaled NO gas concentration with no change in mean arterial blood pressure. These results are contrasted to the rapid return in pulmonary artery pressure to hypoxic baseline after termination of inhaled NO gas (**Figure 13A**). Methemoglobin (Met Hb) concentrations increased from 2.1 ± 0.1 % during baseline to 2.8 ± 0.2% after nitrite nebulization (P <0.05). Note that the exhaled nitric oxide concentrations in **Figure 13A** reach the limit of detection during administration of inhaled nitric oxide (20 ppm). **Figure 13C** shows the change in pulmonary artery pressure (PAP) after aerosolization of nebulized nitrite and during the remaining hour of hypoxia following the termination of nitrite nebulization. **Figure 13D** shows the arterial plasma nitrite concentrations during the course of the experiment. **Figure 13E** shows the relationship between pulmonary artery pressure and exhaled NO after nitrite nebulization during hypoxia. Data are mean ± SEM.

Figure 14 is a multi-column (panel) figure depicting experiment design, biochemical and clinical results in a series of non-human primates that received intravenous nitrite to examine its effects on the development of vasospasm of the cerebral arteries and resulting ischemia. Each of the three columns represents a separate experimental group (control, low nitrite, and high nitrite). This figure describes experimental design (upper row: arrows pointing down marking the events; small arrows pointing up in the middle column representing daily boluses of nitrite), biochemical results (linear graphs: red, nitrite levels in blood; blue, nitrite levels in CSF; green, levels of nitrosylated protein/albumin in CSF; the brown bar graph represents the methemoglobin levels in blood), and mean blood pressure (the last grey bar graph) in samples collected during the experiment.

Figure 15 presents characteristic cerebral arteriograms before SAH (Day 0 (preinfusion); **Figure 15A, 15C**) and on day 7 after SAH (**Figure 15B, 15D**) in two animals: one control treated with intravenous infusion of saline at 2 μl/min for 14 days (**Figure 15A, 15B**) and one treated with intravenous nitrite at 870 μmol/min for 14 days (**Figure 15C, 15D**). In **Figure 15B**, the arrows point to the right middle cerebral artery (R MCA) in spasm. R ICA, the right internal carotid artery, R ACA, the right anterior cerebral artery.

Figure 16 depicts degree of vasospasm of the right middle cerebral artery (R MCA) in each animal from all experimental groups (8 control, 3 low dose, and 3 high dose of nitrite). R MCA vasospasm was assessed as the area of the proximal 14-mm segment of the right MCA by three blinded examiners using a computerized image analysis system (NIH Image 6.21). Arteriographic vasospasm was quantified relative to each animal baseline arteriogram. The mean values for saline vs. nitrite groups are represented by the circles; bars represent standard deviations. Statistical significance p<0.001.

Detailed Description of the Disclosure

I. *Abbreviations*

ANOVA analysis of variance

carboxy-PTIO	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt
DCV	delayed cerebral vasospasm
deoxy-RBC	deoxygenated red blood cells
eNOS	endothelial NO synthase
FiO_2	fractional concentration of inspired oxygen
FBF	forearm blood flow
iNO	inhaled nitric oxide
I/R	ischemia-reperfusion
LCA	main coronary artery
L-NMMA	L-NG-monomethyl-arginine
LV	left ventricle
NO	nitric oxide
NOS	nitric oxide synthase
MAP	mean arterial pressure
MetHb	methemoglobin
oxy-RBC	oxygenated red blood cells
PBS	phosphate buffered saline
pO_2 (or Po_2)	partial oxygen pressure
SAH	subarachnoid hemorrhage
S-NO	S-nitroso-hemoglobin

II. Terms

Unless otherwise noted, terms used herein should be accorded their standard definitions and conventional usage. For example, one of skill in the art can obtain definitions for the terms used herein in dictionaries and reference textbooks, for example: *Stedman's Medical Dictionary* (26th Ed., Williams and Wilkins, Editor M. Spraycar, 1995); *The New Oxford American Dictionary* (Oxford University Press, Eds E. Jewell and F. Abate, 2001); *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 3rd Ed., Cold Spring Harbor Laboratory Press, 2001); and *Hawley's Condensed Chemical Dictionary*, 11th Ed. (Eds. N. I. Sax and R. J. Lewis, Sr., Van Nostrand Reinhold, New York, New York, 1987); *Molecular Biology and Biotechnology: a Comprehensive Desk Reference* (VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8)).

In order to facilitate review of the various embodiments, the following explanations of specific terms are provided:

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals.

Cerebral ischemia or ischemic stroke: A condition that occurs when an artery to or in the brain is partially or completely blocked such that the oxygen demand of the tissue exceeds the oxygen supplied. Deprived of oxygen and other nutrients following an ischemic stroke, the brain suffers damage as a result of the stroke.

Ischemic stroke can be caused by several different kinds of diseases. The most common problem is narrowing of the arteries in the neck or head. This is most often caused by atherosclerosis, or gradual cholesterol deposition. If the arteries become too narrow, blood cells may collect in them and form blood clots (thrombi). These blood clots can block the artery where they are formed (thrombosis), or can dislodge and become trapped in arteries closer to the brain (embolism).

Another cause of stroke is blood clots in the heart, which can occur as a result of irregular heartbeat (for example, atrial fibrillation), heart attack, or abnormalities of the heart valves. While these are the most common causes of ischemic stroke, there are many other possible causes. Examples include use of street drugs, traumatic injury to the blood vessels of the neck, or disorders of blood clotting.

Ischemic stroke is by far the most common kind of stroke, accounting for about 80% of all strokes. Stroke can affect people of all ages, including children. Many people with ischemic strokes are older (60 or more years old), and the risk of stroke increases with older ages. At each age, stroke is more common in men than women, and it is more common among African-Americans than white Americans. Many people with stroke have other problems or conditions which put them at higher risk for stroke, such as high blood pressure (hypertension), heart disease, smoking, or diabetes.

Fetal: A term describing the time period in the latter part of pregnancy when organ systems are functional and blood flow patterns are established for central critical organs, such as the heart, brain and lungs.

Hypoxia: Deficiency in the amount of oxygen reaching body tissues.

Injectable composition: A pharmaceutically acceptable fluid composition comprising at least one active ingredient, for example, a salt of nitrite. The active ingredient is usually dissolved or suspended in a physiologically acceptable carrier, and the composition can additionally comprise minor amounts of one or more non-toxic auxiliary substances, such as emulsifying agents, preservatives, pH buffering agents and the like. Such injectable compositions that are useful for use with the compositions of this disclosure are conventional; appropriate formulations are well known in the art.

Ischemia: A vascular phenomenon in which a decrease in the blood supply to a bodily organ, tissue, or part is caused, for instance, by constriction or obstruction of one or more blood vessels. Ischemia sometimes results from vasoconstriction or thrombosis or embolism. Ischemia can lead to direct ischemic injury, tissue damage due to cell death caused by reduced oxygen supply.

Ischemia/reperfusion injury: In addition to the immediate injury that occurs during deprivation of blood flow, ischemic/reperfusion injury involves tissue injury that occurs after blood flow is restored. Current understanding is that much of this injury is caused by chemical products and free radicals released into the ischemic tissues.

When a tissue is subjected to ischemia, a sequence of chemical events is initiated that may ultimately lead to cellular dysfunction and necrosis. If ischemia is ended by the restoration of blood flow, a second series of injurious events ensue producing additional injury. Thus, whenever there is a transient decrease or interruption of blood flow in a subject, the resultant injury involves two components - the direct injury occurring during the ischemic interval and the indirect or reperfusion injury that follows. When there is a long duration of ischemia, the direct ischemic damage, resulting from hypoxia, is predominant. For relatively short duration ischemia, the indirect or reperfusion mediated damage becomes increasingly important. In some instances, the injury produced by reperfusion can be more severe than the injury induced by ischemia *per se*. This pattern of relative contribution of injury from direct and indirect mechanisms has been shown to occur in all organs.

Methemoglobin: The oxidized form of hemoglobin in which the iron in the heme component has been oxidized from the ferrous (+2) to the ferric (+3) state. This renders the hemoglobin molecule incapable of effectively transporting and releasing oxygen to the tissues. Normally, there is about 1% of total hemoglobin in the methemoglobin form.

Methemoglobinemia: A condition in which a substantial portion of the hemoglobin in the blood of a subject is in the form of methemoglobin, making it unable to carry oxygen effectively to the tissues. Methemoglobinemia can be an inherited disorder, but it also can be acquired through exposure to chemicals such as nitrates (nitrate-contaminated water), aniline dyes, and potassium chlorate. It is not the presence of methemoglobin but the amount that is important in the clinical setting. The following provides rough indications of symptoms associated with different levels of methemoglobin in the blood: < 1.7%, normal; 10-20%, mild cyanosis (substantially asymptomatic, though it can result in "chocolate brown" blood); 30-40%, headache, fatigue, tachycardia, weakness, dizziness; >35%, symptoms of hypoxia, such as dyspnea and lethargy; 50-60%, acidosis, arrhythmias, coma, convulsions, bradycardia, severe hypoxia, seizures; >70% usually results in death.

Neonate: A term describing the human or animal organism in the time period after birth and extending until the adjustments from fetal to newborn life are completed.

Nitrite: The inorganic anion $^-NO_2$ or a salt of nitrous acid (NO_2^-). Nitrites are often highly soluble, and can be oxidized to form nitrates or reduced to form nitric oxide or ammonia. Nitrite may form salts with alkali metals, such as sodium ($NaNO_2$, also known as nitrous acid sodium salt), potassium and lithium, with alkali earth metals, such as calcium, magnesium and barium, with organic bases, such as amine bases, for example, dicyclohexylamine, pyridine, arginine, lysine and the like. Other nitrite salts may be formed from a variety of organic and inorganic bases. In particular embodiments, the nitrite is a salt of an anionic nitrite delivered with a cation, which cation is selected from sodium, potassium, and arginine. Many nitrite salts are commercially available, and/or readily produced using conventional techniques.

Parenteral: Administered outside of the intestine, for example, not via the alimentary tract. Generally, parenteral formulations are those that will be administered through any possible mode except ingestion. This term especially refers to injections, whether administered intravenously, intrathecally, intramuscularly, intraperitoneally, or subcutaneously, and various surface applications including intranasal, intradermal, and topical application, for instance.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the compounds herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Peripheral Vascular Disease (PVD): A condition in which the arteries that carry blood to the arms or legs become narrowed or occluded. This interferes with the normal flow of blood, sometimes causing pain but often causing no readily detectable symptoms at all.

The most common cause of PVD is atherosclerosis, a gradual process in which cholesterol and scar tissue build up, forming plaques that occlude the blood vessels. In some cases, PVD may be caused by blood clots that lodge in the arteries and restrict blood flow. PVD affects about one in 20 people over the age of 50, or 8 million people in the United States. More than half the people with PVD experience leg pain, numbness or other symptoms, but many people dismiss these signs as "a normal part of aging" and do not seek medical help. The most common symptom of PVD is painful cramping in the leg or hip, particularly when walking. This symptom, also known as "claudication," occurs when there is not enough blood flowing to the leg muscles during exercise, such that ischemia occurs. The pain typically goes away when the muscles are rested.

Other symptoms may include numbness, tingling or weakness in the leg. In severe cases, people with PVD may experience a burning or aching pain in an extremity such as the foot or toes while resting, or may develop a sore on the leg or foot that does not heal. People with PVD also may experience a cooling or color change in the skin of the legs or feet, or loss of hair on the legs. In extreme cases, untreated PVD can lead to gangrene, a serious condition that may require amputation of a leg, foot or toes. People with PVD are also at higher risk for heart disease and stroke.

A **"pharmaceutical agent"** or **"drug"** refers to a chemical compound or other composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

Placenta: A vascular organ that provides for metabolic exchange between mother and fetus in mammals. It delivers oxygen, water, and nutrients to the fetus from the mother's blood and secretes the hormones necessary for successful pregnancy. In addition, it carries wastes away from the fetus to be processed in the mother's body.

Preeclampsia: A disease of unknown cause in pregnant women, characterized by hypertension, abnormal blood vessels in the placenta, and protein in the urine. It often but not always occurs with gestational diabetes or in diabetics. Additional symptoms may include water retention, leading to swelling in the face, hands and feet, and greater weight gain. Also called toxemia. Preeclampsia can lead to eclampsia if not treated. The only known cure for preeclampsia is delivery of the child.

Preventing or treating a disease: "Preventing" a disease refers to inhibiting the full development of a disease. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified nitrite salt preparation is one in which the specified nitrite salt is more enriched than it is in its generative environment, for instance within a biochemical reaction chamber. Preferably, a preparation of a specified nitrite salt is purified such that the salt represents at least 50% of the total nitrite content of the preparation. In some embodiments, a purified preparation contains at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or more of the specified compound, such as a particular nitrite salt.

Reperfusion: Restoration of blood supply to tissue that is ischemic, due to decrease in blood supply. Reperfusion is a procedure for treating infarction or other ischemia, by enabling viable ischemic tissue to recover, thus limiting further necrosis. However, it is thought that reperfusion can itself further damage the ischemic tissue, causing reperfusion injury.

Subject: Living multi-cellular organisms, including vertebrate organisms, a category that includes both human and non-human mammals.

Therapeutic: A generic term that includes both diagnosis and treatment.

Therapeutically effective amount of [a vasodilator]: A quantity of compound, such as a nitrite salt, sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to treat or ameliorate relatively high blood pressure, or to measurably decrease blood pressure over a period of time, or to measurably inhibit an increase in blood pressure, in a subject.

An effective amount of a vasodilator may be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount will be dependent on the compound applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the compound. For example, a therapeutically effective amount of an active ingredient can be measured as the concentration (moles per liter or molar-M) of the active ingredient (such as a pharmaceutically-acceptable salt of nitrite) in blood (*in vivo*) or a buffer (*in vitro*) that produces an effect.

By way of example, as described herein it is now shown that pharmaceutically-acceptable salts of nitrite (such as sodium nitrite) are effective as vasodilators at calculated dosages of about 0.6 to about 200 μM final concentration of nitrite in the circulating blood of a subject, which level can be determined empirically or through calculations. Specific levels can be reached, for instance, by providing less than about 200 mg or less nitrite in a single dose, or a dose provided over a period of time (*e.g.*, by infusion or inhalation). For instance, other dosages may be 150 mg, 100 mg, 75 mg, 50 mg or less. Specific example dosages of nitrite salts are provided herein, though the examples are not intended to be limiting. Exact dosage amounts will vary by the size of the subject being treated, the duration of the treatment, the mode of administration, and so forth.

Particularly beneficial therapeutically effective amounts of a vasodilator, such as a pharmaceutically-acceptable nitrite salt (*e.g.*, sodium nitrite), are those that are effective for vasodilation or increasing blood flow, but not so high that a significant or toxic level of methemoglobin is produced in the subject to which the vasodilator is administered. In specific embodiments, for instance, no more than about 25% methemoglobin is produced in the subject. More preferably, no more than 20%, no more than 15%, no more than 10%, no more than 8% or less methemoglobin is produced, for instance as little as 5% or 3% or less, in response to treatment with the vasodilator.

The compounds discussed herein have equal application in medical and veterinary settings. Therefore, the general term "subject being treated" is understood to include all animals (for example, humans, apes, laboratory animals, companion animals, etc.) that are or may be suffering from an aberration in blood pressure, such as hypertension.

Vasoconstriction. The diminution of the caliber or cross-sectional area of a blood vessel, for instance constriction of arterioles leading to decreased blood flow to a body part. This can be caused by a specific **vasoconstrictor**, an agent (for instance a chemical or biochemical compound) that causes, directly or indirectly, constriction of blood vessels. Such an agent can also be referred to as a **vasohypertonic** agent, and is said to have **vasoconstrictive** activity. A representative category of vasoconstrictors is the **vasopressor** (from the term pressor, tending to increase blood pressure), which term is generally used to refer to an agent that stimulates contraction of the muscular tissue of the capillaries and arteries.

Vasoconstriction also can be due to vasospasm, inadequate vasodilatation, thickening of the vessel wall, or the accumulation of flow-restricting materials on the internal wall surfaces or within the wall itself. Vasoconstriction is a major presumptive or proven factor in aging and in various clinical conditions including progressive generalized atherogenesis, myocardial infarction, stroke, hypertension, glaucoma, macular degeneration, migraine, hypertension and diabetes mellitus, among others.

Vasodilation. A state of increased caliber of the blood vessels, or the act of dilation of a blood vessel, for instance dilation of arterioles leading to increased blood flow to a body part. This can be caused by a specific **vasodilator**, an agent (for instance, a chemical or biochemical compound) that causes, directly or indirectly, dilation of blood vessels. Such an agent can also be referred to as a **vasohypotonic** agent, and is said to have **vasodilative** activity.

Vasospasm: Another cause of stroke occurs secondary to spasm of blood vessels supplying the brain. This type of stroke typically follows a subarchnoid aneurismal hemorrhage with a delayed development of vasospasm within 2-3 weeks of the bleeding event. A similar type of stroke may complicate sickle cell disease.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

III. Overview of Several Embodiments

It has been surprisingly discovered that administration of pharmaceutically-acceptable salts of nitrite is useful in the regulation of the cardiovascular system. It has also been surprisingly discovered that nitrite is reduced to nitric oxide *in vivo*, and that the nitric oxide produced thereby is an effective vasodilator. These effects surprisingly occur at doses that do not produce clinically significant methemoglobinemia. These discoveries now enable methods to prevent and treat conditions associated with the cardiovascular system, for example, high blood pressure, pulmonary hypertension, cerebral vasospasm and tissue ischemia-reperfusion injury. These discoveries also provide methods to increase blood flow to tissues, for example, to tissues in regions of low oxygen tension. It is particularly surprising that the nitrite does not need to be applied in an acidified condition in order for it to be effective in regulating the cardiovascular system, and more particularly to act as a vasodilator *in vivo*.

Accordingly, the present disclosure provides in one embodiment a method for decreasing a subject's blood pressure, including administering to the subject sodium nitrite at about 36 μmoles per minute or less into the forearm brachial artery or intravenously.

The present disclosure also provides a method for decreasing a subject's blood pressure, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to decrease (or lower, or reduce) the subject's blood pressure. Another embodiment is a method for treating a subject having a condition associated with elevated blood pressure, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to treat at least one vascular complication associated with the elevated blood pressure. Also provided is a method for treating a subject having a hemolytic condition, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to treat at least one vascular complication associated with the hemolytic condition.

The present disclosure additionally provides a method for increasing blood flow to a tissue of a subject, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite so as to increase blood flow to a tissue of the subject. Also provided is a method for producing an amount of NO in a subject effective the decrease the subject's blood pressure, including administering a pharmaceutically-acceptable nitrite to the subject.

The present disclosure further provides a pharmaceutical composition comprising an effective amount of a pharmaceutically-acceptable nitrite and a carrier.

In some embodiments, the vascular complication is one or more selected from the group consisting of pulmonary hypertension (including neonatal pulmonary hypertension, primary pulmonary hypertension, and secondary pulmonary hypertension), systemic hypertension, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, an ischemic central nervous system event, and death.

In some embodiments, nitrite is administered to neonates to treat pulmonary hypertension.

In some embodiments, the hemolytic condition includes one or more selected from: sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, cardiopulomonary bypass, and hemodialysis.

In some embodiments, the decreased blood flow to the tissue is caused directly or indirectly by at least one of the following conditions: sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, transfusion of hemoglobin, transfusion of red blood cells, cardiopulmonary bypass, coronary disease, cardiac ischemia syndrome, angina, iatrogenic hemolysis, angioplasty, myocardial ischemia, tissue ischemia, hemolysis caused by intravascular devices, hemodialysis, pulmonary hypertension, systemic hypertension, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, and an ischemic central nervous system event.

In some embodiments, the tissue is an ischemic tissue. In some embodiments, the administration is parenteral, oral, bucal, rectal, *ex vivo*, or intraocular. In some embodiments, the administration is peritoneal, intravenous, intraarterial, subcutaneous, inhaled, or intramuscular. In some embodiments, the nitrite is administered to the subject in an environment of low oxygen tension, or acts in an area of the subject's body that displays relatively low oxygen tension. In some embodiments, the nitrite is administered as a pharmaceutically-acceptable salt of nitrite, such as, for instance, sodium nitrite, potassium nitrite, or arginine nitrite. In some embodiments, the nitrite is administered in combination with at least one additional active agent. It is specifically contemplated that, in certain embodiments, that the subject is a mammal, for instance, a human.

The disclosure further provides a method for treating a subject having a condition associated with elevated blood pressure in the lungs, *e.g.* pulmonary hypertension, including administering to the subject an effective amount of pharmaceutically-acceptable nitrite. In some embodiments, this includes treating a subject having neonatal pulmonary hypertension. In some embodiments, this includes treating a subject having primary and/or secondary pulmonary hypertension. In some embodiments for treating subjects having a condition associated with elevated blood pressure in the lungs, the nitrite is nebulized.

The disclosure also provides suggestions for a means of treating hypertension and/or preeclampsia in pregnant women. Such therapy would include action of nitrites on spastic and diseased blood vessels within the placenta.

The disclosure also provides suggestions for treating, *in utero*, fetuses with cardiovascular anomalies, hypertension, and/or misdirected blood flow. In such approaches, nitrite may be administered by introduction into the amniotic cavity either directly or by osmotic minipumps, the latter to achieve sustained release throughout days and weeks of pregnancy.

Thus, there is provided herein a method for inducing vasodilation and/or increasing blood flow in a subject, which method involves administering to the subject an effective amount of a pharmaceutically-acceptable salt of nitrite for a sufficient period of time to induce vasodilation and/or increase blood flow in the subject. Non-limiting examples of pharmaceutically acceptable salts of nitrite include sodium nitrite, potassium nitrite, and arginine nitrite. In examples of the provided methods, the pharmaceutically-acceptable salt of nitrite reacts in the presence of hemoglobin in the subject to release nitric oxide.

It is a specific advantage of methods provided herein that the effective amount of the pharmaceutically-acceptable salt of nitrite administered to the subject does not induce toxic levels of methemoglobin, and in many embodiments does not induced formation of clinically significant amounts of methemoglobin in the subject. Therefore, contemplated herein are methods in which the effective amount of the pharmaceutically-acceptable salt of nitrite, when administered to the subject, induces production in the subject of no more than about 25% methemoglobin; no more than about 20% methemoglobin; no more than about 10% methemoglobin; no more than about 8% methemoglobin; or no more than about 5% methemoglobin. Beneficially, examples of the provided methods induce production of even less than 5% methemoglobin, for instance no more than about 3% methemoglobin, less than 3%, less than 2%, or even less than 1%.

In one specific example of a method for inducing vasodilation and/or increasing blood flow in a subject, sodium nitrite is administered by injection at about 36 μmoles per minute for at least five minutes into the forearm brachial artery of the subject.

The effective amount of the pharmaceutically-acceptable salt of nitrite is administered, in various embodiments, to a circulating concentration in the subject of about 0.6 to 240 μM, measured locally to the site of administration or generally in the subject. It is noted that the local level of nitrite is expected to be higher than the general circulating level particularly in short delivery regimens; in long term delivery regimens, such as delivery using a pump or injector, or by inhalation, the system-wide or general nitrite level is expected to near the level measured near the administration site.

Administration of the pharmaceutically-acceptable nitrite can be, for instance, parenteral, oral, bucal, rectal, *ex vivo*, or intraocular in certain embodiments. In various embodiments, it is also contemplated that the administration of the nitrite can be peritoneal, intravenous, intraarterial, subcutaneous, inhaled, intramuscular, or into a cardiopulmonary bypass circuit. Combinations of two or more routes of administration are also contemplated.

In various embodiments of the method for inducing vasodilation and/or increasing blood flow in a subject, the subject is a mammal. It is particularly contemplated that the subject can be a human.

Combination therapy methods are contemplated, wherein the nitrite is administered in combination with at least one additional agent. By way of non-limiting examples, the additional agent is one or more selected from the list consisting of penicillin, hydroxyurea, butyrate, clotrimazole, arginine, or a phosphodiesterase inhibitor (such as sildenafil).

In another embodiment of the method for inducing vasodilation and/or increasing blood flow in a subject, the subject has elevated blood pressure, and the method is a method for treating at least one vascular complication associated with the elevated blood pressure, or the subject has a hemolytic condition, and the method is a method for treating at least one vascular complication associated with the hemolytic condition. Optionally, the subject may have both elevated blood pressure and a hemolytic condition.

In examples of the methods provided herein, the at least one vascular complication is one or more selected from the group consisting of pulmonary hypertension, systemic hypertension, peripheral vascular disease, trauma, cardiac arrest, general surgery, organ transplantation, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, angina, an ischemia-reperfusion event, an ischemic central nervous system event, and death.

In examples of the methods in which the subject has a hemolytic condition, the hemolytic condition is one or more selected from the group consisting of sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, transfusion of hemoglobin, transfusion of red blood cells, cardiopulmonary bypass, coronary disease, cardiac ischemia syndrome, angina, iatrogenic hemolysis, angioplasty, myocardial ischemia, tissue ischemia, hemolysis caused by intravascular devices, and hemodialysis.

In yet another embodiment of the method for inducing vasodilation and/or increasing blood flow in a subject, the subject has a condition associated with decreased blood flow to a tissue, and the method is a method to increase blood flow to the tissue of the subject. For instance, in examples of this method, the decreased blood flow to the tissue is caused directly or indirectly by at least one condition selected from the group consisting of: sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, transfusion of hemoglobin, transfusion of red blood cells, cardiopulmonary bypass, coronary disease, cardiac ischemia syndrome, angina, iatrogenic hemolysis, angioplasty, myocardial ischemia, tissue ischemia, hemolysis caused by intravascular devices, hemodialysis, pulmonary hypertension, systemic hypertension, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, and an ischemic central nervous system event.

It is specifically contemplated in examples of this method that the tissue is an ischemic tissue, for instance one or more tissues selected from the group consisting of neuronal tissue, bowel tissue, intestinal tissue, limb tissue, lung tissue, central nervous tissue, or cardiac tissue.

Also provided are methods for inducing vasodilation and/or increasing blood flow in a subject having elevated blood pressure, wherein the elevated blood pressure comprises elevated blood pressure in the lungs. By way of example, it is contemplated that such subject in some instances has neonatal pulmonary hypertension, or primary and/or secondary pulmonary hypertension.

In examples of embodiments where the elevated blood pressure, or need for increased blood flow, in the subject comprises elevated blood pressure or need for increased blood flow in the lungs, the pharmaceutically-acceptable salt of nitrite is nebulized.

By way of example, in various embodiments the pharmaceutically-acceptable salt of nitrite is administered to a circulating concentration in the subject of no more than about 100 μM; no more than about 50 μM; no more than about 20 μM; no more than about 16 μM; or less than about 16 μM.

Another embodiment is a method for treating or ameliorating a condition selected from: (a) hepatic or cardiac or brain ischemia-reperfusion injury; (b) pulmonary hypertension; or (c) cerebral artery vasospasm, in a subject by decreasing blood pressure and/or increasing vasodilation in the subject, the method comprising administering sodium nitrite to the subject to decrease the blood pressure and/or increase vasodilation in the subject, thereby treating or ameliorating the condition.

In specific examples of this embodiment, the method is a method for treating or ameliorating hepatic or cardiac or brain ischemia-reperfusion injury. Optionally, the sodium nitrite is administered to the subject via injection, for instance, intravenous injection. In certain examples, the sodium nitrite is administered to a circulating concentration of about 0.6 to 240 μM.

In other specific examples of this embodiment, the method is a method for treating or ameliorating pulmonary hypertension, such as for instance neonatal pulmonary hypertension. Beneficially, in such methods the sodium nitrite can be administered to the subject by inhalation, for instance it can be nebulized. Optionally, in any of these methods, the sodium nitrite is administered at a rate of 270 μmol/minute, though other rates and circulating levels are contemplated.

Also provided in other examples of this embodiment are methods for treating or ameliorating cerebral artery vasospasm. Optionally, the sodium nitrite is administered to the subject via injection, for instance, intravenous injection. In examples of such methods, the sodium nitrite is administered at a rate of about 45 to 60 mg/kg.

In examples of the described methods, optionally the sodium nitrite can be administered in combination with at least one additional agent.

In any of the described methods, it is contemplated that the subject can be a mammal, such as for instance a human.

IV. Sodium Nitrite as an in vivo *vasodilator*

Nitrite anions are present in concentrations of about 150-1000 nM in the plasma and about 10 μM in aortic tissue. This represents the largest vascular storage pool of nitric oxide (NO), provided physiological mechanisms exist to reduce nitrite to NO. The vasodilator properties of nitrite in the human forearm and the mechanisms extant for its bioactivation have been investigated and results are reported herein. Sodium nitrite was infused at about 36 μmoles per minute into the forearm brachial artery of 18 normal volunteers, resulting in a regional nitrite concentration of about 222 μM and an immediate about 175% increase in resting forearm blood flow. Increased blood flow was observed at rest, during NO synthase inhibition and with exercise, and resulted in increased tissue perfusion, as demonstrated by increases in venous hemoglobin-oxygen saturation, partial pressure of oxygen, and pH. Systemic concentrations of nitrite increased to about 16 μM and significantly reduced mean arterial blood pressure. In an additional six subjects, the dose of nitrite was reduced about 2-logs and infused at 360 nmoles per minute, resulting in a forearm nitrite concentration of about 2 μM and an about 22% increase in blood flow.

Nitrite infusions were associated with the formation of erythrocyte iron-nitrosyl-hemoglobin, and to a lesser extent, S-nitroso-hemoglobin across the forearm vasculature. The formation of NO-modified hemoglobin appears to result from the nitrite reductase activity of deoxyhemoglobin, linking tissue hypoxia and nitrite bioactivation.

These results indicate that physiological levels of blood and tissue nitrite represent a major bioavailable pool of NO that contributes to vaso-regulation and provides a mechanism for hypoxic vasodilation via reaction of vascular nitrite with deoxygenated heme proteins. Substantial blood flow effects of nitrite infusion into the brachial artery of normal human subjects results from forearm nitrite concentrations as low as about 0.9μM.

By way of example, as described herein it is now shown that pharmaceutically-acceptable salts of nitrite (such as sodium nitrite) are effective as vasodilators at calculated dosages of about 0.6 to about 200 μM final concentration of nitrite in the circulating blood of a subject. Specific circulating levels (locally or generally in the subject) can be reached, for instance, by providing less than about 200 mg or less nitrite in a single dose, or a dose provided over a period of time (*e.g.*, by infusion or inhalation). For instance, other dosages may be 150 mg, 100 mg, 75 mg, 50 mg or less. Specific example dosages of nitrite salts are provided herein, though the examples are not intended to be limiting. Exact dosage amounts will vary by the size of the subject being treated, the duration of the treatment, the mode of administration, and so forth.

Infusion rates can be calculated, for any given desired target circulating concentration, by using the following equation:

Infusion rate (μM/min) = target concentration (μmol/L, or μM) x Clearance (L/min)
where Clearance (L/min) = 0.015922087 x weight of the subject (kg) $\uparrow$0.8354

The rate of clearance has been calculated based on empirical results, including those reported herein.

By way of example, when sodium nitrite is infused into a human forearm at 36 micromoles (μMol) per minute, the concentration measured coming out of forearm is about 222 μM and about 16 μM in whole body, after 15 minutes infusion. The background level of circulating nitrite in mammals is low, around 150-500 nanoM.

Particularly beneficial therapeutically effective amounts of a vasodilator, such as a pharmaceutically-acceptable nitrite salt (*e.g.*, sodium nitrite), are those that are effective for vasodilation or increasing blood flow, but not so high that a significant or toxic level of methemoglobin is produced in the subject to which the vasodilator is administered. In specific embodiments, for instance, no more than about 25% methemoglobin is produced in the subject. More preferably, no more than 20%, no more than 15%, no more than 10%, no more than 8% or less methemoglobin is produced, for instance as little as 5% or 3% or less, in response to treatment with the vasodilator.

By way of specific example, nitrite can be infused at concentrations less than 40 μMol per minute intravenously or intraarterially, or given by mouth. Importantly, doses used are less than those used for the treatment of cyanide poisoning, which are designed to induce clinically significant methemoglobinemia. Surprisingly, the doses described herein for the treatment/prevention of cardiovascular conditions produce significant and beneficial clinical effects without clinically significant methemoglobin production.

Relatively complex inorganic/organic nitrite compounds and nitrate compounds have been utilized clinically to treat disorders, including angina. These drugs (*e.g.*, glyceryl trinitrate) suffer from tolerance (requiring increases in dosage in order to maintain the same effect), however, and are distinct vasodilators compared to nitrite. For example, the former require cellular thiols for metabolism, whereas nitrite or the nitrite salts discussed herein (*e.g.*, sodium nitrite) do not.

V. A mechanism of iron-nitrosyl- and S-nitroso-hemoglobin formation in vivo

The levels of both iron-nitrosyl- and S-nitroso-hemoglobin formed *in vivo* in this study are striking. During a transit time of less than 10 seconds through the forearm circulation during exercise, infused nitrite (200 μM regional concentration) produced approximately 750 nM iron-nitrosyl-hemoglobin and 200 nM SNO-Hb. The formation of both NO-hemoglobin adducts was inversely correlated with hemoglobin-oxygen saturation, which fell during exercise stress, measured from the antecubital vein by co-oximetry (for iron-nitrosyl-hemoglobin $r=-0.7$, $P<0.0001$; for S-nitroso-hemoglobin $r=-0.45$, $P=0.04$; Figure 4B). Addition of 200 μM nitrite to whole blood at different oxygen tensions (0-100%) recapitulated the *in vivo* data with increasing concentrations of iron-nitrosyl hemoglobin being formed at lower oxygen tensions (for iron-nitrosyl-hemoglobin $r=-0.968$, $P<0.0001$; for S-nitroso-hemoglobin $r=-0.45$, $P=0.07$), strongly suggesting that the NO and SNO formation was dependent on the reaction of nitrite with deoxyhemoglobin.

These data are consistent with the reaction of nitrite with deoxyhemoglobin to form NO and iron-nitrosyl-hemoglobin (Doyle *et al.*, *J Biol Chem, 256*, 12393-12398, 1981). Nitrite is first reduced to form NO and methemoglobin with a rate constant of 2.9 $M^{-1}sec^{-1}$ (measured at 25°C, pH 7.0). This reaction will be pseudo-first order, governed by the amounts (20 mM) of intra-erythrocytic hemoglobin, and limited by the rate of nitrite uptake by the erythrocyte membrane. NO then binds to deoxyhemoglobin to form iron-nitrosyl-hemoglobin, escapes the erythrocyte, or reacts with other higher oxides, such as NO_2, to form N_2O_3 and S-nitroso-hemoglobin.

Equation series 1

$$NO_2^- \text{ (nitrite)} + HbFe^{II} \text{ (deoxyhemoglobin)} + H^+ \rightarrow HbFe^{III} \text{ (methemoglobin)} + NO + OH^-$$

$$NO + HbFe^{II} \text{ (deoxyhemoglobin)} \rightarrow HbFe^{II}NO \text{ (iron-nitrosyl-hemoglobin)}$$

The formation of significant amounts of S-nitroso-hemoglobin *in vivo* during nitrite infusion was also observed. Luschinger and colleagues (*Proc Natl Acad Sci USA, 100*, 461-6, 2003) recently proposed that nitrite reacts with deoxyhemoglobin to make iron-nitrosyl-hemoglobin, with subsequent "transfer" of the NO to the cysteine 93 to form S-nitroso-hemoglobin mediated by reoxygenation and quaternary T to R transition of hemoglobin. However, a direct transfer of NO from the heme to the thiol requires NO oxidation to NO+ and such "cycling" has not been reproduced by other research groups. Fernandez and colleagues have recently suggested that nitrite catalyzes the reductive nitrosylation of methemoglobin by NO, a process that generates the intermediate nitrosating species dinitrogen teraoxide (N_2O_3) (*Inorg Chem, 42*, 2-4, 2003). However, nitrite reactions with hemoglobin provide ideal conditions for NO and S-nitrosothiol generation along the oxygen gradient as nitrite reacts with deoxyhemoglobin to form NO and with oxyhemoglobin to form nitrogen dioxide (NO_2) radical. NO_2 participates in radical-radical reactions ($k=10^9$ $M^{-1}sec^{-1}$) with NO to form N_2O_3 and S-nitrosothiol. Additional chemistry of nitrite with hemoglobin produces reactive oxygen metabolites (such as superoxide and hydrogen peroxide; Watanabe *et al.*, *Acta Med Okayama 35*, 173-8, 1981; Kosaka *et al.*, *Biochim Biophys Acta 702*, 237-41, 1982; and Kosaka *et al.*, *Environ Health Perspect 73*, 147-51, 1987). Chemistry involving such NO radical- oxygen radical reactions provides competitive pathways for S-nitrosothiol formation in the presence of high affinity NO sinks, such as hemoglobin.

VI. Physiological Considerations

The last decade has seen an increase in the understanding of the critical role nitric oxide (NO) plays in vascular homeostasis. The balance between production of NO and scavenging of NO determines NO bioavailability, and this balance is carefully maintained in normal physiology. The homeostatic, vasoregulatory system is apparently fine-tuned to scavenge excess NO to limit gross endocrine actions while allowing for sufficient local NO necessary for regional tonic vasodilation. However, rapid NO scavenging by cell-free hemoglobin disrupts this balance (Reiter *et al.*, *Nat Med* 8, 1383-1389, 2002). Under normal physiological conditions, hemoglobin is rapidly and effectively cleared by the hemoglobin scavenger system. However, chronic hemolytic conditions, such as sickle cell disease, result in the daily release of substantial quantities of hemoglobin into the vasculature, suggesting that cell-free hemoglobin may have major systemic effects on NO bioavailability. A current focus of research attempts to explain and treat the vascular complications common to many chronic hemolytic conditions, such as pulmonary hypertension, cutaneous ulceration and acute and chronic renal failure. Similarly, a number of clinical diseases and therapies such as acute hemolytic crises, hemolysis during cardiopulmonary bypass procedures, transfusion of aged blood, and myoglobinuria following muscle infarction are often complicated by acute pulmonary and systemic hypertension, acute renal failure, intravascular thrombosis, ischemic central nervous system events and/or death.

It is demonstrated herein that nitrite produces vasodilation in humans associated with nitrite reduction to NO by deoxyhemoglobin. Remarkably, systemic levels of 16 μM resulted in systemic vasodilation and decreased blood pressure, and regional forearm levels of only 1-2 μM significantly increased blood flow at rest and with exercise stress. Furthermore, conversion of nitrite to NO and S-nitrosothiol was mediated by reaction with deoxyhemoglobin, providing a mechanism for hypoxia-regulated catalytic NO production by the erythrocyte or endothelial/tissue heme proteins. While high concentrations of hemoglobin in red cells, coupled with the near diffusion-limited reaction rates ($\sim 10^7$ $M^{-1}s^{-1}$) of NO with hemoglobin, seem to prohibit NO from being exported from the red blood cell, the data presented herein argue to the contrary. While not intending to be limiting, perhaps unique characteristics of the erythrocyte membrane, with a submembrane protein and methemoglobin-rich microenvironment, and the relative lipophilic nature of NO, allow compartmentalized NO production at the red blood cell membrane. This, coupled with the small yields of NO necessary for vasodilation, could account for the export of NO despite these kinetic constraints. It is further proposed that *in vivo* chemistry for the conversion of nitrite to NO and S-nitrosothiol by reaction with deoxyhemoglobin and methemoglobin provides a mechanism for hypoxia-regulated catalytic NO production by the erythrocyte or endothelial tissue heme proteins.

Three factors uniquely position nitrite, rather than S-nitrosothiol, as the major vascular storage pool of NO: 1) Nitrite is present in substantial concentrations in plasma, erythrocytes and in tissues (Rodriguez *et al., Proc Natl Acad Sci USA* 100:336-341, 2003). 2) Nitrite is relatively stable, because it is not readily reduced by intracellular reductants, as are S-nitrosothiols (Gladwin *et al., J Biol Chem* 21:21, 2002) and its reaction rate with heme proteins is 10,000 times less than that of authentic NO. 3) Nitrite is only converted to NO by reaction with deoxyhemoglobin (or presumably deoxy-myoglobin, -cytoglobin, and -neuroglobin) and its "leaving group" is the met(ferric)heme protein which will not scavenge and inactivate NO (Doyle *et al., J Biol Chem* 256:12393-12398, 1981). Therefore, this pool provides the ideal substrate for NO generation during hypoxia, providing a novel mechanism for hypoxic vasodilation.

Because a deoxyhemoglobin-nitrite reductase system would result in NO formation in deoxygenating blood, such a system links hemoglobin oxygenation status to NO generation, the principle previously ascribed to S-nitroso-hemoglobin (Jia *et al., Nature* 380:221-226, 1996). Hemoglobin possesses anionic binding cavities that retain nitrite (Gladwin *et al., J Biol Chem* 21:21, 2002) and nitrite is taken up by erythrocytes through the anion exchange protein (AE1 or Band 3) or through the membrane as nitrous acid (a pH dependent process that accelerates nitrite uptake during tissue hypoxia (Shingles *et al., J Bioenerg Biomembr* 29:611-616, 1997; May *et al., Am I Physiol Cell Physiol* 279:C1946-1954, 2000). Such nitrite would provide a steady source of NO, NO_2 and S-nitrosothiol generation that would occur preferentially in hypoxic vascular territories. Because the AE1 protein binds both deoxyhemoglobin and methemoglobin and may channel nitrite, AE1 could serve to localize catalytic NO and S-nitrosothiol generation at the erythrocyte membrane, where the relatively lipophilic NO, NO_2 and N_2O_3 could react in the vicinal lipid bilayer (Figure 5). The erythrocyte membrane is lined by an unstirred outer diffusion barrier and an inner methemoglobin rich protein matrix that might further promote such NO and NO_2 chemistry (Coin *et al., J Biol Chem* 254:1178-1190, 1979; Liu *et al., J Biol Chem* 273:18709-18713, 1998; Han *et al., Proc Natl Acad Sci USA* 99:7763-7768, 2002).

This model is consistent with the *in vitro* observations of Pawloski and colleagues (Pawloski *et al., Nature* 409:622-626, 2001) showing that S-nitrosation of hemoglobin and AE1 occurs in the erythrocyte membrane after treatment of deoxygenated red blood cells with NO solutions (which contain significant-more than 50 μM- contaminating nitrite; Fernandez,*et al.* Inorg Chem 42:2-4, 2003). Further, N_2O_3 generated at the membrane could directly nitrosate the abundant intra-erythrocytic glutathione, eliminating the requirement of transnitrosation reactions with S-nitroso-hemoglobin and thus facilitating rapid export of low molecular weight S-nitrosothiol by simple diffusion across the erythrocyte membrane (Figure 5). A nitrite-hemoglobin chemistry supports a role for the red cell in oxygen-dependent NO homeostasis and provides a mechanism for the observations of multiple research groups that red blood cells and plasma "loaded" with NO, by exposure to NO in high concentration in solution or to NO gas or donors (in equilibria with high concentrations of nitrite), can export NO and induce vasodilation *in vitro* and *in vivo* (Rassaf *et al., J Clin Invest* 109:1241-1248, 2002; Fox-Robichaud *et al., J Glitz Invest* 101:2497-2505, 1998; McMahon *et al., Nat Med* 3:3, 2002; Cannon *et al., J Clin Invest* 108:279-287, 2001; Gladwin *et al., J Biol Chem* 21:21, 2002; Gladwin *et al., Circulation* 107:271-278, 2003; Schechter *et al., N Engl J Med* 348:1483-1485, 2003).

In addition to the reaction of nitrite with deoxyhemoglobin, reactions with deoxy-myoglobin, -cytoglobin and -neuroglobin or with other endothelial cell heme proteins may also be important. Such chemistry would occur between tissue nitrite and deoxy-myoglobin in vascular and skeletal muscle, thus contributing to hypoxic vasodilation and hypoxic potentiation of NO donors. The P_{50} of these globin monomers is approximately 3-5 mm Hg, placing their equilibrium deoxygenation point in the range of tissue pO_2 (0-10 mm Hg) during metabolic stress, such as exercise. Such a low oxygen tension reduces oxygen availability as substrate for NO synthesis, however, the tissue nitrite stores could then be reduced to NO and 5-nitrosothiol, thus sustaining critical vasodilation.

VII. Methods of Use

Therapeutic application of nitrite now can be used to provide selective vasodilation in a subject, and particularly to hypoxemic and ischemic tissue in the subject, and will be useful to treat hemolytic conditions such as sickle cell disease, where free hemoglobin released during hemolysis scavenges NO and disrupts NO-dependent vascular function. Nitrite is expected to not only inhibit the ability of free hemoglobin to scavenge NO by oxidizing it to methemoglobin, but also to generate NO in tissue beds with low oxygen tension. Thus, the applied nitrite will preferentially release nitric oxide at areas of low oxygen tension, thereby providing localized vasodilation and/or increased blood flow.

Nitrites can be administered to a subject to increase blood flow to a tissue of the subject, for example, to increase blood flow to a tissue, for instance a tissue with low oxygen tension; to cause vasodilation; to decrease a subject's blood pressure; to treat a subject having a condition associated with elevated blood pressure; to treat a hemolytic condition; to treat vascular complications associated with treatments or conditions that cause hemolysis; to treat pulmonary hypertension, cerebral vasospasm, or low blood flow to organs (such as ischemia reperfusion injury to organs including brain, heart, kidney, placenta, and liver); and/or to treat organs before and after transplantation.

Nitrite has vasodilatory properties *in vivo*

The vasodilator properties of nitrite and the mechanisms for its bioactivation were investigated as described herein. Sodium nitrite infused at 36 μmoles per minute into the forearm brachial artery of 18 normal volunteers resulted in a regional nitrite concentration of 222 μM and, surprisingly, a 175% increase in resting forearm blood flow. Increased blood flow was observed at rest, during NO synthase inhibition and with exercise. The nitrite infusion also surprisingly resulted in increased tissue perfusion, as demonstrated by increases in venous hemoglobin-oxygen saturation, partial pressure of oxygen, and pH. Increased systemic concentrations of nitrite (16 μM) significantly reduced mean arterial blood pressure.

In an additional ten subjects, the dose of nitrite was reduced 2-logs, resulting in a forearm nitrite concentration of 2 μM at rest and 0.9 μM during exercise (Figure 3). These concentrations of nitrite surprisingly significantly increased blood flow at rest and during NO synthase inhibition, with and without exercise.

Nitrite infusions were associated with the rapid formation of erythrocyte iron-nitrosyl-hemoglobin, and to a lesser extent, S-nitroso-hemoglobin across the forearm vasculature. Formation of these NO-Hb adducts was inversely proportional to the oxyhemoglobin saturation. Additionally, vasodilation of rat aortic rings and the formation of both NO gas and NO-modified hemoglobin from the nitrite reductase activity of deoxyhemoglobin and deoxygenated erythrocytes was observed, a result that links tissue hypoxia, hemoglobin allostery, and nitrite bioactivation. These results indicate that physiological levels of blood and tissue nitrite are a major bioavailable pool of NO that contributes to vaso-regulation and provide a mechanism for hypoxic vasodilation via reaction of vascular nitrite with deoxygenated heme proteins in tissue and/or the erythrocyte.

The findings described herein that administration of nitrite reduces blood pressure and increases blood flow are unexpected and surprising because published reports to date teach the person of ordinary skill in the art that pharmacological levels of nitrites (below about 100-200 μM), when administered to subjects, lack intrinsic vasodilatory properties (Lauer *et al.*, *Proc Natl Acad Sci USA,* 98:12814-9, 2001).

It is also believed that pharmaceutically acceptable salts of nitrite can be infused into patients with hemolytic disease, such as sickle cell disease, to improve blood flow, limit ischemia-reperfusion tissue injury, and oxidize cell-free plasma Hb. These effects should be useful in the treatment of sickle cell vaso-occlusive pain crisis, stroke (brain ischemia) and the acute chest syndrome.

Cytoprotective Effects of Nitrite during Ischemia-reperfusion of the Heart and Liver

The anion nitrite (NO_2^-) forms as a consequence of nitric oxide (NO) oxidation and is present at concentrations of 0.3-1.0 μM in plasma and 1-20 μM in tissue (Gladwin *et al.*, *Proc Natl Acad Sci U S A* 97:11482-11487, 2000; Rodriguez *et al.*, *Proc Natl Acad Sci U S A* 100:336-341, 2003; Rassaf *et al.*, *Nat Med* 9:481-483, 2003; Bryan *et al.*, *Proc Natl Acad Sci U S A*., 2004; Gladwin *et al.*, *J Clin Invest* 113:19-21, 2004). Nitrite has been historically considered an inert metabolic end product with limited intrinsic biological activity (Lauer *et al.*, *Proc Natl Acad Sci U S A* 98:12814-12819, 2001; McMahon, *N Engl J Med* 349:402-405; author reply 402-405, 2003; Pawloski, *N Engl J Med* 349:402-405; author reply 402-405, 2003). Recent data from our group and others suggest that nitrite may be reduced to NO during hypoxia and acidosis (Gladwin *et al.*, *Proc Natl Acad Sci U S A* 97:11482-11487, 2000; Bryan *et al.*, *Proc Natl Acad Sci U S A.*, 2004; Cosby *et al.*, *Nat Med* 9:1498-1505, 2003; Nagababu *et al.*,. *J Biol Chem* 278:46349-46356, 2003; Tiravanti *et al.*, *J Biol Chem* 279:11065-11073, 2004). At extremely low tissue pH and PO_2, nitrite may be reduced to NO by disproportionation (acidic reduction; Zweier *et al.*, *Nat Med* 1:804-809, 1995) or by the enzymatic action of xanthine oxidoreductase (Millar *et al.*, *FEBS Lett* 427:225-228, 1998; Zhang *et al.*, *Biochem Soc Trans* 25:524S, 1997; Godber *et al.*, *J Biol Chem* 275:7757-7763, 2000; Li *et al.*, *J Biol Chem* 276:24482-24489, 2001).

Nitrite represents a circulating and tissue storage form of nitric oxide (NO) whose bioactivation is mediated by the nitrite reductase activities of deoxyhemoglobin. Because the rate of NO generation from nitrite is linearly dependent on reductions in oxygen and pH, we hypothesized that nitrite would be reduced to NO in ischemic tissue and exert NO-dependent protective effects. Solutions of sodium nitrite were administered in the setting of hepatic and cardiac ischemia-reperfusion (I/R) injury in mice. In hepatic I/R, nitrite exerted profound dose dependent protective effects on cellular necrosis and apoptosis with highly significant protective effects observed at near-physiological nitrite concentrations (0.6 μM). In myocardial I/R injury, nitrite reduced cardiac infarct size by 67% and significantly improved post-ischemic left ventricular ejection fraction. Consistent with hypoxia dependent nitrite bioactivation, nitrite was reduced to NO, S-nitrosothiols, N-nitrosamines and iron-nitrosylated heme proteins within 1-30 minutes of reperfusion. Nitrite-mediated protection was dependent on NO generation and independent of eNOS and HO-1. These results suggest that nitrite is a biological storage reserve of NO subserving a critical function in tissue protection from ischemic injury. These studies evince an unexpected and novel therapy for diseases such as myocardial infarction, organ preservation and transplantation, and shock states.

Although reperfusion of ischemic tissues provides oxygen and metabolic substrates necessary for the recovery and survival of reversibly injured cells, reperfusion itself actually results in the acceleration of cellular necrosis (Braunwald *et al.*, *J. Clin. Invest.* 76:1713-1719, 1985). Ischemia-reperfusion is characterized by the formation of oxygen radicals upon reintroduction of molecular oxygen to ischemic tissues resulting in widespread lipid and protein oxidative modifications of cellular proteins, mitochondrial injury, and tissue apoptosis and necrosis (McCord *et al.*, *Adv Myocardiol* 5:183-189, 1985). In addition, following reperfusion of ischemic tissues blood flow may not return uniformly to all portions of the ischemic tissues, a phenomenon that has been termed the "no-reflow" phenomenon (Kloner *et al.*, *J Clin Invest* 54:1496-1508, 1974). Reductions in blood flow following reperfusion are thought to contribute to cellular injury and necrosis (Kloner *et al.*, *J Clin Invest* 54:1496-1508, 1974). The sudden re-introduction of blood into ischemic tissue also results in a dramatic increase in calcium delivery to the previously ischemic tissue (*i.e.*, "calcium paradox") resulting in massive tissue disruption, enzyme release, reductions in high energy phosphate stores, mitochondrial injury, and necrosis (Nayler, *Amer. J. Path.* 102:262, 1981; Shen *et al.*, *Amer. J. Path* 67:417-440, 1972). Recent studies have also indicated that the ischemia-reperfusion injury is also characterized by an inappropriate inflammatory response in the microcirculation resulting in leukocyte-endothelial cell interactions that are mediated by the upregulation of both leukocyte and endothelial cell adhesion molecules (Lefer *et al.*, *Cardiovasc Res* 32:743-751, 1996; Entman *et al.*, *Faseb J* 5:2529-2537, 1991). Intensive research efforts have been focused on ameliorating various pathophysiological components of ischemia-reperfusion injury to limit the extent of tissue injury and necrosis.

NO, NO donors, and NO synthase activation or transgenic over-expression have been shown to exert protective effects on this process in a number of models (Lefer *et al.*, *New Horiz* 3:105-112, 1995; Lefer *et al.*, *Circulation* 88:2337-2350, 1993; Nakanishi *et al.*, *Am J Physiol* 263:H1650-1658, 1992; Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004; Jones *et al.*, *Proc Natl Acad Sci U S A* 100:4891-4896. 2003; Kanno *et al.*, *Circulation* 101:2742-2748, 2000), but in other models appears harmful (Flogel *et al.*, *J Mol Cell Cardiol* 31:827-836. 1999; Menezes *et al.*, *Am J Physiol* 277:G144-151, 1999; Woolfson *et al.*, *Circulation* 91:1545-1551, 1995; Schulz, R. *et al.*, *Cardiovasc Res* 30:432-439, 1995). Evaluation of these studies suggests a critical effect of dose and duration of NO exposure, resulting in a narrow therapeutic safety window for NO in ischemia-reperfusion pathophysiology (Bolli, *J. Mol. Cell. Cardio.* 33:1897-1918, 2001; Wink *et al.*, *Am J Physiol Heart Circ Physiol* 285:H2264-2276, 2003). An additional limitation is that NO formation from NO synthase requires oxygen as substrate, a molecule whose availability becomes limited during ischemia.

We therefore considered the use of nitrite in this context for the following reasons: (1) It is a naturally occurring substance with no potentially toxic "leaving group" (2), it is selectively reduced to NO in tissues with low oxygen tension and low pH (Bryan *et al.*, *Proc Natl Acad Sci U S A.*, 2004; Cosby *et al.*, *Nat Med* 9:1498-1505, 2003; Nagababu *et al.*, *J Biol Chem* 278:46349-46356, 2003; Tiravanti *et al.*, *J Biol Chem* 279:11065-11073, 2004; Doyle *et al.*, *J Biol Chem* 256:12393-12398, 1981; Luchsinger *et al.*, *Proc Natl Acad Sci US A* 100:461-466, 2003), (3) its activation does not require molecular oxygen (Cosby *et al.*, *Nat Med* 9:1498-1505, 2003), and (4) NO is known to maintain heme proteins in a reduced and liganded state (Herold *et al.*, *Free Radic Biol Med* 34:531-545, 2003; Herold *et al.*, *J Biol Inorg Chem* 6:543-555, 2001; Fernandez *et al.*, *Inorg Chem* 42:2-4, 2003), limit free iron and heme mediated oxidative chemistry (Kanner *et al.*, *Arch Biochem Biophys* 237:314-321, 1985; Kanner *et al.*, *Lipids* 20:625-628, 1985; Kanner *et al.*, *Lipids* 27:46-49, 1992), transiently inhibit cytochome c oxidase and mitochondrial respiration (Torres *et al.*, *FEBS Lett* 475:263-266, 2000; Brown *et al.*, *FEBS Lett* 356:295-298, 1994; Cleeter *et al.*, *FEBS Lett* 345:50-54, 1994; Rakhit *et al.*, *Circulation* 103:2617-2623, 2001), and modulate apoptotic effectors (Mannick *et al.*, *Science* 284:651-654, 1999), all mechanisms that might participate in cytotoxicity following severe ischemia.

Nitric oxide has been shown to quench oxygen free radicals in a transient ischemia and reperfusion injury animal models (Mason *et al.*, *J Neurosurg* 93: 99-107, 2000), significantly limiting volume of stroke (Pluta *et al.*, *Neurosurgery*, 48:884-892, 2001). Therefore, nitrite via releasing NO in the area of reperfusion may also have the same beneficial effect on stroke via limiting oxygen free radicals presence after reperfusion.

Furthermore, the selective opening of blood-tumor barrier by NO facilitates penetration of chemotherapeutic agents into the brain tumor (Weyerbrock *et al.*, *J. Neurosurgery*, 99:728-737, 2003); it is believed that this will also enhance penetration of other agents, particularly therapeutic agents such as radiation therapy, brain cancer. Therefore, due to hypoxic conditions within the brain tumor it is possible that nitrite can also selectively open the blood-tumor barrier providing beneficial effect in combination with chemotherapy.

Inhaled Nebulized Nitrite is a Pulmonary Vasodilator

Persistent pulmonary hypertension of the newborn occurs with an incidence of 0.43–6.8/1,000 live births and is associated with mortality rates between 10–20% (Walsh-Sukys *et al.*, *Pediatrics* 105, 14-20, 2000). Survivors may develop neurodevelopmental and audiological impairment (46%), cognitive delays (30%), hearing loss (19%) and a high rate of rehospitalization (22%) (Lipkin *et al.*, *J Pediatr* 140, 306-10, 2002).

Pulmonary hypertension occurs as a primary or idiopathic disease (Runo & Loyd, *Lancet* 361:1533-44, 2003; Trembath & Harrison, *Pediatr Res* 53:883-8, 2003), as well as secondary to a number of systemic and pulmonary diseases (Rubin, *N Engl J Med* 336:111-7, 1997). Regardless of etiology, pulmonary hypertension is associated with substantial morbidity and mortality. Newborn infants and adults with pulmonary disease often develop systemic hypoxemia, reduced oxyhemoglobin saturation and increased pulmonary vascular resistance (Rubin, *N Engl J Med* 336:111-7, 1997; Haworth, *Heart* 88:658-64, 2002). Therapeutically administered inhaled nitric oxide (NO) decreases pulmonary vascular resistance in newborns and adults and improves ventilation-to-perfusion matching and oxygenation; in newborns, inhaled NO reduces chronic lung damage and reduces the need for extracorporeal membrane oxygenation. Randomized placebo-controlled trials of inhaled NO therapy for term and near-term newborns with severe hypoxic respiratory failure demonstrated an improvement in hypoxemia and reduced need for extracorporal membrane oxygenation (Clark *et al.*, *N Engl J Med* 342, 469-74, 2000; Roberts *et al.*, *N Engl J Med* 336, 605-10, 1997; The Neonatal Inhaled Nitric Oxide Study Group. *N Engl J Med* 336, 597-604, 1997). A recent randomized placebo-controlled trial in premature infants with respiratory distress syndrome indicated that treatment with inhaled NO reduced the combined endpoint of death and chronic lung disease (Schreiber *et al.*, *N Engl J Med* 349, 2099-107, 2003).

Despite the encouraging results regarding treatment of persistent pulmonary hypertension of the newborn with inhaled NO, the therapy does have several significant limitations (Martin, *N Engl J Med* 349, 2157-9, 2003): considerable cost (Jacobs *et al.*, *Crit Care Med* 30, 2330-4, 2002; Pierce *et al.*,. *Bmj* 325, 336, 2002; Subhedar *et al.*, *Lancet* 359, 1781-2, 2002; Angus *et al.*, *Pediatrics* 112, 1351-60, 2003), technical difficulties involved in adapting NO delivery systems for neonatal transport (Kinsella *et al.*, *Pediatrics* 109, 158-61, 2002), and the lack of availability in small community hospitals and developing countries. In addition, NO reacts with oxygen, forming the toxic nitrogen dioxide, and thus must be stored and delivered in nitrogen at high flow rates. The gas and delivery systems are costly and the requisite delivery technology is not universally available. Therefore, alternative NO-based therapies for the treatment of pulmonary hypertension are highly desirable.

The relationship between nitrite and nitric oxide has been appreciated for close to a century, with Haldane and later Hoagland recognizing that iron-nitrosylated myoglobin (NO bound to heme) formed as an end-product during nitrite-based meat curing (Gladwin, *J Clin Invest* 113, 19-21, 2004). More than fifty years ago, Furchgott and Bhadrakom reported that nitrite vasodilated aortic ring preparations *in vitro* (Furchgott & Bhadrakom, *J Pharmacol Exp Ther* 108, 129-43, 1953); this observation was later explored by Ignarro's group in experiments evaluating the role of soluble guanylyl cyclase in endothelium-dependent vasodilation (Ignarro *et al.*, *J Pharmacol Exp Ther* 218, 739-49, 1981). However, the high concentrations of nitrite, typically in the millimolar range, required to elicit vasodilation in aortic ring *in vitro* bioassays precluded consideration of nitrite as a physiological vasodilator (Lauer *et al.*, *Proc Natl Acad Sci U S A* 98, 12814-9, 2001; Pawloski, *N Engl J Med* 349, 402-5; author reply 402-5, 2003; McMahon, *N Engl J Med* 349, 402-5; author reply 402-5, 2003).

Two decades later, in human physiological studies, we observed artery-to-vein differences for nitrite across the human forearm with increased extraction occurring during NO inhalation and exercise stress with concomitant NO synthase inhibition (Gladwin *et al.*, *Proc Natl Acad Sci U S A* 97, 11482-7, 2000). This finding suggested that nitrite was being metabolized across the forearm with increased consumption during exercise. Based on these observations along with data from a number of investigators that identified mechanisms for non-enzymatic (nitrite disproportionation) (Zweier *et al.*, *Nat Med* 1, 804-9, 1995) and enzymatic (xanthine oxidoreductase) (Zweier *et al.*, *Nat Med* 1, 804-9, 1995; Millar *et al.*, *FEBS Lett* 427, 225-8, 1998; Tiravanti *et al.*, *J Biol Chem* 279:11065-11073, 2004; Li *et al.*, *J Biol Chem*, 279(17):16939-16946, 2004) reduction of nitrite to NO, we hypothesized that nitrite is reduced *in vivo* to NO in tissues under conditions of low Po_2 or pH. We found support for this hypothesis in studies of normal human volunteers wherein nitrite infusion into the forearm resulted in marked vasodilation even under basal conditions at near-physiological nitrite concentrations (Example 1; Cosby *et al.*, *Nat Med* 9, 1498-505, 2003). The mechanism of this vasodilation was consistent with a reaction of nitrite with deoxygenated hemoglobin to form NO, methemoglobin (Cosby *et al.*, *Nat Med* 9, 1498-505, 2003; Nagababu *et al.*, *J Biol Chem* 278, 46349-56, 2003) and other NO adducts.

This nitrite reductase activity of deoxyhemoglobin was extensively characterized by Doyle and colleagues in 1981 (Doyle *et al.*, *J Biol Chem* 256, 12393-8, 1981): nitrite appears to react with deoxyhemoglobin and a proton to form NO and methemoglobin. Such chemistry is ideally suited for hypoxic generation of NO from nitrite, as the reaction is enhanced by hemoglobin deoxygenation and acid, providing a graded production of NO from nitrite linked to physiological changes in oxygen and pH/CO_2. The observation in this current example that inhaled nitrite generates iron-nitrosyl-hemoglobin, exhaled NO gas, and produces vasodilation in proportion to decreasing levels of oxygenation and pH further indicates that nitrite is a bioavailable storage pool of NO and that hemoglobin may have a physiological function as a nitrite reductase, potentially contributing to hypoxic vasodilation (see Example 1). In addition to these mechanistic considerations, this example supports another therapeutic application of nitrite, extending beyond its well-established role in the treatment of cyanide poisoning.

We show herein (Example 3) that this biochemical reaction can be harnessed for the treatment of neonatal pulmonary hypertension, an NO-deficient state characterized by pulmonary vasoconstriction, right-to-left shunt pathophysiology, ventilation/perfusion inhomogeneity and systemic hypoxemia. We delivered inhaled sodium nitrite by aerosol to newborn lambs with hypoxic and normoxic pulmonary hypertension. Inhaled nitrite elicited a rapid and sustained reduction (~60%) in hypoxia induced pulmonary hypertension, a magnitude approaching that of the effects of 20 ppm NO gas inhalation and which was associated with the immediate appearance of increasing levels of NO in expiratory gas. Pulmonary vasodilation elicited by aerosolized nitrite was deoxyhemoglobin- and pH-dependent and was associated with increased blood levels of hemoglobin iron-nitrosylation. Significantly, from a therapeutic standpoint, short term delivery of nitrite, dissolved in saline, via nebulization produced selective and sustained pulmonary vasodilation with no appreciable increase in blood methemoglobin levels. These data support the paradigm that nitrite is a vasodilator acting via conversion to NO, a process coupled to hemoglobin deoxygenation and protonation, and further evince a novel, simple and inexpensive potential therapy for neonatal pulmonary hypertension.

Aerosolized nitrite is an effective vasodilatory in the described newborn lamb model (Example 3). It can be readily administered by nebulization, and appears to exhibit a wide therapeutic-to-safety margin, with limited systemic hemodynamic changes and methemoglobin production. This presents an attractive therapeutic option to inhaled NO. Nitrite is an ideal "NO producing" agent in that it 1) is a naturally occurring compound in blood, alveolar lining fluid, and tissue, and 2) has no parent-compound leaving group, such as the diazenium diolates, that requires extensive toxicological study prior to translation to human disease.

Inhaled nitrite is a potent and selective vasodilator of pulmonary circulation of the newborn lamb. This further supports the paradigm that nitrite is an NO-dependent vasodilator whose bioactivation is coupled to hemoglobin deoxygenation and protonation. This has clinical applications in veterinary and medical situations, including pulmonary hypertension and other pulmonary syndromes with apparent NO deficiencies. Based on the data presented herein, it is believed that inhaled nitrite will have efficacy in all known and tested applications of inhaled NO.

Prevention of Cerebral Artery Vasospasm after Subarachnoid Hemorrhage

Further, it has been discovered that nitrite infusion can be used to prevent cerebral artery vasospasm after aneurismal hemorrhage (Example 4). Subarachnoid hemorrhage (SAH) due to the rupture of intracranial aneurysms affects 28,000 Americans annually. Almost 70% of patients with aneurysmal SAH develop severe spasm of the cerebral arteries on the seventh day after SAH.

Despite aggressive medical therapy, neurological deficits resulting from vasospasm continue to be a major cause of morbidity and mortality. Although the etiology of cerebral vasospasm is poorly understood, there is increasing evidence that erythrocyte hemolysis in the cerebrospinal fluid and decreased availability of nitric oxide (NO), a potent vasodilator, plays a significant role. Reversal of vasospasm by NO or NO prodrugs has been documented in several animal models.

Delayed cerebral vasospasm (DCV) remains the single cause of permanent neurological deficits or death in at least fifteen percent of patients following otherwise successful endovascular or surgical treatment for ruptured intracranial aneurysm. Decreased bioavailability of nitric oxide (NO) has been mechanistically associated with the development of DCV. A primate model system for cerebral artery vasospasm was used to determine whether infusions of nitrite, a naturally occurring anion that reacts with deoxyhemoglobin to form NO and S-nitrosothiol, might prevent DCV via reactions with perivascular hemoglobin.

As described in Example 4, nitrite infusions (45 mg/kg and 60 mg/kg per day) that produced blood levels of nitrite ranging from 10-60 microM with no clinically significant methemoglobin formation (<5%) were associated with increases in plasma cerebrospinal fluid nitrite and modest increases in blood methemoglobin concentrations (2% or less) without systemic hypotension, and significantly reduced the severity of vasospasm (Figures 15 and 16). No animals infused with sodium nitrite developed significant vasospasm; mean reduction in the R MCA area on day 7 after SAH was 8±9% versus 45±5%; $P < 0.001$) Pharmacological effects of nitrite infusion were associated with bioconversion of cerebrospinal fluid nitrite to S-nitrosothiol, a potent vasodilating NO donor intermediate of nitrite bioactivation. There was no clinical or pathological evidence of nitrite toxicity.

Subacute sodium nitrite infusions prevent DCV in a primate model of SAH, and do so without toxicity. These data evince a novel, safe, inexpensive, and rationally designed therapy for DCV, a disease for which no current preventative therapy exists.

The results presented herein suggest that sodium nitrite therapy may prevent tissue injury produced by metabolic products of hemoglobin, either by vascular spasm, or by other mechanisms of tissue injury by these metabolic products.

Treatment or Amelioration of Gestational or Fetal Cardiovascular Malconditions

Based on results presented herein, it is believed that nitrite, particularly pharmaceutically acceptable salts of nitrite as described herein, can be used to treat hypertension and preeclampsia during pregnancy. Such therapy would include action of nitrites on spastic and diseased blood vessels within the placenta.

Also suggested are methods for treating fetuses in utero, particularly those afflicted with cardiovascular anomalies, hypertension, and misdirected blood flow. It is believed that it may be possible to add nitrites to the amniotic fluid, and thus indirectly to the fetus, to achieve vasodilation and redistribution of blood flow before birth. By this means, fetal cardiovascular system development and function could be altered, for instance with promotion of blood flow to the brain and heart. To be effective longer term, it is envisioned that embodiments of such fetal therapy would include the introduction of one or more mini-osmotic pumps, containing nitrite (*e.g.*, sodium nitrite), into the amniotic cavity to thereby achieve sustained, slow release. For instance, such minipumps could be used to achieve sustained release throughout days and weeks of pregnancy.

Also suggested are methods for treating fetuses in whom plasma nitrite levels may be depressed by immune incompatibility and associated hemolytic anemias. Such fetal treatment may be extended into the neonatal period. Administrated in the fetal period may include implantation of nitrite-charged osmotic minipumps into the amniotic cavity and could include aerosol inhalation after birth.

VIII. Formulations and Administration

Nitrites, including their salts, are administered to a subject in accordance to methods provided herein, in order to decrease blood pressure and/or increase vasodilation in a subject. Administration of the nitrites in accordance with the present disclosure may be in a single dose, in multiple doses, and/or in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the nitrites may be essentially continuous over a preselected period of time or may be in a series of spaced doses. The amount administered will vary depending on various factors including, but not limited to, the condition to be treated and the weight, physical condition, health, and age of the subject. Such factors can be determined by a clinician employing animal models or other test systems that are available in the art.

To prepare the nitrites, nitrites are synthesized or otherwise obtained and purified as necessary or desired. In some embodiments of the disclosure, the nitrite is a pharmaceutically-acceptable salt of nitrite, for example, sodium nitrite. In some embodiments of the disclosure, the nitrite is not ethyl nitrite. In some embodiments of the disclosure, the sodium nitrite is not on a medical devise, for example, not on a stent. In some embodiments of the disclosure, the nitrite is not in the form of a gel. The nitrites can be adjusted to the appropriate concentration, and optionally combined with other agents. The absolute weight of a given nitrite included in a unit dose can vary. In some embodiments of the disclosure, the nitrite is administered as a salt of an anionic nitrite with a cation, for example, sodium, potassium, or arginine.

One or more suitable unit dosage forms including the nitrite can be administered by a variety of routes including topical, oral (for instance, in an enterically coated formulation), parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), rectal, intraamnitic, dermal, transdermal, intrathoracic, intrapulmonary and intranasal (respiratory) routes.

The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods known to the pharmaceutical arts. Such methods include the step of mixing the nitrite with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system. By "pharmaceutically acceptable" it is meant a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious or unsuitably harmful to the recipient thereof. The therapeutic compounds may also be formulated for sustained release, for example, using microencapsulation (see WO 94/ 07529, and U.S. Patent No. 4,962,091).

The nitrites may be formulated for parenteral administration (*e.g.*, by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion containers or in multi-dose containers. Preservatives can be added to help maintain the shelve life of the dosage form. The nitrites and other ingredients may form suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the nitrites and other ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, *e.g.*, sterile, pyrogen-free water, before use.

These formulations can contain pharmaceutically acceptable carriers and vehicles that are available in the art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents such as acetone, ethanol, isopropyl alcohol, glycol ethers such as the products sold under the name "Dowanol," polyglycols and polyethylene glycols, C_1-C_4 alkyl esters of short-chain acids, ethyl or isopropyl lactate, fatty acid triglycerides such as the products marketed under the name "Miglyol," isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

It is possible to add other ingredients such as antioxidants, surfactants, preservatives, film-forming, keratolytic or comedolytic agents, perfumes, flavorings and colorings. Antioxidants such as t-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α-tocopherol and its derivatives can be added.

The pharmaceutical formulations of the present disclosure may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are available in the art. Examples of such substances include normal saline solutions such as physiologically buffered saline solutions and water. Specific non-limiting examples of the carriers and/or diluents that are useful in the pharmaceutical formulations of the present disclosure include water and physiologically acceptable buffered saline solutions, such as phosphate buffered saline solutions. Merely by way of example, the buffered solution can be at a pH of about 6.0-8.5, for instance about 6.5-8.5, about 7-8.

The nitrites can also be administered via the respiratory tract. Thus, the present disclosure also provides aerosol pharmaceutical formulations and dosage forms for use in the methods of the disclosure. In general, such dosage forms include an amount of nitrite effective to treat or prevent the clinical symptoms of a specific condition. Any attenuation, for example a statistically significant attenuation, of one or more symptoms of a condition that has been treated pursuant to the methods of the present disclosure is considered to be a treatment of such condition and is within the scope of the disclosure.

For administration by inhalation, the composition may take the form of a dry powder, for example, a powder mix of the nitrite and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, *e.g.*, gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator, or a metered-dose inhaler (see, for example, the pressurized metered dose inhaler (MDI) and the dry powder inhaler disclosed in Newman, S. P. in *Aerosols and the Lung*, Clarke, S. W. and Davia, D. eds., pp. 197-224, Butterworths, London, England, 1984).

Nitrites may also be administered in an aqueous solution, for example, when administered in an aerosol or inhaled form. Thus, other aerosol pharmaceutical formulations may include, for example, a physiologically acceptable buffered saline solution. Dry aerosol in the form of finely divided solid compound that is not dissolved or suspended in a liquid is also useful in the practice of the present disclosure.

For administration to the respiratory tract, for example, the upper (nasal) or lower respiratory tract, by inhalation, the nitrites can be conveniently delivered from a nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may include a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Nebulizers include, but are not limited to, those described in U.S. Patent Nos. 4,624,251; 3,703,173; 3,561,444; and 4,635,627. Aerosol delivery systems of the type disclosed herein are available from numerous commercial sources including Fisons Corporation (Bedford, Mass.), Schering Corp. (Kenilworth, NJ) and American Pharmoseal Co. (Valencia, CA). For intra-nasal administration, the therapeutic agent may also be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker). The nitrites may also be delivered via an ultrasonic delivery system. In some embodiments of the disclosure, the nitrites may be delivered via an endotracheal tube. In some embodiments of the disclosure, the nitrites may be delivered via a face mask.

The present disclosure further pertains to a packaged pharmaceutical composition such as a kit or other container. The kit or container holds a therapeutically effective amount of a pharmaceutical composition of nitrite and instructions for using the pharmaceutical composition for treating a condition.

IX. Combination Therapies

Furthermore, the nitrite may also be used in combination with other therapeutic agents, for example, pain relievers, anti-inflammatory agents, antihistamines, and the like, whether for the conditions described or some other condition. By way of example, the additional agent is one or more selected from the list consisting of penicillin, hydroxyurea, butyrate, clotrimazole, arginine, or a phosphodiesterase inhibitor (such as sildenafil).

Generally, it is believed that therapies that have been suggested or demonstrated to be effective when combined with NO therapy, may also be effective when combined with nitrite administration. All combination therapies that have been are being studied with NO therapy (inhaled or otherwise) are likely to be worthy of study in combination with nitrite therapy. See, for instance, Uga et al., *Pediatr. Int.* 46 (1): 10-14, 2004; Gianetti *et al.*, *J Thorac. Cardiov. Sur.* 127 (1): 44-50, 2004; Stubbe et al., *Intens. Care Med.* 29 (10): 1790-1797, 2003; Wagner *et al.*, Eur. Heart J 23: 326-326 Suppl. 2002; Park *et al.*, Yonesi Med J 44 (2):219-226, 2003; Kohele, *Israel Med. Assoc. J.* 5:19-23, 2003, for discussions of combination therapies used with NO.

Furthermore, pharmaceutically-acceptable nitrite salts (such as, for instance, sodium nitrite) may be used in combinations with drugs and agents that limit the elimination rate of administered nitrites. This combination could serve to prolong the duration of action of nitrite and would include antagonists and inhibitors of enzymes affecting the elimination of nitrites or their conversion to NO.

Alternatively, the nitrite may be used in combinations with drugs and agents that augment the action of nitrites. This combination could serve to increase the strength of responses to administered nitrites.

Recombinant tissue plasminogen activator (rt-PA) and urokinase are the only drugs that have proven to open occluded brain arteries in ischemic stroke. It is believed possible that using nitrite via quenching oxygen free radicals produced in response to reperfusion may provide an additional beneficial effect.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the invention to the particular features or embodiments described.

Example 1

Nitrite has vasodilatory properties *in vivo*

This example provides a demonstration that nitrite, administered by infusion to the forearm of human subjects, is an effective vasodilator.

Methods

Human subjects protocol.

The protocol was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute, and informed consent was obtained from all volunteer subjects. Nine men and nine women, with an average age of 33 years (range 21 - 50 years), participated in the study. An additional 10 subjects returned three-six months later for a second series of experiments with low dose nitrite infusion. Volunteers had a normal hemoglobin concentration, and all were in excellent general health without risk factors for endothelial dysfunction (fasting blood sugar >120 mg/dL, low-density lipoprotein cholesterol >130 mg/dL, blood pressure >145/95 mmHg, smoking within two years, cardiovascular disease, peripheral vascular disease, coagulopathy, or any other disease predisposing to vasculitis or Raynaud's phenomenon). Subjects with G6PD deficiency, known cytochrome B5 deficiency or a baseline methemoglobin level > 1% were excluded (no screened subjects met these exclusion criteria). Lactating and pregnant females were excluded (one subject with positive HCG levels was excluded). No volunteer subject was allowed to take any medication (oral contraceptive agents allowed), vitamin supplements, herbal preparations, nutriceuticals or other "alternative therapies" for at least one month prior to study and were not be allowed to take aspirin for one week prior to study.

Forearm blood flow measurements

Brachial artery and antecubital vein catheters were placed into the arm, with the intra-arterial catheter connected to a pressure transducer for blood pressure measurements and an infusion pump delivering normal saline at 1 mL/min. After 20 minutes of rest, baseline arterial and venous blood samples were obtained and forearm blood flow measurements were made by strain gauge venous-occlusion plethysmography, as previously reported (Panza *et al.*, *Circulation, 87*, 1468-74, 1993). A series of 7 blood flow measurements were averaged for each blood flow determination. A series of measurements termed Parts I and II were performed in randomized order to minimize a time effect on the forearm blood flow response during nitrite infusion.

Measurement of blood flow and forearm nitrite extraction during NO blockade and repetitive exercise

Part I: Following 20 minutes of 0.9% NaCl (saline) solution infusion at 1 mL/min into the brachial artery, arterial and venous blood samples were obtained for the assays described below and forearm blood flow measured. Exercise was performed by repetitive hand-grip at one-third of the predetermined maximum grip strength using a hand-grip dynamometer (Technical Products Co.) (Gladwin *et al.*, *Proc Natl Acad Sci U S A, 97*, 9943-8, 2000; Gladwin *et al.*, *Proc Natl Acad Sci U S A, 97*, 11482-11487, 2000; Cannon *et al.*, *J Clin Invest, 108*, 279-87, 2001). Each contraction lasted for 10 seconds followed by relaxation for 5 seconds. Following 5 minutes of exercise, forearm blood flow measurements were obtained during relaxation phases of exercise, and arterial and venous samples collected. Following a 20-minute rest period with continued infusion of saline into the brachial artery, repeated baseline blood samples and forearm blood flow measurements were obtained. L-NMMA was then infused at a rate of 1 mL/min (8 μmol/min) into the brachial artery. Following 5 minutes of L-NMMA infusion, forearm blood flow was measured, and arterial and venous blood samples obtained. Forearm exercise was then initiated in that arm during continued L-NMMA infusion. Forearm blood flow was measured and blood samples obtained after 5 minutes of exercise during continued L-NMMA infusion (Figure 1).

Part II: After a 30 minute rest period with continued infusion of saline, baseline measurements were obtained, the saline infusion was then stopped, and infusion of nitrite ($NaNO_2$ 36 μmol/ml in 0.9% saline) at 1 ml/min was started. Sodium nitrite for use in humans was obtained from Hope Pharmaceuticals (300 mg in 10 ml water) and 286 mg was diluted in 100 ml 0.9% saline by the Pharmaceutical Development Service to a final concentration of 36 μmol/ml. For the final 9 subjects studied, 0.01-0.03 mM sodium bicarbonate was added to the normal saline, so as to titrate pH to 7.0-7.4. The nitrite solution was light protected and nitrite levels and free NO gas in solution measured by reductive chemiluminescence after all experiments (Gladwin *et al.*, *J Biol Chem, 21*, 21, 2002). Only 50.5 ± 40.5 nM NO was present in nitrite solutions and was unaffected by bicarbonate buffering. There was no correlation between NO levels in nitrite solutions and blood flow effects of nitrite (r = -0.23; P=0.55). After 5 minutes of nitrite infusion, forearm blood flow measurements and blood samples were obtained, with brief interruption of the nitrite infusion to obtain the arterial sample. With continued nitrite infusion, exercise was performed as described previously, with forearm blood flow measurements and blood samples obtained as described above. The nitrite infusion was stopped and saline infusion re-started during the subsequent 30-minute rest period. Following second baseline measurements, the nitrite infusion was re-initiated, along with L-NMMA at 8 μmol/min. Five minutes later, forearm blood flow measurements were performed and blood samples obtained followed by 5 minutes of exercise with continuation of nitrite and L-NMMA infusions. Final forearm blood flow measurements and blood samples obtained. At all time points during part II, blood samples were obtained from the contralateral arm antecubital vein for determination of methemoglobin and systemic levels of NO-modified hemoglobin (Figure 2, 3, and 4). The total dose of sodium nitrite infused was 36 μmol/min x 15 minutes x 2 infusions = 1.08 mmol = 75 mg (MW $NaNO_2$ = 69).

In additional studies in 10 subjects the same stages of Parts I and II protocol were followed with infusion of low dose nitrite ($NaNO_2$ 0.36 μmol/ml in 0.9% saline, infused at 1 ml/min).

Arterial and venous pH, pO_2, and pCO_2, were measured at the bedside using the i-STAT system (i-STAT Corporation, East Windsor, NJ) and methemoglobin concentration and hemoglobin oxygen saturation measured by co-oximetry.

Measurement of red blood cell S-nitroso-hemoglobin and iron-nitrosyl-hemoglobin.

S-nitroso-hemoglobin is unstable in the reductive red blood cell environment and rapidly decays in a temperature and redox dependent fashion, independent of oxygen tension (Gladwin *et al.*, *J Biol Chem,* 21:21, 2002). To stabilize the S-nitroso-hemoglobin for measurement, the red blood cell must be rapidly oxidized with ferricyanide. Before and during nitrite infusions, blood was drawn from both the brachial artery and antecubital vein and the whole blood immediately (at the bedside to eliminate processing time) lysed 1:10 in an NO-hemoglobin "stabilization solution" of PBS containing 1% NP-40 (to solubilize membranes), 8 mM NEM (to bind free thiol and prevent artefactual S-nitrosation), 0.1 mM DTPA (to chelate trace copper), and 4 mM ferricyanide and cyanide (to stabilize S-nitrosohemoglobin and prevent artefactual ex-vivo iron-nitrosylation during processing). The samples were desalted across a 9.5 mL bed volume Sephadex G25 column to eliminate nitrite and excess reagents and partially purify hemoglobin (99% hemoglobin preparation). The hemoglobin fraction was quantified by the method of Drabkin, and hemoglobin fractions reacted with and without mercuric chloride (1:5 $HgCl_2$:heme ratio- used to differentiate S-nitrosothiol which is mercury labile versus iron-nitrosyl which is mercury stable) and then in 0.1 M HCL/0.5%sulfanilamide (to eliminate residual nitrite; Marley *et al.*, *Free Radic Res, 32*, 1-9, 2000). The samples were then injected into a solution of tri-iodide (I_3^-) in-line with a chemiluminescent nitric oxide analyzer (Sievers, Model 280 NO analyzer, Boulder, CO). The mercury stable peak represents iron-nitrosyl-hemoglobin. This assay is sensitive and specific for both S-nitroso-hemoglobin and iron-nitrosyl-hemoglobin to 5 nM in whole blood (0.00005% S-NO per heme) (Gladwin *et al.*, *J Biol Chem, 21*, 21, 2002).

Analysis was initially performed using red blood cell pellet, however, despite placing the sample in ice and immediately separating plasma from erythrocyte pellet, NO formed in the venous blood *ex vivo*. To measure the true *in vivo* levels, whole blood was mixed at the bedside 1:10 in the "NO-hemoglobin stabilization solution". Plasma S-nitroso-albumin formation was negligible during nitrite infusion so this bedside whole blood assay was used to limit processing time and thus more accurately characterize the *in vivo* chemistry. In a series of validation experiments, both S-nitroso-hemoglobin and iron-nitrosyl-hemoglobin were stable in the "NO-hemoglobin stabilization solution" for 20 minutes at room temperature with no artifactual formation or decay of NO-modified species (n=6).

Chemiluminescent detection of NO gas released from deoxyhemoglobin and deoxygenated erythrocytes following nitrite addition.

To determine whether free NO radical can form from the reaction of nitrite and deoxyhemoglobin, 100 and 200 μM nitrite was mixed with 5 mL of 660 and 1000 μM deoxygenated erythrocytes in a light protected reaction vessel purged with helium or oxygen (both 21% and 100%) in-line with a chemiluminescent NO analyzer (Seivers, Boulder, CO). After allowing equilibration for 5 minutes, nitrite was injected and the rate of NO production measured. Nitrite was injected into PBS as a control and into 100 μM hemoglobin to control for the hemolysis in the 660 and 1000 μM deoxygenated erythrocyte solutions. At the end of all experiments the visible absorption spectra of the supernatant and erythrocyte reaction mixture was analyzed and hemoglobin composition deconvoluted using a least-squares algorithm. There was less than 100 μM hemolysis in the system, no hemoglobin denaturation, and significant formation of iron-nitrosyl-hemoglobin. The NO production from erythrocyte suspensions exceeded that produced from the hemolysate control, consistent with NO export from the erythrocyte.

Statistical analysis.

An *a priori* sample size calculation determined that 18 subjects would be necessary for the study to detect a 25% improvement in forearm blood flow during nitrite infusion when forearm NO synthesis had been inhibited by L-NMMA compared with normal saline infusion control values (alpha=0.05, power=0.80). Two-sided P values were calculated by paired t-test for the pair-wise comparisons between baseline and L-NMMA infusion values, between baseline and exercise values, and between nitrite and saline control values at comparable time-points of the study. Repeated measures ANOVA were performed for artery-to-vein gradients of NO species during basal, L-NMMA infusion, and exercise conditions. Measurements shown are mean ± SEM.

Results and Discussion

Eighteen healthy subjects (9 males, 9 females; age range 21 to 50 years) were enrolled in a physiological study to determine if nitrite is a vasodilator and to examine nitrite's *in vivo* chemistry. Part I of the protocol was designed to measure the normal hemodynamic and metabolic responses to exercise and to inhibition of NO synthesis within the forearm as a control for Part II of the protocol, in which these interventions were performed during nitrite infusion. Initial baseline measurements included a mean blood pressure of 85.6 ± 3.7 mm Hg and forearm blood flow of 4.0 ± 0.3 ml/min per 100 mL tissue (Figure 1A). Repetitive hand-grip forearm exercise increased blood flow approximately 600% over resting values, and significantly decreased ipsilateral venous hemoglobin oxygen saturation, $p0_2$, and pH, consistent with increased oxygen consumption and CO_2 generation. Following a 20-minute rest period, repeat hemodynamic measurements showed an approximate 10% higher forearm blood flow, but no change in systemic blood pressure or forearm venous hemoglobin oxygen saturation, $p0_2$ and pH values compared with the initial baseline values (Figure 1B). The NO synthase inhibitor L-NMMA was then infused into the brachial artery at 8 μmol/min for 5 minutes, significantly reducing forearm blood flow by approximately 30% and significantly reducing venous hemoglobin oxygen saturation, $p0_2$ and pH values. Repeated forearm exercise during continued L-NMMA infusion increased blood flow, but to a significantly lower peak value compared with exercise alone ($P<0.001$). In addition, hemoglobin oxygen saturation, $p0_2$ and pH were significantly lower during exercise with L-NMMA than with exercise without regional NO synthase inhibition ($P<0.001$, $P<0.005$ and $P=0.027$, respectively). Mean arterial blood pressure was unchanged during all components of Part I of the protocol.

Figure 1 depicts hemodynamic and metabolic measurements at baseline and during exercise, without (Figure 1A) and with (Figure 1B) inhibition of NO synthesis in 18 subjects. Mean arterial pressure (MAP), forearm blood flow (FBF), and venous oxyhemoglobin saturation, partial pressure of oxygen (pO_2), and pH are shown for all experimental conditions. These interventions and measurements (part I of the protocol) served as a control for Part II of the protocol, in which these interventions were performed during nitrite infusion.

To determine whether nitrite has vasoactivity in humans, in Part II of the protocol sodium nitrite in bicarbonate-buffered normal saline (final concentration 36 μmol/ml) was infused into the brachial arteries of these 18 subjects to achieve an estimated intravascular concentration of approximately 200 μM (Lauer *et al., Proc Natl Acad Sci U S A, 98*, 12814-9, 2001). Following repeat baseline measurements and infusion of sodium nitrite at 1 mL/min for 5 minutes, nitrite levels in the ipsilateral antecubital vein increased from 3.32 ± 0.32 to 221.82 ± 57.59 μM (Figure 2A). Forearm blood flow increased 175% over resting values; venous hemoglobin oxygen saturation, $p0_2$ and pH levels significantly increased over pre-infusion values, consistent with increased perfusion of the forearm.

Systemic levels of nitrite were 16 μM as measured in the contralateral arm and were associated with a systemic effect of decreased mean blood pressure of approximately 7 mm Hg. Consistent with immediate NO generation from nitrite during an arterial-to-venous transit, iron-nitrosylated-hemoglobin in the ipsilateral antecubital vein increased from 55.7 ± 11.4 to 693.4 ± 216.9 nM during the nitrite infusion. During forearm exercise with continuation of the nitrite infusion, blood flow increased further, with evidence of metabolic stress by virtue of reduction in forearm venous hemoglobin oxygen saturation, $p0_2$ and pH levels from baseline values. Venous nitrite levels declined, consistent with increased blood flow to the forearm diluting the concentration of infused nitrite. Despite decreasing forearm nitrite concentrations during exercise, iron-nitrosyl-hemoglobin levels increased (Figure 2A).

Following cessation of nitrite infusion and substitution of saline as the intra-arterial infusate for 30 minutes, repeat baseline measurements showed persistent elevations in systemic levels of nitrite, iron-nitrosyl-hemoglobin and methemoglobin (Figure 2B) over values obtained prior to the infusion of nitrite almost one hour before. In addition, persistence of a vasodilator effect was also apparent, as forearm blood flow was significantly higher (4.79 ± 0.37 versus 3.94 ± 0.38 mL/min per 100 mL tissue, P=0.003) and systemic blood pressure significantly lower (82.1 ± 3.7 versus 89.2 ± 3.5 mm Hg, P=0.002) than initial pre-nitrite infusion values. During re-infusion into the brachial artery of sodium nitrite 36 μmol/ml, combined with L-NMMA 8 μmol/min in order to again inhibit regional synthesis of NO, similar vasodilator effects of nitrite on resting and exercise forearm blood flow were seen as during nitrite infusion without L-NMMA (Figure 2B). This stands in contrast to the vasoconstrictor effect of NO synthase inhibition with L-NMMA observed in Part I of the protocol (Figure 1B). Venous nitrite and iron-nitrosyl-hemoglobin levels followed similar patterns during NO inhibition as during the initial nitrite infusion.

Figure 2 depicts the effects of infusion of sodium nitrite ($NaNO_2$) in bicarbonate-buffered normal saline (0.9%; final concentration 36 μmol/ml) into the brachial arteries of 18 healthy subjects at 1 ml/min for 5 minutes at baseline and continued during exercise. Figure 2A depicts the effects without inhibition of NO synthesis. Figure 2B depicts the effects with inhibition of NO synthesis. Values for mean arterial blood pressure (MAP), forearm blood flow (FBF), venous oxyhemoglobin saturation, partial pressure of oxygen (pO_2) and pH, venous nitrite, venous iron-nitrosyl-hemoglobin and venous methemoglobin are shown for all experimental interventions.

As a test of the physiological relevance of vascular nitrite as a vasodilator, nitrite concentrations were decreased by 2-logs to 400 nmol/mL. An infusion of 1 mL/min for five minutes in 10 subjects significantly increased forearm blood flow in all ten subjects from 3.49 ± 0.24 to 4.51 ± 0.33 ml/min per 100 mL tissue (Figure 3A; P=0.0006). Blood flow significantly increased at rest and during NO synthase inhibition with and without exercise (Figure 3B; $P<0.05$ during all conditions). Mean venous nitrite levels increased from 176 ± 17 nM to 2564 ± 462 nM following a five-minute infusion and exercise venous nitrite levels decreased to 909 ± 113 nM (secondary to dilutional effects of increased flow during exercise; Figure 3C). Again, the vasodilator effects of nitrite were paralleled with an observed formation of both iron-nitrosyl-hemoglobin and S-nitroso-hemoglobin across the forearm circulation (Figure 3D; described below). These data indicate that basal levels of nitrite, from 150-1000 nM in plasma to 10,000 nM in vascular tissue, contribute to resting vascular tone and hypoxic vasodilation.

Figure 3 depicts the effects of infusion of low-dose sodium nitrite in bicarbonate-buffered normal saline into the brachial arteries of 10 healthy subjects at baseline and during exercise, without and with inhibition of NO synthesis. Figure 3A depicts forearm blood flow at baseline and following a five-minute in fusion of $NaNO_2$ (0.36 μmol/ml in 0.9% saline, infused at 1 ml/min). Figure 3B depicts forearm blood flow with and without low-dose nitrite infusion at baseline and during L-NMMA infusion with and without exercise stress. Figure 3C depicts venous levels of nitrite from the forearm circulation at the time of blood flow measurements. Figure 3D depicts venous levels of S-nitroso-hemoglobin (S-NO) and iron-nitrosyl-hemoglobin (Hb-NO) at baseline and following nitrite infusion during exercise stress.

The vasodilatory property of nitrite during basal blood flow conditions, when tissue pO_2 and pH are not exceedingly low, was unexpected. These results indicate that the previously hypothesized mechanisms for nitrite reduction, nitrite disproportionation and xanthine oxidoreductase activity, both of which require extremely low pO_2 and pH values not typically encountered in normal physiology, are complemented *in vivo* by additional factors that serve to catalyze nitrite reduction. While ascorbic acid and other reductants, present in abundance in blood, can provide necessary electrons for nitrous acid reduction, such that the reaction might occur at physiologically attainable pH levels, it is herein reported that deoxyhemoglobin effectively reduces nitrite to NO, within one half-circulatory time. This mechanism provides a graded production of NO along the physiological oxygen gradient, tightly regulated by hemoglobin oxygen desaturation.

Intravascular formation of NO and S-nitrosothiol by reaction of nitrite with intraerythrocytic deoxyhemoglobin

Before and during nitrite infusions, blood was drawn from both the brachial artery and antecubital vein and the whole blood immediately (at the bedside to eliminate processing time) lysed 1:10 in an NO-hemoglobin "stabilization solution" and the iron-nitrosyl-hemoglobin and S-nitroso-hemoglobin content determined by tri-iodide-based reductive chemiluminescence and electron paramagnetic resonance spectroscopy as described in Methods. The baseline levels of S-nitroso-hemoglobin and iron-nitrosyl-hemoglobin were at the limits of detection (<50 nM or 0.0005% NO per heme) with no artery-to-vein gradients. Following nitrite infusion in Part II of the protocol venous levels of both iron-nitrosyl-hemoglobin and S-nitroso-hemoglobin rose strikingly (Figure 4A). The formation of both NO-hemoglobin adducts occurred across the vascular bed, a half-circulatory time of less than 10 seconds. The rate of NO formation, measured as iron-nitrosyl and S-nitroso-hemoglobin and quantified by subtraction of the arterial from the venous levels with the difference being multiplied by blood flow, increased greatly during exercise, despite a significant decrease in the venous concentration of nitrite secondary to increasing blood flow diluting the regional nitrite concentration (Figure 4A; P=0.006 for iron-nitrosyl-hemoglobin and P=0.02 for S-nitroso-hemoglobin by repeated measures ANOVA).

Figure 4A depicts formation of iron-nitrosyl-hemoglobin (black squares) and S-nitroso-hemoglobin (red circles) during nitrite infusion at baseline, during nitrite infusion and during nitrite infusion with exercise, quantified by subtraction of the arterial from the venous levels and multiplying the result by blood flow. The formation of both NO-hemoglobin adducts was inversely correlated with hemoglobin-oxygen saturation in the human circulation during nitrite infusion (for iron-nitrosyl-hemoglobin $r=-0.7$, $p<0.0001$, for S-nitroso-hemoglobin $r=-0.45$, $p=0.04$) (Figure 4B). Hemoglobin oxygen saturation was measured from the antecubital vein by co-oximetry. Asterix in all figures signify $P<0.05$ by paired t test or repeated measures analysis of variance.

To determine whether free NO radical can form from the reaction of nitrite and deoxyhemoglobin, 100 and 200 μM nitrite was reacted with deoxygenated erythrocytes (5 mL volume containing a total of 660 and 1000 μM in heme) in a light protected reaction vessel purged with helium in-line with a chemiluminescent NO analyzer (Seivers, Boulder, CO.). As shown in Figure 5A and 5B, the injection of nitrite into a solution of deoxygenated erythrocytes resulted in the liberation of NO into the gas phase. There was no release from nitrite in buffer control under the same conditions, and significantly less NO was released upon nitrite addition to oxygenated erythrocytes (21% and 100% oxygen). The observed rate (determined by the assessment of the area under the curve of increased steady-state NO generation following nitrite injection calculated over 120 seconds) of NO production in the 5 mL reaction volume was consistent with 47 pM NO production per second (corresponding to an estimated 300 to 500 pM NO production per second in whole blood). While NO formation rates in this experimental system may not be extrapolated to rates of NO formation *in vivo*, the experiments are consistent with two important concepts: 1) A fraction of free NO can escape auto-capture by the remaining heme groups; this is likely only possible because nitrite is only converted to NO by reaction with deoxyhemoglobin and its "leaving group" is the met(ferric)heme protein which will limit scavenging and inactivation of NO (Doyle *et al.*, *J Biol Chem, 256*, 12393-12398, 1981); and 2) The rate of NO production is increased under anaerobic conditions, consistent with a nitrite-deoxyhemoglobin reaction.

Example 2

Cytoprotective Effects of Nitrite during Ischemia-reperfusion of the Heart and Liver

As demonstrated in Example 1, nitrite is reduced to NO by reaction with deoxyhemoglobin along the physiological oxygen gradient, a chemistry whose rate is oxygen and pH dependent and that potentially contributes to hypoxic vasodilation. Based on that unexpected discovery, we proposed that hypoxia-dependent NO production from nitrite in ischemic tissue might limit ischemia-reperfusion injury. This example provides a demonstration that infusions of sodium nitrite are effective to provide cytoprotection during ischemia-reperfusion of the heart and liver.

Although reperfusion of ischemic tissues provides oxygen and metabolic substrates necessary for the recovery and survival of reversibly injured cells, reperfusion itself actually results in the acceleration of cellular necrosis (Braunwald *et al.*, *J. Clin. Invest.* 76:1713-1719, 1985). Ischemia-reperfusion is characterized by the formation of oxygen radicals upon reintroduction of molecular oxygen to ischemic tissues resulting in widespread lipid and protein oxidative modifications of cellular proteins, mitochondrial injury, and tissue apoptosis and necrosis (McCord *et al.*, *Adv Myocardiol* 5:183-189, 1985). In addition, following reperfusion of ischemic tissues blood flow may not return uniformly to all portions of the ischemic tissues, a phenomenon that has been termed the "no-reflow" phenomenon (Kloner *et al.*, *J Clin Invest* 54:1496-1508, 1974). Reductions in blood flow following reperfusion are thought to contribute to cellular injury and necrosis (Kloner *et al.*, *J Clin Invest* 54:1496-1508, 1974). The sudden re-introduction of blood into ischemic tissue also results in a dramatic increase in calcium delivery to the previously ischemic tissue (*i.e.*, "calcium paradox") resulting in massive tissue disruption, enzyme release, reductions in high energy phosphate stores, mitochondrial injury, and necrosis (Nayler, *Amer. J. Path.* 102:262, 1981; Shen *et al.*, *Amer. J. Path* 67:417-440, 1972). Recent studies have also indicated that the ischemia-reperfusion injury is also characterized by an inappropriate inflammatory response in the microcirculation resulting in leukocyte-endothelial cell interactions that are mediated by the upregulation of both leukocyte and endothelial cell adhesion molecules (Lefer *et al.*, *Cardiovasc Res* 32:743-751, 1996; Entman *et al.*, *Faseb J* 5:2529-2537, 1991). Intensive research efforts have been focused on ameliorating various pathophysiological components of ischemia-reperfusion injury to limit the extent of tissue injury and necrosis.

NO, NO donors, and NO synthase activation or transgenic over-expression have been shown to exert protective effects on this process in a number of models (Lefer *et al.*, *New Horiz* 3:105-112, 1995; Lefer *et al.*, *Circulation* 88:2337-2350, 1993; Nakanishi *et al.*, *Am J Physiol* 263:H1650-1658, 1992; Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004; Jones *et al.*, *Proc Natl Acad Sci U S A* 100:4891-4896. 2003; Kanno *et al.*, *Circulation* 101:2742-2748, 2000), but in other models appears harmful (Flogel *et al.*, *J Mol Cell Cardiol* 31:827-836. 1999; Menezes *et al.*, *Am J Physiol* 277:G144-151, 1999; Woolfson *et al.*, *Circulation* 91:1545-1551, 1995; Schulz, R. *et al.*, *Cardiovasc Res* 30:432-439, 1995). Evaluation of these studies suggests a critical effect of dose and duration of NO exposure, resulting in a narrow therapeutic safety window for NO in ischemia-reperfusion pathophysiology (Bolli, *J. Mol. Cell. Cardio.* 33:1897-1918, 2001; Wink *et al.*, *Am J Physiol Heart Circ Physiol* 285:H2264-2276, 2003). An additional limitation is that NO formation from NO synthase requires oxygen as substrate, a molecule whose availability becomes limited during ischemia.

We therefore considered the use of nitrite in this context for the following reasons:

(1) It is a naturally occurring substance with no potentially toxic "leaving group",

(2) it is selectively reduced to NO in tissues with low oxygen tension and low pH (Bryan *et al.*, *Proc Natl Acad Sci USA.*, 2004; Cosby *et al.*, *Nat Med* 9:1498-1505, 2003; Nagababu *et al.*, *J Biol Chem* 278:46349-46356, 2003; Tiravanti *et al.*, *J Biol Chem*

279:11065-11073, 2004; Doyle *et al., J Biol Chem* 256:12393-12398, 1981; Luchsinger *et al., Proc Natl Acad Sci USA* 100:461-466, 2003),

(3) its activation does not require molecular oxygen (Cosby *et al., Nat Med* 9:1498-1505, 2003), and

(4) NO is known to maintain heme proteins in a reduced and liganded state (Herold *et al., Free Radic Biol Med* 34:531-545, 2003; Herold *et al., J Biol Inorg Chem* 6:543-555, 2001; Fernandez *et al., Inorg Chem* 42:2-4, 2003), limit free iron and heme mediated oxidative chemistry (Kanner *et al., Arch Biochem Biophys* 237:314-321, 1985; Kanner *et al., Lipids* 20:625-628, 1985; Kanner *et al., Lipids* 27:46-49, 1992), transiently inhibit cytochome c oxidase and mitochondrial respiration (Torres *et al., FEBS Lett* 475:263-266, 2000; Brown *et al., FEBS Lett* 356:295-298, 1994; Cleeter *et al., FEBS Lett* 345:50-54, 1994; Rakhit *et al., Circulation* 103:2617-2623, 2001), and modulate apoptotic effectors (Mannick *et al., Science* 284:651-654, 1999), all mechanisms that might participate in cytotoxicity following severe ischemia.

We evaluated the effects of nitrite therapy, compared with vehicle and nitrate controls, in well characterized murine models of hepatic and myocardial ischemia-reperfusion injury. The following description provides strong evidence for a profound protective effect of nitrite on cellular necrosis and apoptosis, which is believed to be mediated by a hypoxia-dependent bioconversion of nitrite to NO and nitros(yl)ated proteins.

Materials and Methods

Chemicals and Reagents: Sodium nitrite (S-2252) and sodium nitrate (S-8170) were obtained from the Sigma Chemical Co. (St. Louis, MO). Sodium nitrite and sodium nitrate were dissolved in phosphate buffered saline and the pH was adjusted to 7.4. In all experiments a final volume of 50 μL of sodium nitrite or sodium nitrate were administered to the mice to achieve final concentrations of circulating nitrite of 0.6 to 240 μM assuming a total circulating blood volume of 2mL. Carboxy-PTIO [2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt], a direct intravascular NO scavenger, was utilized to inhibit NO dependent effects following hepatic I/R injury. Carboxy-PTIO (Alexis Biochemicals) was dissolved in phosphate buffered saline and administered intravenously at a dose of 1 mg/Kg in a volume of 50 μL at 30 minutes prior to hepatic ischemia. Zinc(II) Deuteroporphyrin IX-2,4-bisethyleneglycol (ZnDBG) (Alexis Biochemicals) , a heme oxygenase-1 inhibitor was injected i.p. at a dose of 10 mg/Kg in a volume of 50 μL at 30 minutes prior to the induction of hepatic ischemia.

Animals: All of the mice utilized in the present studies were C57BL6/J at 8-10 weeks of age obtained from the Jackson Laboratories (Bar Harbor, ME). In additional experiments of hepatic I/R injury we utilized mice completely deficient (-/-) in endothelial nitric oxide synthase (eNOS). eNOS-/- mice were originally generously donated from Dr. Paul Huang (Mass. General Hospital) and generated in our breeding colony at LSU-Health Sciences Center. eNOS-/- mice were utilized at 8-10 weeks of age.

Hepatic Ischemia-Reperfusion (I/R) Protocol: The hepatic I/R protocol is depicted in Figure 6A and has been described previously (Hines *et al.*, *Biochem Biophys Res Commun* 284:972-976, 2001; Hines *et al.*, *Am J Physiol Gastrointest Liver Physiol* 284:G536-545, 2001). Mice were anesthetized with the combination of ketamine (100 mg/kg) and zylazine (8 mg/kg) and a midline laparotomy was performed to expose the liver. Mice were then injected with heparin (100 μg/kg, i.p.) to prevent blood clotting. The left lateral and median lobes of the liver were rendered ischemic by completely clamping the hepatic artery and the portal vein using microaneurysm clamps. This experimental model results in a segmental (70%) hepatic ischemia. This method of partial ischemia prevents mesenteric venous congestion by allowing portal decompression throughout the right and caudate lobes of the liver. The liver was then repositioned in the peritoneal cavity in its original location for 45 minutes. The liver was kept moist using gauze soaked in 0.9% normal saline. In addition, body temperature was maintained at 37°C using a heat lamp and monitoring body temperature with a rectal temperature probe. Sham surgeries were identical except that hepatic blood flow was not reduced with a microaneurysm clamp. The duration of hepatic ischemia was 45 minutes in all experiments, following which the microaneurysm clamps were removed. The duration of hepatic reperfusion was 5 hours in the studies of serum liver transaminase levels (*i.e.*, AST or ALT) and 24 hours for the studies of liver histopathology (such as hepatocellular infarction).

Liver Enzyme Determinations: Serum samples were analyzed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using a spectrophotometric method (Sigma Chemical Co., St. Louis, MO) (Harada *et al.*, *Proc Natl Acad Sci U S A* 100:739-744, 2003). These enzymes are liver specific and are released from the liver during injury (Hines *et al.*, *Biochem Biophys Res Commun* 284:972-976, 2001; Hines *et al.*, *Am J Physiol Gastrointest Liver Physiol* 284:G536-545, 2001).

Liver Histopathology Studies: Histopathology of liver tissue was performed as previously reported (Hines *et al.*, *Biochem Biophys Res Commun* 284:972-976, 2001). Liver tissue was fixed in 10% buffered formalin for 24 hours, embedded in paraffin, and 10 μM sections stained with hematoxylin and eosin. Histopathology scoring was performed in a double blinded manner on random high power fields using the following criteria:

0- no hepatocellular damage,

1- mild injury characterized by cytoplasmic vacuolization and focal nuclear pyknosis,

2- moderate injury with dilated sinusoids, cytosolic vacuolization, and blurring of intercellular borders,

3- moderate to severe injury with coagulative necrosis, abundant sinusoidal dilation, RBC extravasation into hepatic chords, and hypereosinophilia and margination of neutrophils,

4- severe necrosis with loss of hepatic architecture, disintegration of hepatic chords, hemorrhage, and neutrophil infiltration.

Hepatocellular apoptosis was determined using the TUNEL staining kit from Roche according to the manufacturer's recommendations. Briefly, liver tissue from various treatments was fixed in buffered formalin and 10 μm sections were prepared. Sections were permeabilized on ice for 2 minutes and incubated in 50 μL TUNEL solution for 30 minutes at 37°C. Sections were then treated with 50 μL substrate solution for 10 min. and mounted under glass coverslips. The number of apoptotic nuclei was determined from 5 random 40x fields per specimen. A total of six specimens per treatment group (16 slides per group) were analyzed and compared using one-way analysis of variance with Bonferroni's post-testing.

Myocardial Ischemia-Reperfusion (I/R) Protocol: Surgical ligation of the left main coronary artery (LCA) was performed similar to methods described previously (Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004). Briefly, mice were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and pentobarbital sodium (50 mg/kg). The animals were then attached to a surgical board with their ventral side up. The mice were orally intubated with PE-90 polyethylene tubing connected to PE-240 tubing and then connected to a Model 683 rodent ventilator (Harvard Apparatus, Natick, MA). The tidal volume was set at 2.2 milliliters and the respiratory rate was set at 122 breaths per minute. The mice were supplemented with 100% oxygen via the ventilator side port. A median sternotomy was performed using an electric cautery and the proximal left main coronary artery was visualized and completely ligated with 7-0 silk suture mounted on a tapered needle (BV-1 ethicon). In the initial experiments of myocardial infarct size coronary occlusion was maintained for 30-minutes followed by removal of suture and reperfusion for 24 hours. In additional experiments of cardiac function, the proximal LCA was completely occluded for 45 minutes followed by suture removal and reperfusion for 48 hours. In these experiments, two-dimensional echocardiography was performed at baseline and again at 48 hours of reperfusion.

Myocardial Infarct Size Determination: At 24 hours of reperfusion, the mice were anesthetized as described previously, intubated, and connected to a rodent ventilator. A catheter (PE-10 tubing) was placed in the common carotid artery to allow for Evans Blue dye injection. A median sternotomy was performed and the left main coronary artery was re-ligated in the same location as before Evans Blue dye (1.2 mL of a 2.0% solution, Sigma Chemical Co.) was injected into the carotid artery catheter into the heart to delineate the ischemic zone from the nonischemic zone. The heart was rapidly excised and serially sectioned along the long axis in five, 1 mm thick sections that were then incubated in 1.0% 2,3,5-triphenyltetrazolium chloride (Sigma Chemical Co.) for 5 minutes at 37°C to demarcate the viable and nonviable myocardium within the risk zone. Each of the five, 1 mm thick myocardial slices were weighed and the areas of infarction, risk, and nonischemic left ventricle were assessed by a blinded observer using computer-assisted planimetry (NIH Image 1.57). All of the procedures for the left ventricular area-at-risk and infarct size determination have been previously described (Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004).

Echocardiographic Assessment of Left Ventricular Function: Transthoracic echocardiography of the left ventricle using a 15 MHz linear array transducer (15L8) interfaced with a Sequoia C256 (Acuson) was performed in additional groups of mice (n=9 vehicle and n=10 nitrite) subjected to 45 minutes of myocardial ischemia and 48 hours of reperfusion. Two-dimensional echocardiography was performed at baseline and at 48 hours of reperfusion as described previously (Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004; Jones *et al.*, *Proc Natl Acad Sci U S A* 100:4891-4896. 2003). Ventricular parameters were measured using leading-edge technique. M-mode (sweep speed = 200 mm/sec) echocardiograms were captured from parasternal, short and long-axis 2D views of the left ventricle (LV) at the mid-papillary level. LV percent fractional shortening (FS) was calculated according to the following equation: LV%FS = ((LVEDD-LVESD)/LVEDD) x 100. All data were calculated from 10 cardiaccycles per experiment.

HO-1 Western Blot Analysis of homogenized liver tissue samples (50 μg total protein) was performed using mouse anti-HO-1 mAb (Stressgen, Victoria, BC) at a 1:3,000 dilution and goat anti-mouse secondary Ab (Amersham Biosciences, Piscataway, NJ) at a 1:3,000 dilution.

Blood and Tissue Nitrite Determination: For blood nitrite measurements, 160 μL of whole blood was mixed with 40 μL of a nitrite stabilizing solution containing 80 mM ferricyanide, 20 mM N-ethylmaleimide (NEM), 200 μL diethylenetriaminepentaacetic acid (DTPA), and 0.2% NP-40 (concentrations provided are after mixing with whole blood). The nitrite in whole blood was then measured using tri-idodide-based reductive chemiluminescence as previously described and validated (Gladwin *et al.*, *J Biol Chem* 21:21, 2002; Yang *et al.*, *Free Radic Res* 37:1-10, 2003).

Liver tissue was homogenized using an amended protocol published by Bryan and colleagues (Bryan *et al.*, *Proc Natl Acad Sci U S A.*, 2004). Harvested liver tissue was blotted dry on filter paper, weighed, and homogenized immediately in ice-cold NEM (10 mmol/L)/ DTPA (2 mmol/L) containing buffer (3:1 dilution - w/v). The buffer/tissue mix was then homogenized with a Wheaton glass-glass homogenizer. Tissue homogenates were kept on ice and analyzed within 5 minutes. The homogenate was subsequently either injected directly into triiodine to measure the sum of nitrite, mercury stable (Rx-NO) and mercury-labile (RS-NO) NO-adducts. To determine the levels of specific NO-adducts (Rx-NO and RS-NO), the sample was reacted with and without 5 mM mercuric chloride (RS-NO becomes nitrite in presence of mercuric chloride and Rx-NO is stable) and both treated with acid sulfanilamide (0.5%) to eliminate nitrite.

Statistical Analyses: Data were analyzed by two-way analysis of variance (ANOVA) with post hoc Bonferroni analysis using StatView software version 5.0 (SAS Institute, Carey, North Carolina). Data are reported as means ± standard error of the mean (SEM) with differences accepted as significant when $p < 0.05$.

Results

Intraperitoneal nitrite limits hepatic ischemia-reperfusion (I/R) injury: Intraperitoneal delivery of 1.2 - 480 nmoles of sodium nitrite (0.6 μM to 240 μM estimated final concentration in a 2 mL total blood volume of the mouse) during hepatic ischemia dose-dependently limited serum elevations of liver transaminases, aspartate amino transferase (AST) and alanine amino transferase (ALT) (Figures 6B and 6C), with a peak effect occurring at a calculated systemic concentration of 24 μM (48 nmoles added nitrite). In sharp contrast, treatment with saline or sodium nitrate (48 nmoles) did not exert any protective effects in the setting of hepatic I/R injury. Additional studies were performed to evaluate the effects of nitrite treatment on hepatocellular injury in mice following *in vivo* hepatic ischemia (45 minutes) and more prolonged reperfusion (24 hours; Figure 6D, 6E, and 6F). The administration of nitrite at a final blood concentration of 24 μM (48 nmoles) significantly reduced hepatocellular injury at 24 hours of reperfusion compared with saline and nitrate treated animals. In addition, nitrite therapy also significantly ($p < 0.001$) attenuated the extent of hepatocellular apoptosis following 45 minutes of hepatic ischemia and 24 hours of reperfusion (Figure 6F). The extent of hepatic cell apoptosis in nitrite treated animals subjected to I/R was similar to that observed in sham operated control animals (p = NS).

Intraventricular Nitrite Limits Myocardial Ischemia-Reperfusion Injury: To determine whether the potent cytoprotective effects of nitrite on liver ischemia-reperfusion injury were generalizable to other organ systems, studies were next performed to evaluate the potential cardioprotective effects of acute nitrite therapy in the setting of coronary artery occlusion and reperfusion. The experimental protocol for the myocardial I/R studies is depicted in Figure 7A. Administration of nitrite (48 nmoles) into the left ventricular cavity at 5 minutes prior to reperfusion significantly ($p < 0.001$) limited myocardial infarct size (Figures 7B and 7C) compared to 48 nmoles nitrate treatment. Despite similar myocardial areas-at-risk (p = NS between groups), myocardial infarct size per area-at-risk and per left ventricle were both reduced by 67% with nitrite therapy compared to nitrate.

In additional studies, mice were subjected to 45 minutes of myocardial ischemia and 48 hours of reperfusion to evaluate the effects of nitrite treatment on left ventricular performance (Figures 7D and 7E). In these studies, both myocardial ejection fraction (Figure 7D) and myocardial fractional shortening (Figure 7E) were measured using two-dimensional echocardiography at baseline and following myocardial infarction and reperfusion. Myocardial ejection fraction was similar between the vehicle and nitrite treated study groups at baseline. Following myocardial infarction and reperfusion, ejection fraction was significantly ($p < 0.001$ vs. baseline value) lower in the saline vehicle group, yet remained essentially unchanged in the nitrite treated animals (p = NS vs. baseline). Additionally, ejection fraction was significantly ($p < 0.02$) greater in the nitrite group compared to the vehicle group. Similar observations were made for fractional shortening with no significant group differences at baseline. However, following myocardial infarction and reperfusion, left ventricular fractional shortening was significantly ($p < 0.001$ vs. baseline) depressed in the vehicle group, but not in the nitrite group (p = NS vs. baseline) and was significantly ($p <0.02$) greater in the nitrite group compared to the vehicle group.

Nitrite-Mediated Cytoprotection is Associated with an Acute Ischemic Reduction of Nitrite to NO and S- and N-nitrosated Proteins within the Liver: Consistent with previously described reduction of nitrite to NO and S-nitrosothiols in a reaction with deoxyhemoglobin and deoxygenated heme proteins (Bryan *et al.*, *Proc Natl Acad Sci U S A.*, 2004; Cosby *et al.*, *Nat Med* 9:1498-1505, 2003; Nagababu *et al.*, *J Biol Chem* 278:46349-46356, 2003; Doyle *et al.*, *J Biol Chem* 256:12393-12398, 1981), one minute after reperfusion the levels of nitrite in the livers of saline (control) treated mice subjected to ischemia decreased from 1.75 μM to undetectable ($p < 0.001$ vs. sham group) and levels of mercury stable NO modified proteins (likely N-nitrosamines and iron-nitrosyl proteins; RxNO) increased to approximately 750 nM (Figure 8A; $p < 0.001$). Interestingly, with nitrite treatment there was a significant ($p < 0.01$ vs. saline treated controls) increase in post-reperfusion liver levels of nitrite (Figure 8B), S-nitrosothiols (Figure 8C) and N-nitrosamines (Figure 8D) in the nitrite treated mice. These data are consistent with the thesis that nitrite is bioactivated during hypoxic stress and consistent with recent studies of Bryan and colleagues demonstrating an acute conversion of tissue nitrite to RSNO and RxNO after a systemic anoxic insult (*Proc Natl Acad Sci U S A.*, 2004). The low levels of nitrite that are cytoprotective (1.2 nmoles at lowest dose – Figure 6B and 6C) and the reductive decomposition of "native" liver nitrite in the saline treated control animals (Figure 8A) suggest that this may be a natural mechanism for hypoxic NO production and cytoprotection. Consistent with the near-physiological amounts of nitrite given, blood nitrite levels were not significantly elevated (594 ± 83 nM to 727 ± 40 nM; n=3; p=0.16) in mice treated with 48 nmoles of nitrite, the most effective dose.

Cytoprotective effects of Nitrite are NO dependent, NO synthase Independent and Heme Oxygenase Independent: Further supporting a mechanism involving the hypoxic reduction of nitrite to NO, the NO inhibitor PTIO completely inhibited protective effects of nitrite in full factorial design experiments (Figure 9A). In contrast, significant nitrite cytoprotection was observed in endothelial NO synthase (eNOS) deficient mice (Figure 9B; $p < 0.001$), suggesting that NO production from nitrite during ischemia-reperfusion is eNOS independent. While heme oxygenase 1 protein expression is significantly induced following ischemia-reperfusion in this model, and appears to confer protection (Figure 9C and 9D), in mice pre-treated with ZnDPBG (a specific and potent heme oxygenase 1 inhibitor) nitrite significantly limited tissue injury suggesting a heme oxygenase-independent effect (Figure 9C; $p < 0.05$).

Discussion

In this example, nitrite treatment significantly increased the levels of liver nitrite and nitros(yl)ated species (RSNO and RXNO), compared with saline and nitrate treated controls, and conferred a dramatic dose-dependent cytoprotective effect, limiting necrosis, apoptosis, and preserving organ function. Remarkably, the levels of nitrite added were near-physiological, with a protective effect observed at even 1.2 nmoles added nitrite (a calculated blood level of 600 nM), suggesting that this may represent an endogenous protective mechanism that buffers severe metabolic or pathophysiological stress.

Recent data suggest that nitrite concentrations vary between blood and different organs and are typically in the high nanomolar to low micromolar range. However, until recently the high concentrations required to vasodilate aortic ring preparations led to its dismissal as an important biologically active molecule. Indeed, Furchgott *et al.* (*J. Pharmaco. Exper. Thera.* 108:129-143, 1953) demonstrated in 1953 that 100 μM nitrite stimulated vasodilation of aortic ring preparations, a process later shown to be mediated by activation of soluble guanylate cyclase (Kimura *et al., J Biol*

Chem 250:8016-8022, 1975; Mittal *et al.*, *J Biol Chem* 253:1266-1271, 1978; Ignarro *et al.*, *Biochim Biophys Acta* 631:221-231, 1980; Ignarro *et al.*, *J Pharmacol Exp Ther* 218:739-749, 1981). From a physiological standpoint, the *in vivo* conversion of nitrite to NO was thought to be limited to the stomach and severely ischemic heart, where acidic reduction or disproportionation at very low pH produces gastric mucosal vasodilation (Gladwin *et al.*, *J Clin Invest* 113:19-21, 2004; Bjorne *et al.*, *J Clin Invest* 113:106-114, 2004) and apparent cardiac tissue injury and heme iron-nitrosylation (at high nitrite concentrations in ischemic *ex vivo* heart preparations; Tiravanti *et al.*, *J Biol Chem* 279:11065-11073, 2004), respectively. While xanthine oxidoreductase dependent nitrite reduction can occur at very low oxygen tensions, NO production from this system is only detectable in the presence of high concentrations of superoxide dismutase (Li *et al.*, *J Biol Chem* 279:16939-16946, 2004; Li *et al.*, *Biochemistry* 42:1150-1159, 2001).

As described in Figure 6 and Cosby *et al.* (*Nat Med* 9:1498-1505, 2003), infusions of sodium nitrite into the human circulation produced significant vasodilation at both pharmacological and near-physiological concentrations. The bioactivation of nitrite appeared to be mediated by a nitrite reductase activity of deoxygenated hemoglobin, ultimately forming NO and iron-nitrosylated hemoglobin, and to a lesser extent S-nitrosated protein species. Based on these data, a role for circulating nitrite in mediating hypoxic vasodilation was proposed, with the oxygen sensor in this case being hemoglobin (Cosby *et al.*, *Nat Med* 9:1498-1505, 2003). It is now proposed that a similar nitrite reductase activity of deoxyhemoglobin, deoxymyoglobin and/or other deoxygenated heme proteins, accounts for the formation of nitros(yl)ated proteins and apparent NO-dependent cytoprotection observed during liver and cardiac ischemia in the present example.

Though the precise mechanism of how nitrite confers tissue protection is unclear, a critical role for NO is implicated from data shown in Figure 3 and 9A. Previous studies of NO and ischemia-reperfusion have yielded conflicting reports regarding the effects of NO on the severity of I/R injury, with some studies suggesting that NO actually contributed to reperfusion injury (Woolfson *et al.*, *Circulation* 91:1545-1551, 1995; Wink *et al.*, *Am J Physiol Heart Circ Physiol* 285:H2264-2276, 2003). Our laboratory has previously demonstrated that NO donors as well as the NO precursor, L-arginine, protect against myocardial I/R injury (Lefer *et al.*, *New Horiz* 3:105-112, 1995; Nakanishi *et al.*, *Am J Physiol* 263:H1650-1658, 1992; Pabla *et al.*, *Am J Physiol* 269:H1113-1121, 1995). More recently, we demonstrated that the severity of myocardial I/R injury is markedly exacerbated in eNOS-/- mice (Jones *et al.*, *Am J Physiol* 276:H1567-1573, 1999) whereas mice with eNOS overexpression are protected against myocardial infarction and subsequent congestive heart failure (Jones *et al.*, *Am J Physiol Heart Circ Physiol* 286:H276-282, 2004; Jones *et al.*, *Proc Natl Acad Sci U S A* 100:4891-4896. 2003; Jones *et al.*, *Am J Physiol* 276:H1567-1573, 1999).

Conflicting data on the effects of NO on ischemia-reperfusion injury may be related to the dose of NO and the conditions during ischemia and reperfusion (Bolli, *J. Mol. Cell. Cardio.* 33:1897-1918, 2001). It is now well appreciated that very high, non-physiological levels of NO (*i.e.*, high micromolar and millimolar) actually promote cellular necrosis and apoptosis (Dimmeler *et al.*, *Nitric Oxide* 4:275-281, 1997), while the demonstrated cytoprotective effects of NO typically involve nanomolar or low micromolar concentrations of NO (Lefer *et al., New Horiz* 3:105-112, 1995; Lefer *et al., Circulation* 88:2337-2350, 1993; Bolli, *J. Mol. Cell. Cardio.* 33:1897-1918, 2001). Additionally, studies investigating NO and NO-releasing agents under *in vitro* conditions of I/R have consistently reported deleterious effects of NO (Bolli, *J. Mol. Cell. Cardio.* 33:1897-1918, 2001), in contrast to *in vivo* studies of I/R that reported beneficial effects of NO therapy (Lefer *et al., New Horiz* 3:105-112, 1995; Lefer *et al., Circulation* 88:2337-2350, 1993). How NO mediates protection is also not clear, with multiple mechanisms being reported, including sGC activation, inhibition of cytochrome C oxidase and inhibition of deleterious mitochondrial calcium uptake (Torres *et al., FEBS Lett* 475:263-266, 2000; Brown *et al., FEBS Lett* 356:295-298, 1994; Cleeter *et al., FEBS Lett* 345:50-54, 1994; Rakhit *et al., Circulation* 103:2617-2623, 2001). While these data suggest that the effects of nitrite occur secondary to NO formation, the ultimate mechanism of nitrite-dependent cytoprotection is currently unknown (Luchsinger *et al., Proc Natl Acad Sci US A* 100:461-466, 2003; Fernandez *et al., Inorg Chem* 42:2-4, 2003; Han *et al., Proc Natl Acad Sci U S A* 99:7763-7768, 2002; Crawford *et al., Blood* 101:4408-4415, 2003).

An intriguing possibility is the intermediate formation of S-nitrosothiols, known to form via reactions of nitrite with deoxyhemoglobin and possibly tissue heme proteins (Bryan *et al., Proc Natl Acad Sci U S A.*, 2004; Cosby *et al., Nat Med* 9:1498-1505, 2003; Nagababu *et al., J Biol Chem* 278:46349-46356, 2003). Consistent with hypoxia dependent formation of S-nitrosothiols in red blood cells and tissues from nitrite, hepatic levels of these species were significantly higher following reperfusion (one-to-thirty minutes) in livers exposed to ischemia and nitrite. Within the relative reductive environment intracellularly, S-nitrosothiols formed via nitrite readily will be reduced to NO and activate sGC. Alternatively, S-nitrosation and subsequent effects on activity of critical proteins important in I/R induced injury and apoptotic cell death may lead to protection (Mannick *et al., Science* 284:651-654, 1999).

In addition, the data reported here reveal a dynamic regulation of hepatic RxNO's, a pool of mercury stable NO-modified proteins that include N-nitrosamines and iron-nitrosyls (Bryan *et al., Proc Natl Acad Sci U S A.*, 2004; Gladwin *et al., J Biol Chem* 21:21, 2002; Rassaf *et al., Free Radic Biol Med* 33:1590-1596, 2002), during ischemia-reperfusion. In saline treated groups, RxNO levels increase at 1 minutes of reperfusion and then decrease after 30 minutes reperfusion, whereas sustained elevation in RxNO levels are observed in nitrite treated mice, suggesting that maintenance of RxNO's could be important in protecting tissues from I/R injury.

In conclusion, the data presented in this example demonstrate a remarkable function for the relatively simple inorganic anion nitrite as a potent inhibitor of liver and cardiac ischemia-reperfusion injury and infarction, as shown in a mouse model system. The effects of nitrite appear NO-dependent, with a rapid conversion of nitrite to NO and nitros(yl)ated proteins following reperfusion. Considering the known safety of nitrite as a naturally occurring anion and as an FDA approved therapeutic for cyanide poisoning, these data evince a novel, safe, and inexpensive therapy for ischemia-reperfusion injury. Such a therapy could be used to prevent or modulate organ dysfunction following, for instance, coronary and peripheral vasculature reperfusion, high risk abdominal surgery (such as aortic aneurism repair that leads to renal acute tubular necrosis), cardiopulmonary resuscitation, and perhaps most importantly, solid organ transplantation.

Example 3

Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator

This example provides a description of use of inhaled, nebulized nitrite (specifically, sodium nitrite) to treat neonatal pulmonary hypertension.

Based on the results presented above, it is now known that the blood anion nitrite contributes to hypoxic vasodilation via a heme-based, nitric oxide (NO) generating reaction with deoxyhemoglobin and potentially other heme proteins. This biochemical reaction can be harnessed for the treatment of neonatal pulmonary hypertension, an NO-deficient state characterized by pulmonary vasoconstriction, right-to-left shunt pathophysiology, ventilation/perfusion inhomogeneity and systemic hypoxemia. As shown in this example, inhaled sodium nitrite was delivered by aerosol to newborn lambs with hypoxic and normoxic pulmonary hypertension. Inhaled nitrite elicited a rapid and sustained reduction (~60%) in hypoxia induced pulmonary hypertension, a magnitude approaching that of the effects of 20 ppm NO gas inhalation and which was associated with the immediate appearance of increasing levels of NO in expiratory gas. Pulmonary vasodilation elicited by aerosolized nitrite was deoxyhemoglobin- and pH-dependent and was associated with increased blood levels of hemoglobin iron-nitrosylation. Significantly, from a therapeutic standpoint, short term delivery of nitrite, dissolved in saline, via nebulization produced selective and sustained pulmonary vasodilation with no appreciable increase in blood methemoglobin levels. These data support the paradigm that nitrite is a vasodilator acting via conversion to NO, a process coupled to hemoglobin deoxygenation and protonation, and further evince a novel, simple and inexpensive therapy for neonatal pulmonary hypertension.

The effect of nebulized sodium nitrite versus saline, or inhaled NO, on both hypoxia-induced and drug-induced pulmonary hypertension was compared in newborn lambs. As described in this example, inhaled nitrite forms expired NO gas and circulating iron-nitrosyl-hemoglobin, and selectively vasodilates the pulmonary circulation. This vasoactivity is associated with the level of hemoglobin desaturation and blood pH in the physiologic range, supporting the physiological and therapeutic paradigm of hemoglobin as a deoxygenation-linked nitrite reductase.

Methods

Animal protocols were approved by the Institutional Animal Research Committee of Loma Linda University and were in accordance with the National Institutes of Health guidelines for use of experimental animals.

Animal preparation: Following induction of anesthesia with intravenous thiopental sodium (20 mg/Kg), the newborn lambs were orotracheally intubated and anesthesia maintained with 1% halothane until catheters were placed surgically. Thereafter halothane was discontinued and anesthesia maintained with morphine (0.1 mg/kg/hr). After paralysis with vecuronium (0.1 mg/kg/hr) the lungs were mechanically ventilated with initial settings of pressures: 22/6 cm H_2O, frequency: 25 breaths per minute, FiO_2: 0.21, and inspiratory time: 0.6 seconds (Sechrist Model 100, Sechrist Industries, Anaheim CA, USA). Initially and throughout the normoxic experiments, ventilator settings of frequency, peak inspiratory pressure, and FiO_2 were adjusted to maintain $SaO_2 > 95\%$, PaO_2 at 90-150 Torr, and $PaCO_2$ at 35-45 Torr.

A catheter was placed in the right brachial artery to sample pre-ductal blood for gases and chemical analysis. A pediatric thermodilution catheter was passed through a femoral vein to the pulmonary artery to measure cardiac output, pulmonary artery and pulmonary capillary wedge pressure (5.0 Pediatric Swan-Ganz® thermodilution catheter, Baxter Healthcare Corporation, Irvine, CA, USA).

Catheters were placed in the femoral artery and vein for monitoring blood pressure, heart rate, and for administration of fluids and drugs. A thermocouple was placed in the femoral vein to monitor core-body temperature which was maintained at 39 C by using a warming blanket and heat lamp throughout the experiments.

After completion of the experiments, the lambs were euthanized with a proprietary euthanasia solution (Euthasol, Western Medical Supply, Arcadia, CA, USA). In selected experiments necropsy was performed to verify the position of catheters (which were correctly positioned in all cases) and to determine that the ductus arteriosus was closed (which was closed in all cases).

Hemodynamic measurements: Mean arterial pressure, mean pulmonary artery pressure, and central venous pressure were measured continuously, and pulmonary capillary wedge pressure was measured intermittently by using calibrated pressure transducers (COBE Laboratories, Lakewood, CO) zeroed at the midthoracic level. Cardiac output was measured at 15-minute intervals throughout the studies by thermodilution using a Com-2 thermodilution module (Baxter Medical, Irvine, CA, USA). Five-ml injections of ice-cold saline were used. Determinations were carried out in triplicate and results were averaged for each sampling time point. Pulmonary vascular resistance and systemic vascular resistance were calculated by using standard formulas.

Blood gas and methemoglobin analysis: Arterial and mixed venous pH, PCO_2, and PO_2 were measured in blood samples (0.3 ml) collected at intervals throughout the experiments. Blood gases were measured (ABL3, Radiometer, Copenhagen, Denmark) and oxyhemoglobin saturation and hemoglobin concentration were measured using a hemoximeter (OSM2 Hemoximeter, Radiometer, Copenhagen, Denmark). Arterial and mixed venous methemoglobin concentrations were analyzed by photometry with the OSM2 Hemoximeter using the same arterial sample as in the blood gas determinations.

Delivery of aerosolized nitrite, saline, or NO gas: Five milliliters of either aqueous sodium nitrite (1 mM solution) or saline were placed in a jet nebulizer (Hudson RCI Micro Mist Nebulizer (Hudson Respiratory Care; Temucula, CA), driven at a constant flow rate of 8 L/minute in all experiments. The sodium nitrite solution was nebulized at a rate of 270 μmol/minute. Aerosols were delivered to the inspiration loop of the ventilator. Using a jet nebulizer, it is generally thought that <10% of a nebulized drug deposits in the lung (Coates *et al.*, *Chest* 119, 1123-30, 2001). This is the result of the dead volume of the nebulizer and the loss of drug during the expiratory phase. Lung deposition depends on particle size distribution, which is under the influence of air flow, filling volume, drug solution, and ambient temperature (Flavin *et al.*, *Pediatr Pulmonol* 2, 35-9, 1986; Suarez & Hickey, *Respir Care* 45, 652-66, 2000; Clay *et al.*, *Thorax* 38, 755-9, 1983; Clay *et al.*, *Lancet* 2, 592-4, 1983). This is a simple, inexpensive, and widely available clinical nebulizer system, though other systems could be used.

NO gas was introduced into the inspiratory limb of the breathing circuit. The inspired concentration of NO was continuously measured by chemiluminescence (CLD 700 AL, Eco Physics Inc, Ann Arbor, MI) in the inspiratory limb of the ventilator loop.

Inhalation of nitrite or saline aerosols during hypoxic- induced pulmonary vasoconstriction. Seven lambs were studied in order to demonstrate that nebulized nitrite is a selective pulmonary vasodilator in hypoxic newborn lambs. After anesthesia and instrumentation, the lambs were allowed to recover for 30 to 90 minutes while relevant hemodynamic parameters were monitored. After baseline measurements were obtained, a 30-minute period of pulmonary hypertension was induced by decreasing the FiO_2 of the inspired gas to 0.12 for 30 minutes. Ten minutes after initiation of hypoxia, either saline or sodium nitrite aerosols were administered for the remainder of the hypoxic period. After a one-hour recovery period, a second 30-minute period of hypoxia was induced again with either saline or sodium nitrite aerosols administered during the last 20 minutes. Arterial blood samples for blood gases and analytical assays were drawn and cardiac output measurements were performed at regular intervals.

Inhalation of nitrite during U46619-induced pulmonary hypertension in normoxic conditions. Six additional lambs were studied in order to evaluate the effects of nitrite nebulization on normoxic pulmonary hypertension. Stable normoxic pulmonary hypertension was induced by an infusion of a stable endoperoxide analog of thromboxane (U46619 - 9, 11-dideoxy-11α-epoxymethano-prostaglandin $F_{2\alpha}$, Cayman Chemicals, Ann Arbor, MI). The drug was dissolved in saline and was administered at a rate of 2 μg/kg/min into the femoral venous catheter for 30 minutes. Nitrite was nebulized for inhalation during the last 20 minutes of the infusion (Figure 11).

Comparison of inhaled nitrite and NO gas during hypoxic-induced pulmonary vasoconstriction: efficacy and duration of effect. This protocol was designed to compare the efficacy of nitrite with the clinical standard, 20 ppm inhaled NO gas. This concentration of NO gas is at the upper end of the therapeutic dose given to infants with primary pulmonary hypertension (Kinsella & Abman, *Semin Perinatol* 24, 387-95, 2000; Kinsella *et al.*, *Lancet* 340, 819-20, 1992), and has also been shown to be effective in reversing hypoxic vasoconstriction in newborn lambs (Frostell *et al.*, *Circulation* 83, 2038-47, 1991). A second purpose was to determine the duration of effect of a short nitrite nebulization versus NO gas inhalation on hemodynamic and physiological measurements during prolonged hypoxic-induced pulmonary vasoconstriction. After baseline measurements were performed, the lambs were made hypoxic as described above for 35 minutes.

Ten minutes after initiation of hypoxia, a 20-minute period of NO gas inhalation was initiated (20 ppm), with continuation of hypoxia for 5 minutes after cessation of NO gas delivery. Lambs were then allowed to recover for one hour. Again, after baseline measurements were made, a second 90-minute period of hypoxia was initiated. Ten minutes after initiation of hypoxia, sodium nitrite aerosol was administered for 20 minutes, with continuation of hypoxia for 60 minutes after cessation of nitrite aerosolization (Figure 13).

Measurement of exhaled NO. Exhaled NO concentration was measured with a chemiluminescence NO analyzer (NOA 280, Sievers Instruments, Inc., Boulder, CO). The chemiluminescence analyzer was calibrated with NO-free air and NO gas (45 parts per million) according to the manufacturer's recommendations. NO was sampled though a Teflon sidearm attached to a sampling port at the proximal end of the endotracheal tube through which flow passed to the analyzer at 250 ml/min.

In selected early experiments, nitrite was nebulized through a ventilator circuit with no lamb connected while NO was measured with the chemiluminescence NO analyzer. In no experiments did nitrite nebulization through the disconnected circuit result in an increase in NO concentration in the ventilated air.

Measurement of plasma nitrite and iron-nitrosyl-hemoglobin. Blood was drawn from both the brachial artery and central venous catheter and rapidly processed. Plasma was separated after centrifugation, frozen immediately on dry ice, and then stored at –70 C until assayed for nitrite using the chemiluminescence methodologies (Sievers model 280 NO-analyzer) as previously described (Cosby *et al.*, *Nat Med* 9, 1498-505, 2003; Gladwin *et al.*, *J Biol Chem* 277, 27818-28, 2002; Yang *et al.*, *Free Radic Res* 37, 1-10, 2003). The frozen red blood cell pellet was thawed, reacted in 8 mM NEM, 100 µM DTPA, and 4 mM ferricyanide, incubated for 5 minutes, and passed through a Sephadex G25 column (Yang *et al.*, *Free Radic Res* 37, 1-10, 2003; Xu *et al.*, *Proc Natl Acad Sci U S A* 100, 11303-8, 2003). The hemoglobin fraction from the G25 column was quantified by the method of Drabkin (*J. Biol. Chem.* 112, 51-65, 1935)and reacted in 0.1 M HCl/0.5%sulfanilamide to eliminate residual nitrite. The samples were then injected into a solution of tri-iodide (I_3^-) in-line with a chemiluminescent nitric oxide analyzer (Sievers, Model 280 NO analyzer, Boulder, CO). NO gas is striped in the tri-iodide solution stoichiometrically from iron-nitrosyl-hemoglobin (Yang *et al.*, *Free Radic Res* 37, 1-10, 2003).

Electron paramagnetic resonance spectroscopy of whole blood. This was carried out at 110K using a Bruker 4131VT temperature controller on an EMX 10/12 EPR spectrometer system set at 9.4 GHz, 10 mW, 5 G modulation, 0.08 s time constant, and 84 s scan time over 600 G. Each curve represents a single 84-second scan. Concentrations of iron-nitrosyl-hemoglobin were calculated by comparing the peak-to-peak heights to a standard sample.

Data acquisition and analysis. Mean arterial pressure, pulmonary artery pressure, central venous pressure, heart rate, exhaled NO concentration, and core body temperature were measured continuously. Analog signals were digitized at 100 Hz and stored using an analogue-to-digital converter (PowerLab SP, ADInstruments, Colorado Springs, CO) and data acquisition software (Chart v 5.02 for Macintosh, ADInstruments, Colorado Springs, CO). Following the experiments, arterial blood pressure, central venous pressure, heart rate, and exhaled NO measurements were averaged into 60-second blocks.

Statistical analysis. Serial measurements of physiological variables were compared by two-way ANOVA with repeated measures with group and time as the factors. Significance of differences was evaluated with a Dunnett's post-test. Significant differences from the baseline period were evaluated using one-way-ANOVA with repeated measures with individual animals and time as the factors. Significance of differences was further evaluated with a Newman-Keul's post-test. The calculations were done using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was assumed with $P < 0.05$. Data are presented as mean ± SEM.

Results

Pulmonary vasodilatory properties of aerosolized nitrite during hypoxic-induced pulmonary vasoconstriction

In order to determine the effect of nebulized nitrite on hypoxic pulmonary hypertension, seven newborn lambs (2-10 days of age) were instrumented under general anesthesia and maintained on mechanical ventilators and morphine infusion. Following baseline stabilization, the lambs were subjected to a 30-minute period of hypoxia by lowering FiO_2 to 0.12. Nebulized nitrite or saline was administered for the last 20 minutes of the hypoxic period. Initiation of hypoxia (arterial HbO_2 ~55%) was associated with rapid increases in mean pulmonary artery pressure (from 21 ± 1 to 34 ± 2 mmHg, $P < 0.01$) (Figure 10A, 10B) and pulmonary vascular resistance (20% ($P < 0.01$)), and decreased systemic vascular resistance (~20% ($P < 0.01$)). Inhalation of nebulized nitrite but not saline (Figure 10A, 10B) resulted in a selective decrease in pulmonary artery pressure by ~60% ($P < 0.01$) (Figure 10A, 10C) and reduced pulmonary artery resistance by ~70% ($P < 0.05$) but had no measurable effect on mean arterial blood pressure (Figure 10A, 10C) or systemic vascular resistance when compared to control animals. The decrease in pulmonary artery pressure with nitrite nebulization was associated with a progressive increase in exhaled NO from 3 ± 1 to 15 ± 4 ppb (Figure 10A, 10C). Cardiac output, arterial oxyhemoglobin saturation, and methemoglobin levels did not change measurably after nitrite inhalation as compared to values during the preceding ten minutes of hypoxia (Figure 10A). Arterial Po_2 could not change appreciably in our system as this was experimentally clamped.

Pulmonary vasodilating properties of aerosolized nitrite during normoxic drug-induced pulmonary vasoconstriction

In order to contrast the effects of nebulized nitrite on pulmonary artery pressure in the presence of normal deoxyhemoglobin with those in the presence of reduced oxygenated hemoglobin, the effects of nebulized nitrite were studied in a separate group of six lambs subjected to pulmonary hypertension under normoxic conditions. Stable normoxic (SaO_2 ~98%) pulmonary hypertension was induced by infusion of the endoperoxide analog of thromboxane (U46619). Intravenous infusion of U46619 at a rate of 2 μg/kg/min for 30 minutes was associated with rapid increases in pulmonary artery pressure from 24 ± 1 to 51 ± 4 mmHg ($P < 0.001$) (Figure 11). Ten minutes after the infusion began, addition of inhalation of nebulized nitrite resulted in a selective decrease in pulmonary artery pressure by 23 ± 6% ($P < 0.05$ compared to infusion baseline), but had no effect on mean arterial blood pressure or systemic vascular resistance (Figure 11). The decrease in pulmonary artery pressure with nitrite nebulization was associated with a progressive increase in exhaled NO from 4.8 ± 1.2 to 10.1 ± 2.0 ppb ($P < 0.05$ compared to baseline, Figure 11). Figure 2 shows a comparison of the effects of nitrite inhalation after 20 minutes on hypoxic versus drug-induced normoxic pulmonary vasoconstriction. The changes in mean pulmonary artery pressure and exhaled NO were significantly larger with nitrite treatment during hypoxic conditions. Overall the effects of nitrite inhalation on normoxic (thromboxane-induced) pulmonary hypertension were less than those observed with hypoxic pulmonary hypertension (Figures 10, 11, 12A), consistent with a model of hypoxemic and possibly acidemic potentiation of nitrite's vasoactivity.

pH and oxygen dependence of the nitrite reductase activity of deoxyhemoglobin

We hypothesize that the biochemical conversion of nitrite to NO requires both deoxyhemoglobin and protonation. Thus, data from both the normoxic and hypoxic experiments were used to study the influence of hemoglobin saturation and pH on NO production from nitrite. Measurements of exhaled NO gas and NO-modified hemoglobin (iron-nitrosyl-hemoglobin) were used as both dosimeters of NO production and as a measure of the direct byproducts of the nitrite reductase reaction of nitrite and hemoglobin to produce NO. Figure 12 shows that iron-nitrosyl-hemoglobin, measured by tri-iodide based reductive chemiluminescence (Figure 12B) and electron paramagnetic resonance (Figure 12C), was markedly increased by nitrite inhalation during hypoxia but not with drug-induced normoxic pulmonary vasoconstriction. As shown in Figure 12D, change in mean pulmonary artery pressure during hypoxia after inhalation of nebulized sodium nitrite was related to blood pH, with increased vasodilation associated with decreasing pH ($r = 0.57$ $P = 0.055$).

Comparison with inhaled NO and duration of effect.

We next compared the efficacy of nitrite with the current therapeutic standard, inhaled NO gas. After initiation of hypoxia, lambs were subjected to (20 ppm) inhaled NO gas or nebulized nitrite for 20 minutes. The data in Figure 13 show the duration and magnitude of the effect of NO gas inhalation (Figure 13A) or nitrite nebulization (Figure 13B, 13C) on hemodynamic and metabolic measurements during hypoxia. Although both treatments resulted in a pronounced reduction in hypoxic pulmonary hypertension, the response to inhaled NO gas was slightly more rapid and pulmonary pressure more nearly approached baseline when contrasted to the 60-70% correction in pressure elicited by nitrite. Systemically, mean arterial blood pressure and resistance was reduced to a similar extent with both treatments during hypoxia. However, with the relative chemical stability of the nitrite anion compared with NO gas, there was sustained vasodilation for more than 60 minutes (the duration of the hypoxic challenge) after discontinuation of nitrite inhalation, whereas the termination of NO gas delivery abolished the vasodilating effect in a matter of seconds (Figure 13A, 13B). The relatively sustained effect of nitrite nebulization might be therapeutically advantageous by allowing for intermittent therapy analogous to the treatment of asthma with beta-adrenergic agonists by meter dose inhaler. The time course of nitrite inhalation-induced pulmonary vasodilation and plasma nitrite levels are shown (Figure 13C, 13D). In this experiment which tracked biochemical changes for a longer period that in Figure 10 methemoglobin (MetHb) concentrations increased from 2.1 ± 0.1 % during baseline to 2.8 ± 0.2% after nitrite nebulization ($P < 0.05$).

Discussion

A principle finding of this example is that a brief period of inhalation of nebulized sodium nitrite solution produces rapid and selective pulmonary vasodilation during hypoxic-induced pulmonary hypertension in newborn lambs. The significant reduction in pulmonary artery pressure following nitrite nebulization was sustained when hypoxia was continued for more than an hour after termination of nitrite nebulization. In none of the experiments did nitrite inhalation produce systemic hypotension, and methemoglobin elevation was minimal. From a mechanistic standpoint, nitrite administration was associated with NO production, measured by exhaled NO gas and NO-modified hemoglobin, with responses in proportion to levels of hemoglobin-oxygen desaturation and decreases in blood pH. These data support the paradigm that nitrite is an NO-dependent vasodilator whose bioactivation is coupled to hemoglobin deoxygenation and protonation.

Inhaled NO gas is the current standard for the treatment of pulmonary hypertension. Figure 13 provides a comparison of the effects of NO gas at 20 ppm with those of aerosolized nitrite. In about 5 minutes the NO gas effectively ablated about 80% of hypoxic-induced pulmonary hypertension, an effect that was short lived but which could be reproduced when it was given again 20 minutes later. Aerosolized sodium nitrite removed about 60% of hypoxic-induced pulmonary hypertension. This response was consistently observed in each of the lambs studied and it persisted throughout the one-hour period of hypoxia that was maintained after the nitrite aerosol was discontinued. The changes in pulmonary blood flow were accompanied by corresponding changes in the calculated resistance to blood flow through the lungs, indicating that changes were in the pulmonary vasculature rather than secondary to changes in cardiac output or systemic effects that might have altered perfusion pressures.

We demonstrate herein that aerosolized nitrite is an NO producing agent in the newborn lamb that can be readily administered by nebulization and appears to exhibit a wide therapeutic-to-safety margin, with limited systemic hemodynamic changes and methemoglobin production. This presents an attractive therapeutic option to inhaled NO. Nitrite is an ideal "NO producing" agent in that it 1) is a naturally occurring compound in blood, alveolar lining fluid, and tissue, and 2) has no parent-compound leaving group, such as the diazenium diolates, that requires extensive toxicological study prior to translation to human disease, and 3) it is already approved for human use in cyanide antidote kits. These advantages are to be counterbalanced against possible problems that might occur with more prolonged delivery, including alveolar nitrite accumulation, systemic vasodilation, and the development of methemoglobinemia.

In conclusion, the data presented in this example suggest that inhaled nitrite is a potent and selective vasodilator of pulmonary circulation of the newborn lamb and further support the paradigm that nitrite, and particularly salts of nitrite, such as sodium nitrite, is an NO-dependent vasodilator whose bioactivation is coupled to hemoglobin deoxygenation and protonation. In none of our studies did inhaling nitrite produce systemic hypotension or elevate methemoglobin levels.

Example 4

Use of nitrite infusions for the prevention of cerebral artery vasospasm after subarachnoid hemorrhage

This example describes a method for using nitrite infusion to prevent cerebral artery vasospasm after intracranial hemorrhage.

Subarachnoid hemorrhage (SAH) due to the rupture of intracranial aneurysms affects 28,000 Americans annually. Almost 70% of patients with aneurysmal SAH develop severe spasm of the cerebral arteries on the seventh day after SAH. Despite aggressive medical therapy, neurological deficits resulting from vasospasm continue to be a major cause of morbidity and mortality. Although the etiology of cerebral vasospasm is poorly understood, there is increasing evidence that erythrocyte hemolysis in the cerebrospinal fluid and decreased availability of nitric oxide (NO), a potent vasodilator, plays a significant role. Reversal of vasospasm by NO or NO prodrugs has been documented in several animal models.

Despite half a century of research and clinical trials, delayed cerebral vasospasm (DCV) remains the single cause of permanent neurological deficits or death in at least fifteen percent of patients following otherwise successful endovascular or surgical treatment for ruptured intracranial aneurysm. Decreased bioavailability of nitric oxide (NO) has been mechanistically associated with the development of DCV. This work was carried out to determine whether infusions of nitrite, a naturally occurring anion that reacts with deoxyhemoglobin to form NO and S-nitrosothiol, might prevent DCV via reactions with perivascular hemoglobin.

Methods

An autologous arterial blood clot was placed around the right middle cerebral artery (R MCA) of 14 anesthetized *Cynomolgus* monkeys at day 0. Sodium nitrite solution ($NaNO_2$, 135 mg/daily and 180 mg/daily, which approximates 45 mg/kg and 60 mg/kg per day) in 0.9% saline (n=6) or saline alone (n=8) was infused intravenously for 14 days in awake animals via an ambulatory MiniMed Infusion Pump, at 2 μl/minute. Cerebral arteriogram was performed before clot placement and on days 7 and 14, for assessment of DCV. Arteriographic vasospasm was defined as a 25% or greater reduction in the proximal 14 mm of the R MCA area as measured on the AP projection of the cerebral arteriogram (blinded assessment). Mean arterial blood pressure was measured and blood samples were collected daily from day 0; the cerebral spinal fluid samples were collected on day 0, 7, and 14.

Results

In control animals, cerebral spinal fluid nitrite levels decreased from 3.1±1.5 μM to 0.4±0.1 μM at 7 days and 0.4±0.4 μM at 14 days (Figure 14), and all eight animals developed significant vasospasm of the R MCA (Figures 15 and 16), complicated by stroke and death in one animal.

Nitrite infusions were associated with increases in plasma cerebrospinal fluid nitrite and blood methemoglobin concentrations without systemic hypotension (Figure 14), and significantly reduced the severity of vasospasm (Figures 15 and 16; no animals developed significant vasospasm; mean reduction in the R MCA area on day 7 after SAH was 8±9% versus 45±5%; $P < 0.001$). Pharmacological effects of nitrite infusion were associated with bioconversion of cerebrospinal fluid nitrite to S-nitrosothiol, a potent vasodilating NO donor intermediate of nitrite bioactivation. There was no clinical or pathological evidence of nitrite toxicity.

Conclusions

Subacute sodium nitrite infusions prevent DCV in a primate model of SAH, and do so without toxicity. These data evince a novel, safe, inexpensive, and rationally designed therapy for DCV, a disease for which no current preventative therapy exists.

While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments, and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

CLAIMS

1. A method for treating or ameliorating a condition selected from:

(a) hepatic or cardiac or brain ischemia-reperfusion injury;

(b) pulmonary hypertension; or

(c) cerebral artery vasospasm,

in a subject by decreasing blood pressure and/or increasing vasodilation in the subject, the method comprising administering sodium nitrite to the subject to decrease the blood pressure and/or increase vasodilation in the subject, thereby treating or ameliorating the condition.

2. The method of claim 1, which is a method for treating or ameliorating hepatic or cardiac or brain ischemia-reperfusion injury.

3. The method of claim 2, wherein administering sodium nitrite to the subject is intravenous.

4. The method of claim 2 or 3, wherein the sodium nitrite is administered to a circulating concentration of about 0.6 to 240 μM.

5. The method of claim 1, which is a method for treating or ameliorating pulmonary hypertension.

6. The method of claim 5, wherein the pulmonary hypertension is neonatal pulmonary hypertension.

7. The method of claim 5 or 6, wherein administering sodium nitrite to the subject is by inhalation.

8. The method of claim 7, wherein the sodium nitrite is nebulized.

9. The method of any one of claims 5 through 8, wherein the sodium nitrite is administered at a rate of 270 μmol/minute.

10. The method of claim 1, which is a method for treating or ameliorating cerebral artery vasospasm.

11. The method of claim 10, wherein administering sodium nitrite to the subject is intravenous.

12. The method of claim 10 or 11, wherein the sodium nitrite is administered at a rate of about 45 to 60 mg/kg.

13. The method of any one of claims 1-12, wherein the sodium nitrite is administered in combination with at least one additional agent.

14. The method of any one of claims 1-13, wherein the subject is a mammal.

15. The method of any one of claims 14, wherein the subject is a human.

Figure 1A **Figure 1B**

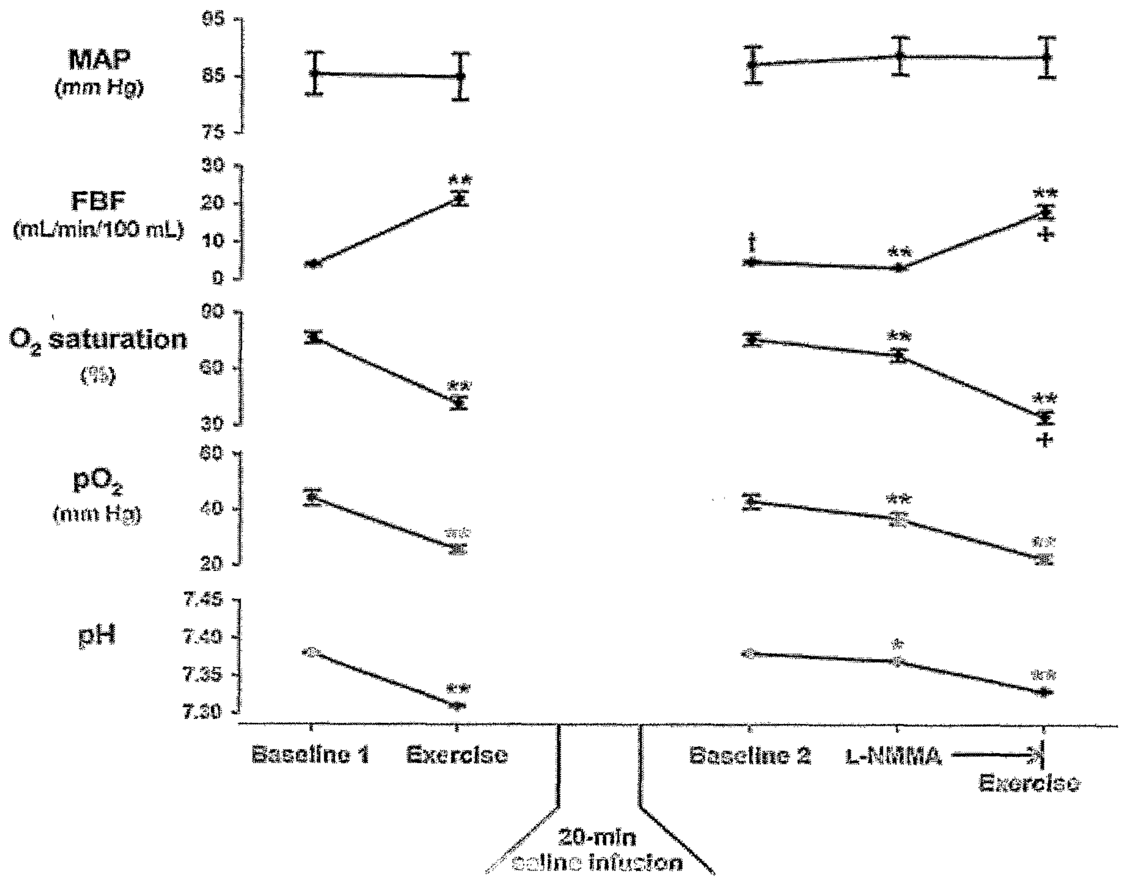
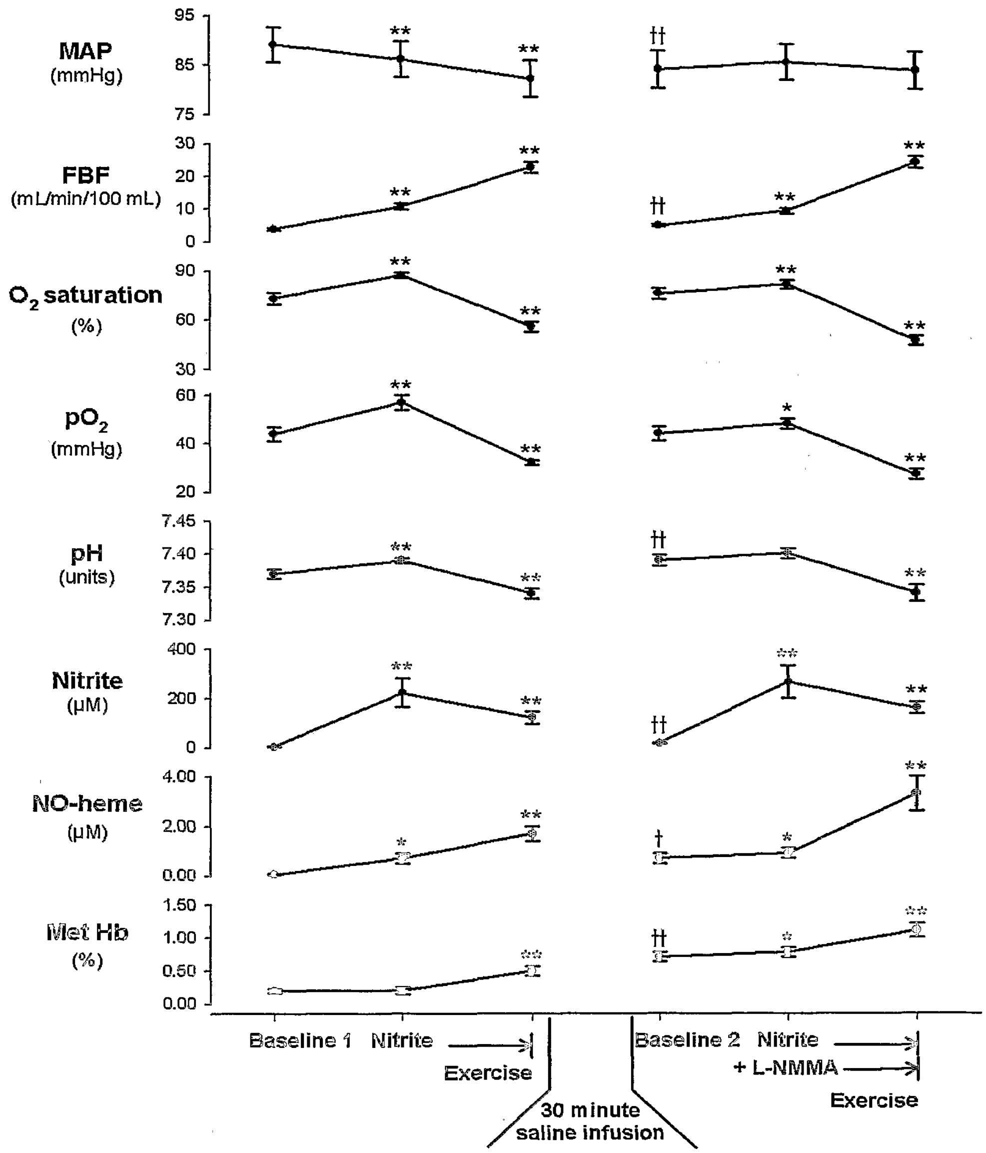


Figure 2A **Figure 2B**



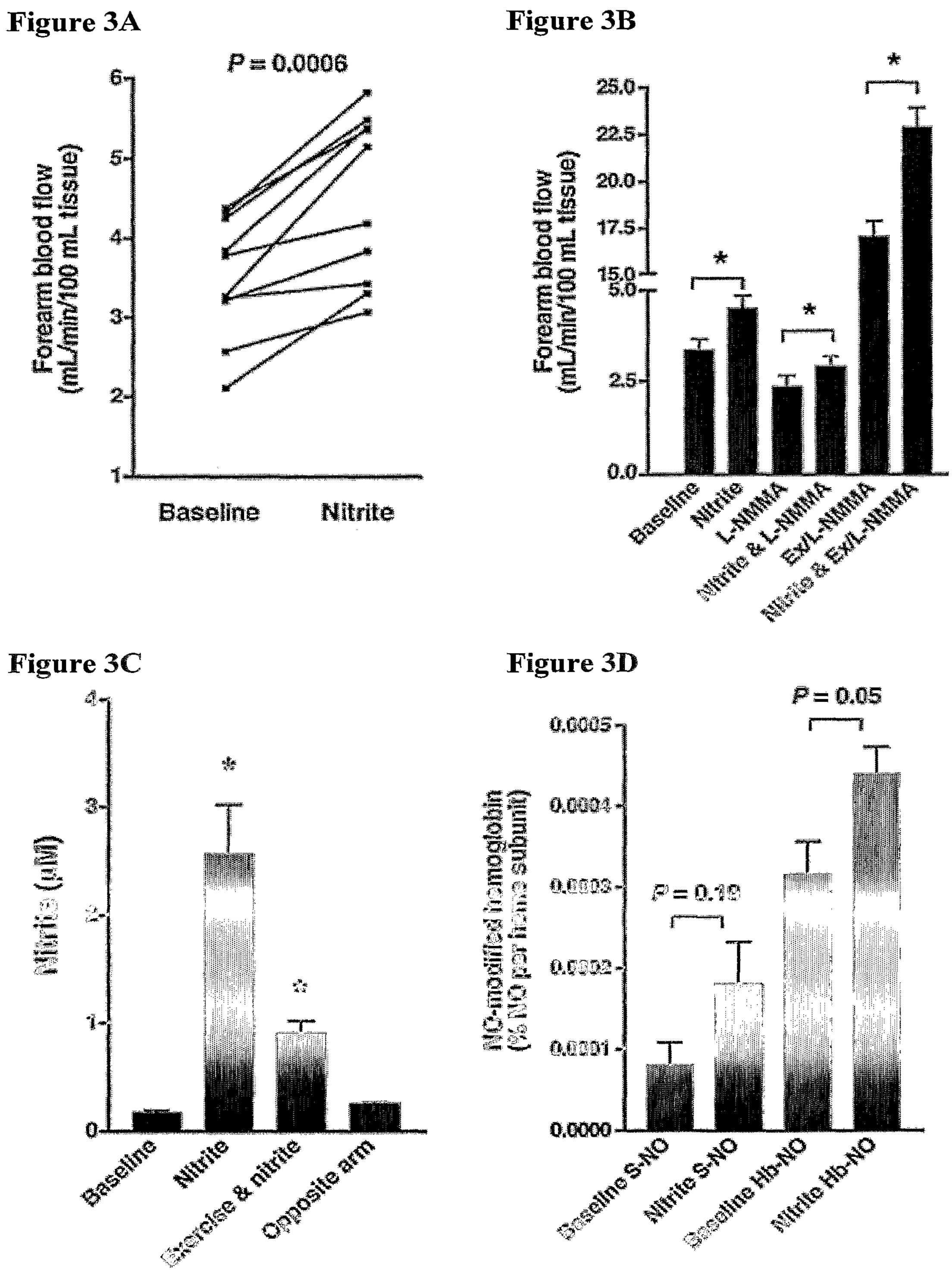


Figure 3A
P = 0.0006
Forearm blood flow (mL/min/100 mL tissue)
Baseline
Nitrite
Figure 3B
Forearm blood flow (mL/min/100 mL tissue)
Baseline
Nitrite
L-NMMA
Nitrite & L-NMMA
Ex/L-NMMA
Nitrite & Ex/L-NMMA
Figure 3C
Nitrite (μM)
Baseline
Nitrite
Exercise & nitrite
Opposite arm
Figure 3D
P = 0.05
P = 0.19
NO-modified hemoglobin (% NO per heme subunit)
Baseline S-NO
Nitrite S-NO
Baseline Hb-NO
Nitrite Hb-NO

Figure 4A

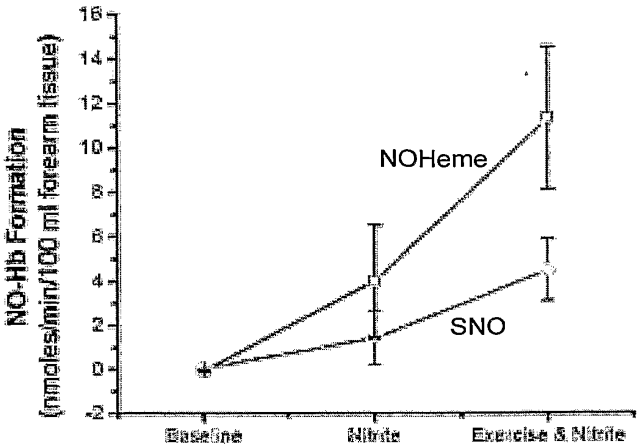


Figure 4B

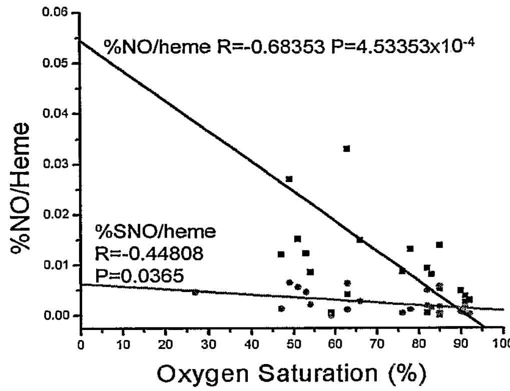


Figure 5A

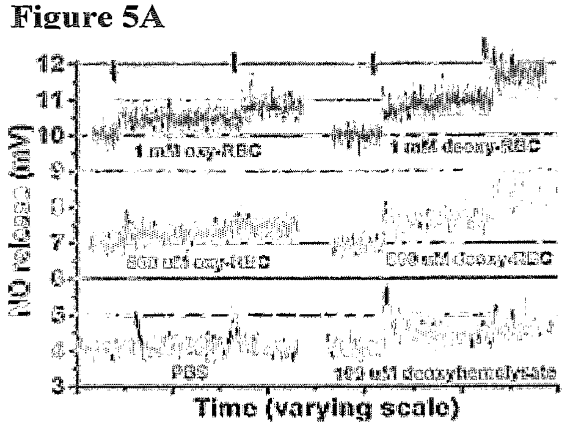


Figure 5B

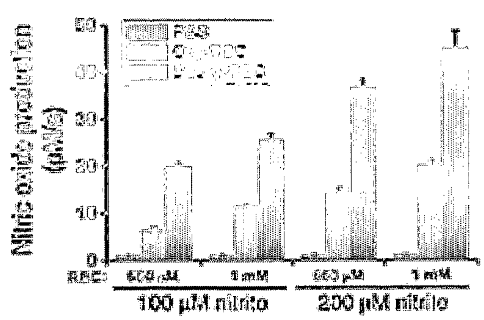


Figure 6A

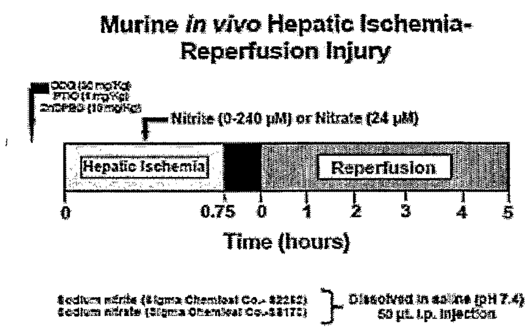


Figure 6B

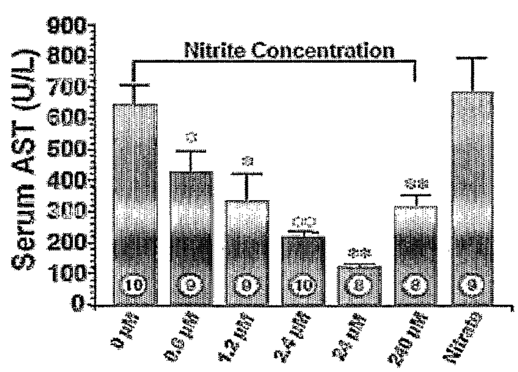


Figure 6C

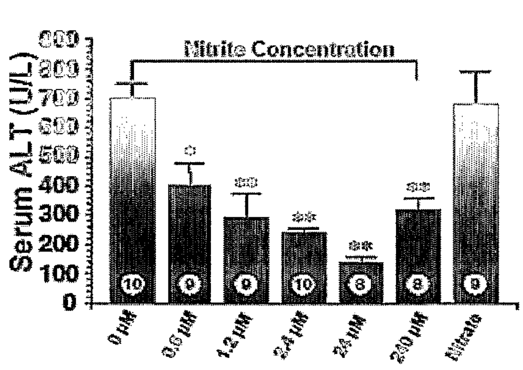


Figure 6D

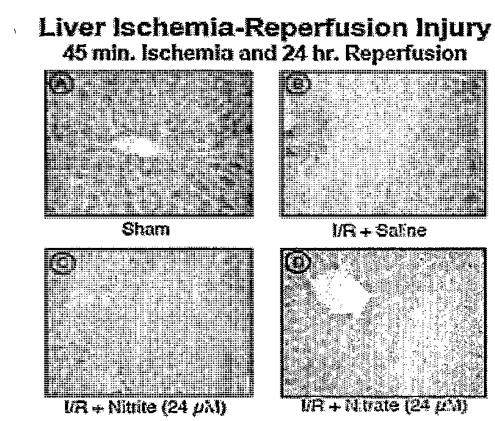


Figure 6E

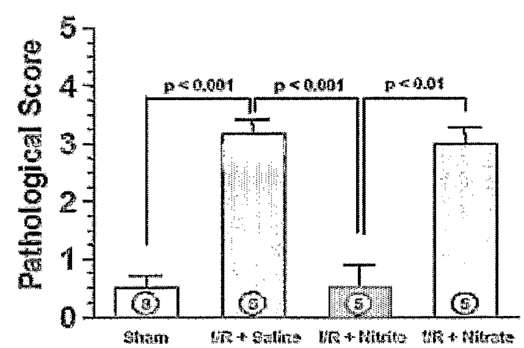


Figure 6F

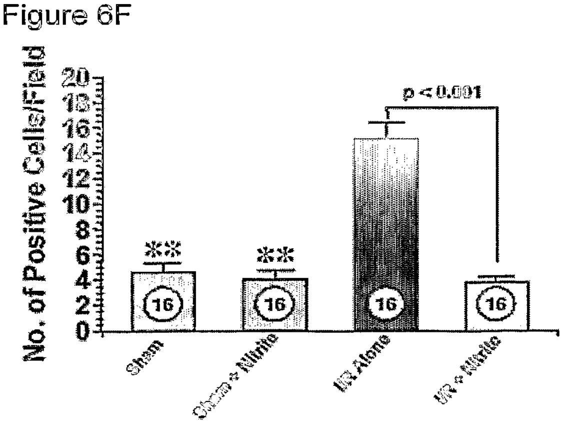


Figure 7A

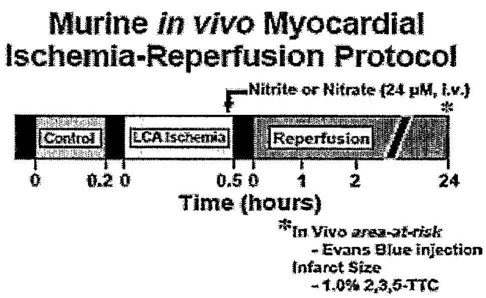


Figure 7B

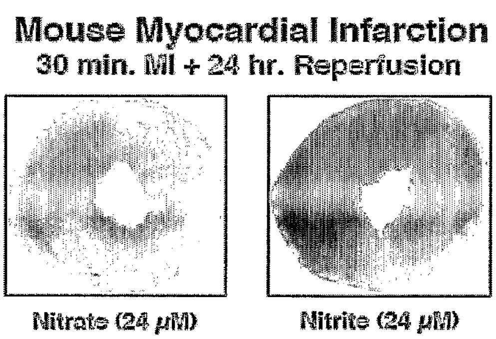


Figure 7C

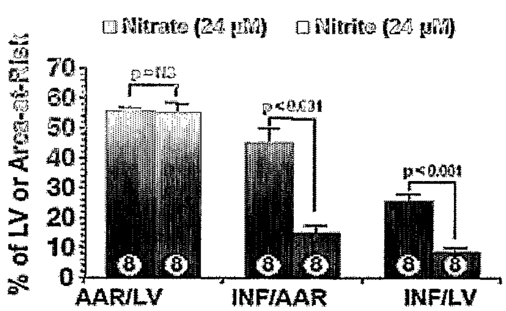


Figure 7D

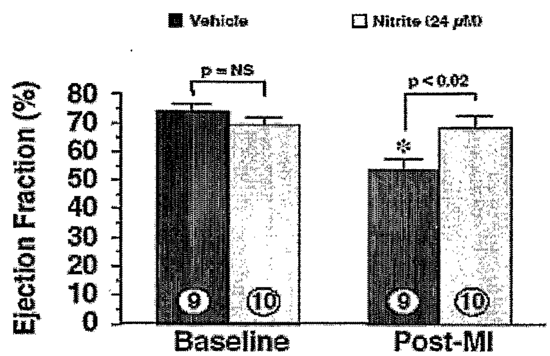


Figure 7E

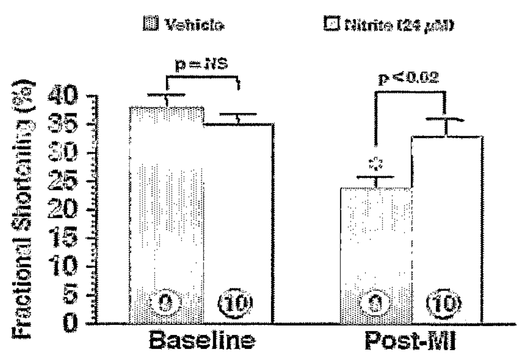


Figure 8B

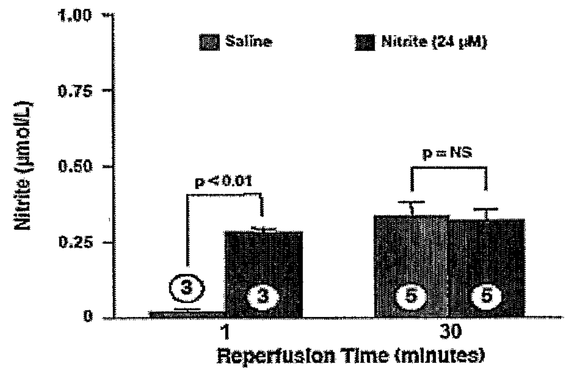


Figure 8A

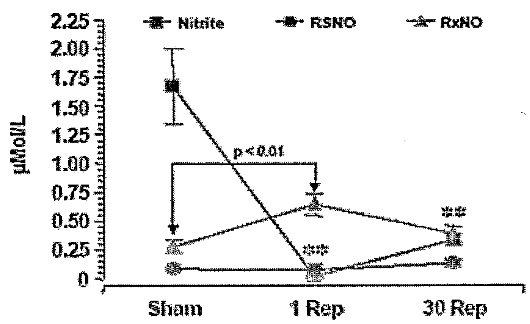


Figure 8C

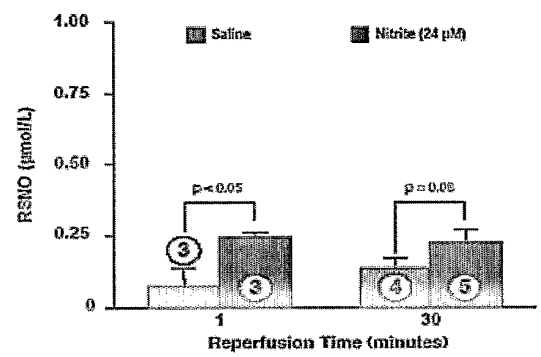


Figure 8D

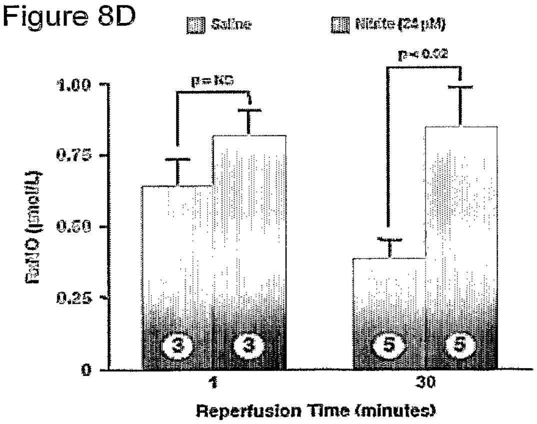


Figure 9A

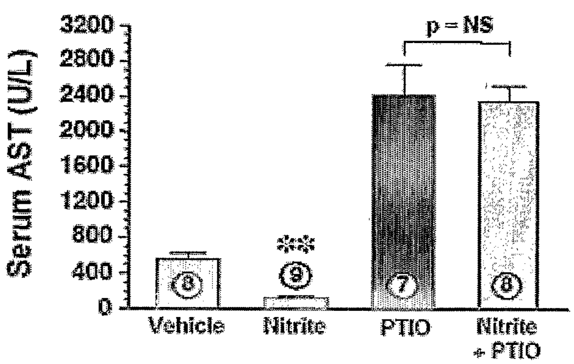


Figure 9B

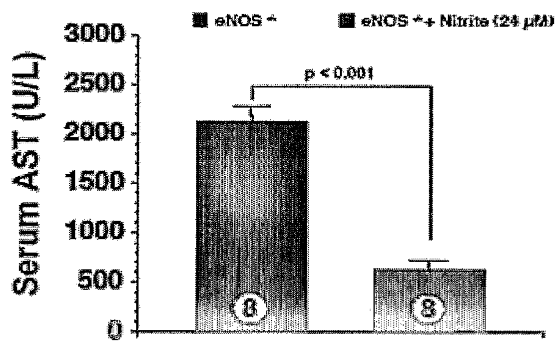


Figure 9C

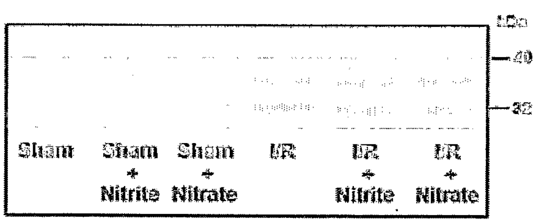


Figure 9D

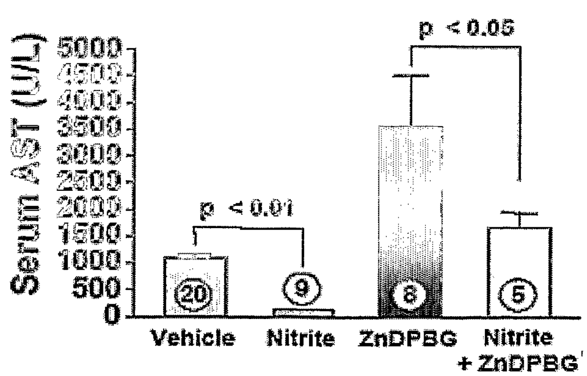


Figure 10A

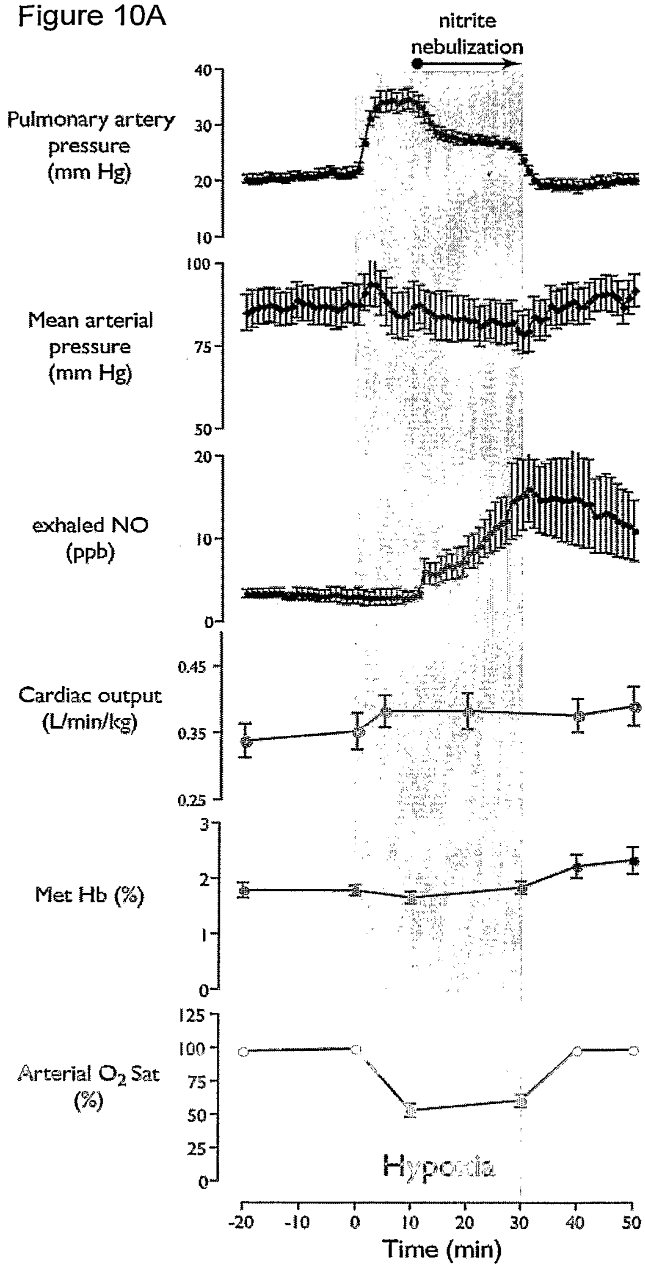


Figure 10B

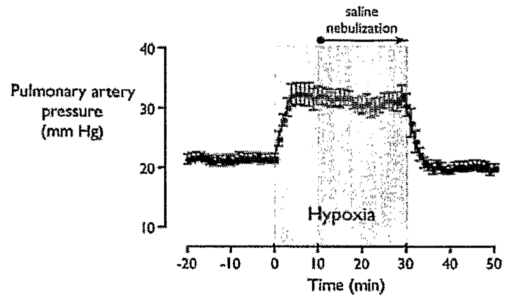
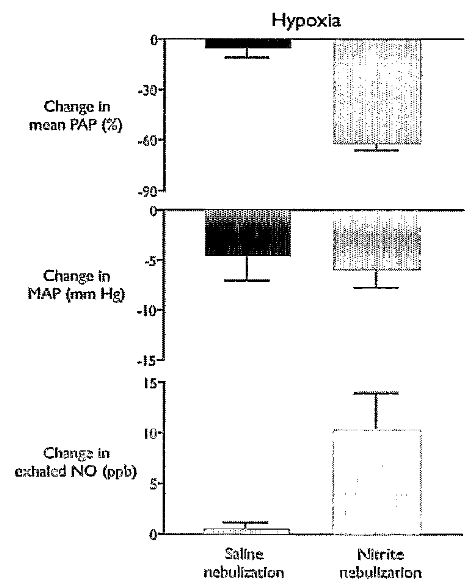


Figure 10C



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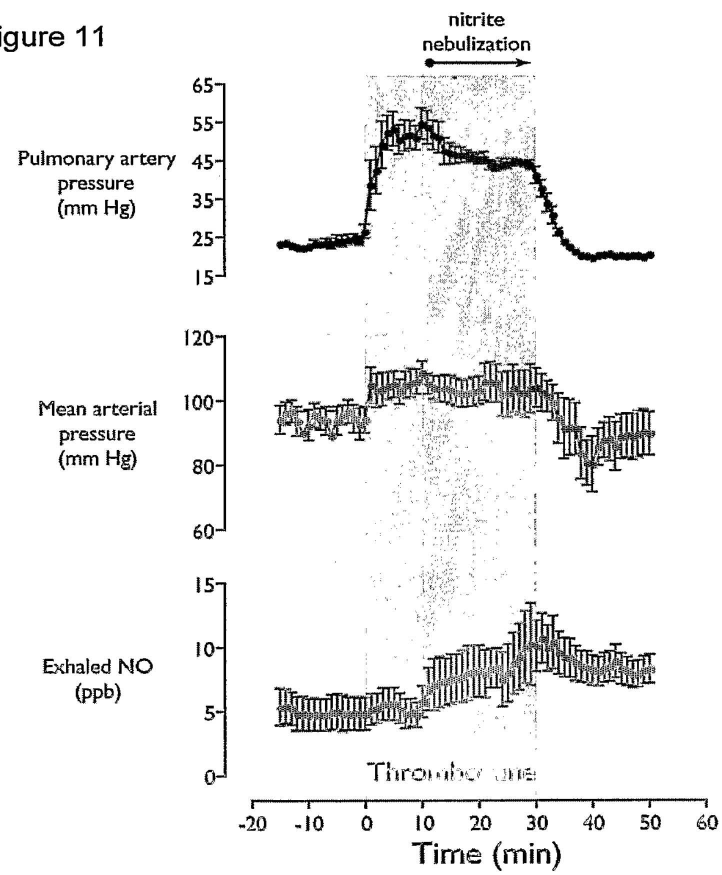

nitrite
nebulization
Pulmonary artery
pressure
(mm Hg)
65
55
45
35
25
15
Mean arterial
pressure
(mm Hg)
120
100
80
60
Exhaled NO
(ppb)
15
10
5
0
-20
-10
0
10
20
30
40
50
60
Time (min)

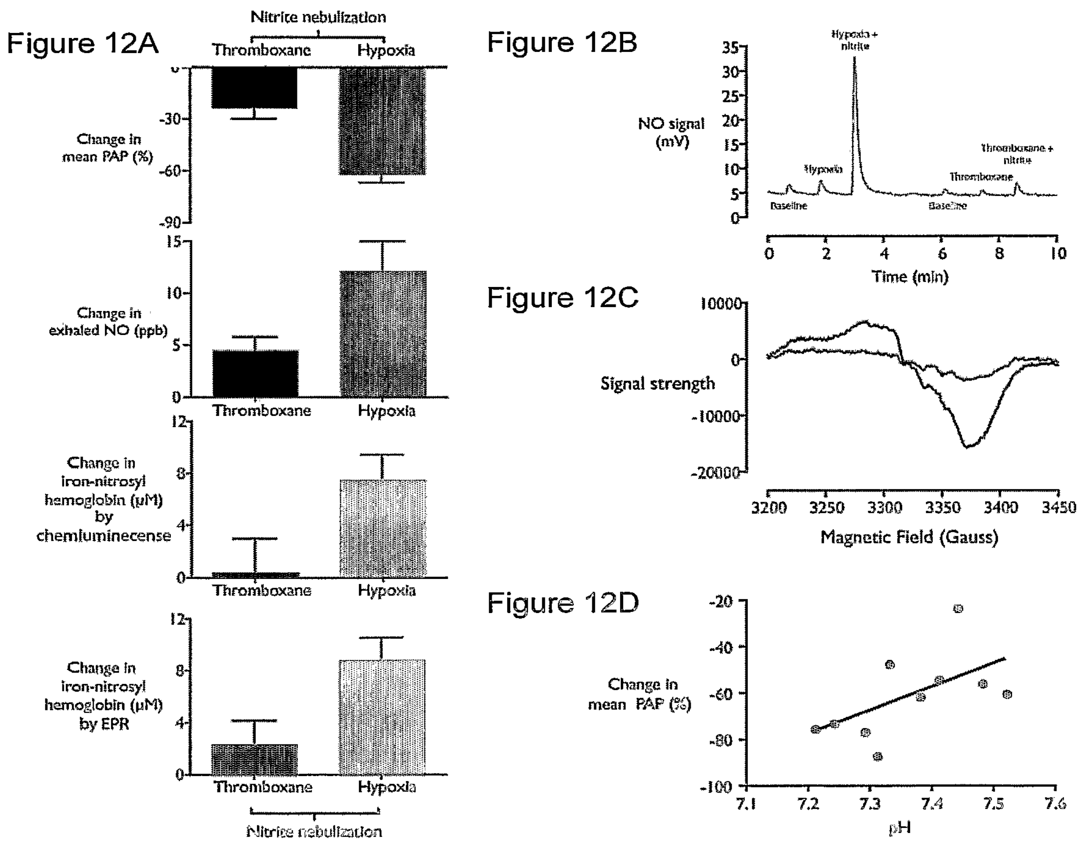


Figure 12A
Nitrite nebulization
Thromboxane
Hypoxia
Change in mean PAP (%)
Change in exhaled NO (ppb)
Change in iron-nitrosyl hemoglobin (µM) by chemiluminecense
Change in iron-nitrosyl hemoglobin (µM) by EPR
Figure 12B
NO signal (mV)
Hypoxia + nitrite
Baseline
Thromboxane
Thromboxane + nitrite
Time (min)
Figure 12C
Signal strength
Magnetic Field (Gauss)
Figure 12D
Change in mean PAP (%)
pH

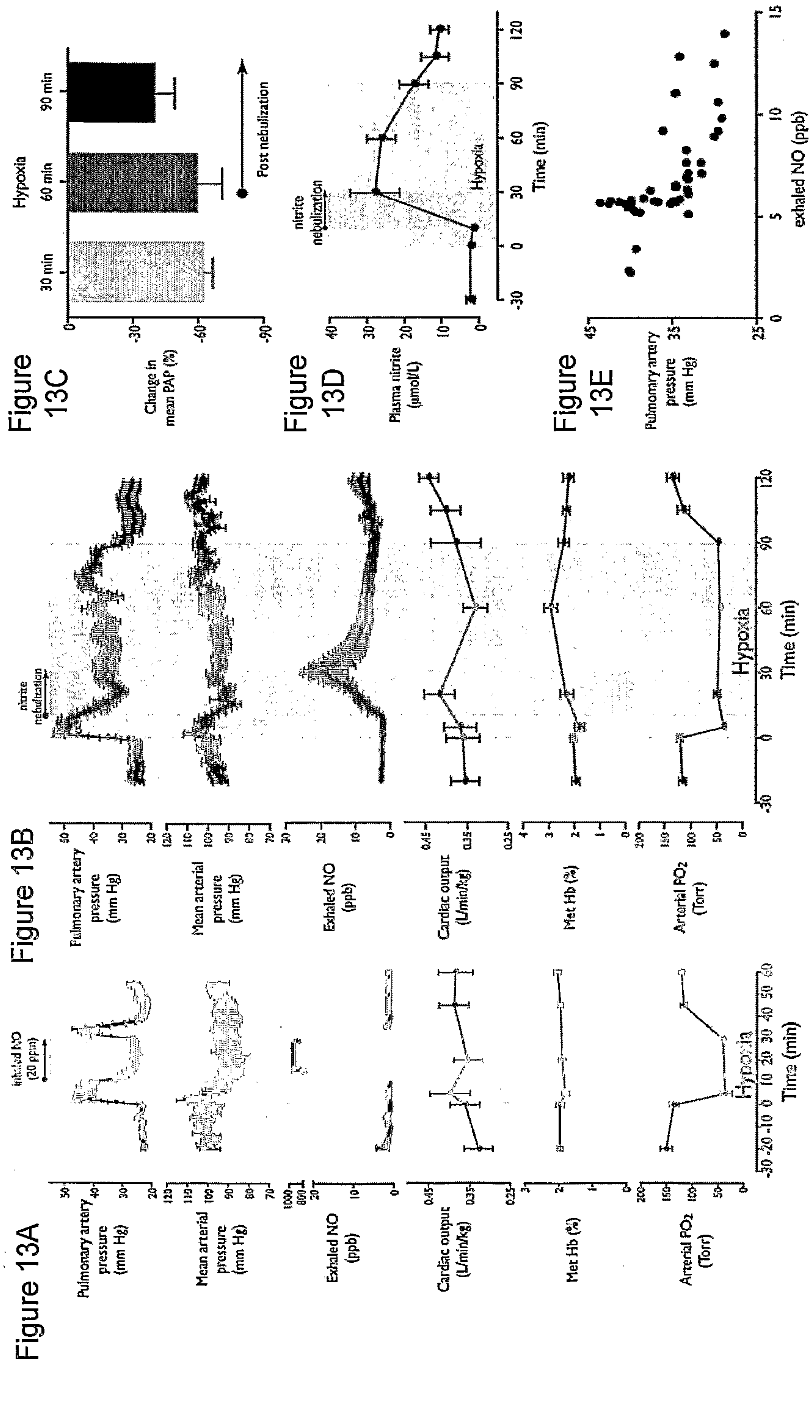
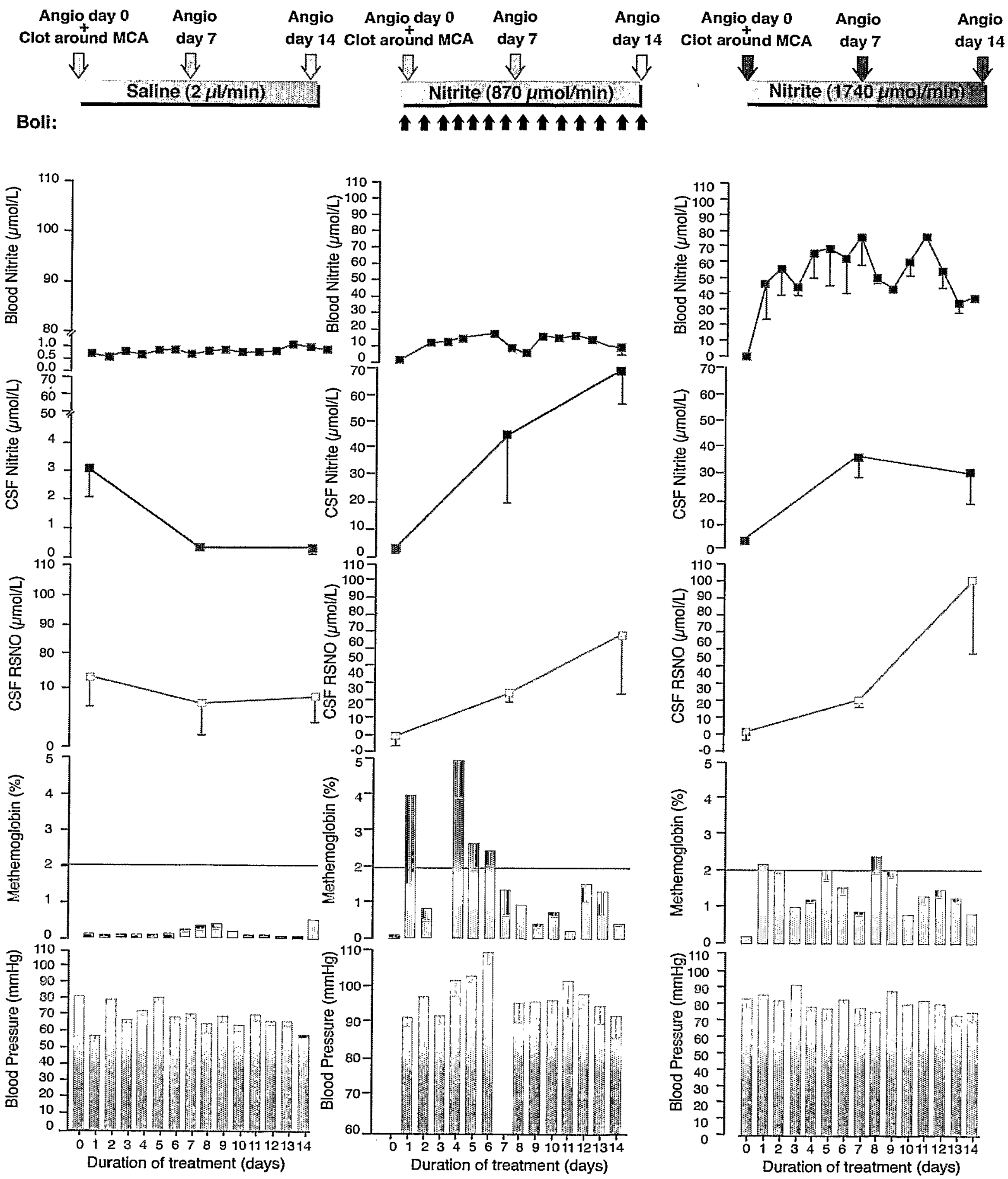


Figure 13A
Pulmonary artery pressure (mm Hg)
Mean arterial pressure (mm Hg)
Exhaled NO (ppb)
Cardiac output (L/min/kg)
Met Hb (%)
Arterial PO2 (Torr)
Inhaled NO (20 ppm)
Hypoxia
Time (min)
Figure 13B
Pulmonary artery pressure (mm Hg)
Mean arterial pressure (mm Hg)
Exhaled NO (ppb)
Cardiac output (L/min/kg)
Met Hb (%)
Arterial PO2 (Torr)
nitrite nebulization
Hypoxia
Time (min)
Figure 13C
Change in mean PAP (%)
Hypoxia
30 min
60 min
90 min
Post nebulization
Figure 13D
Plasma nitrite (µmol/L)
nitrite nebulization
Hypoxia
Time (min)
Figure 13E
Pulmonary artery pressure (mm Hg)
exhaled NO (ppb)

Figure 14


Angio day 0 + Clot around MCA
Angio day 7
Angio day 14
Saline (2 μl/min)
Nitrite (870 μmol/min)
Nitrite (1740 μmol/min)
Boli:
Blood Nitrite (μmol/L)
CSF Nitrite (μmol/L)
CSF RSNO (μmol/L)
Methemoglobin (%)
Blood Pressure (mmHg)
Duration of treatment (days)

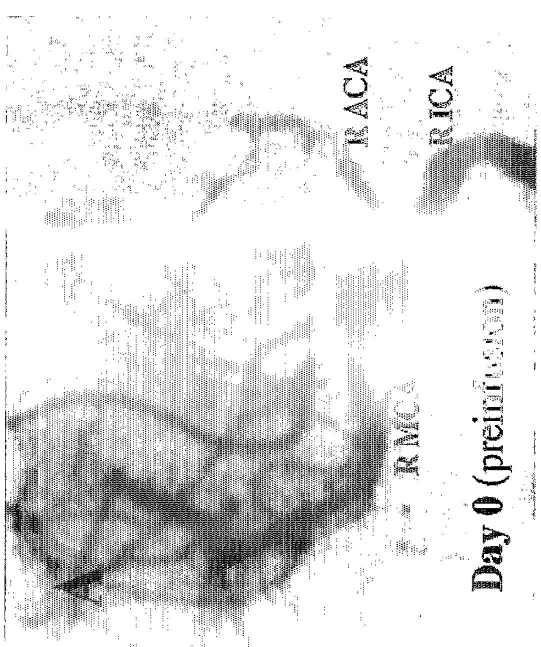


Figure 15A

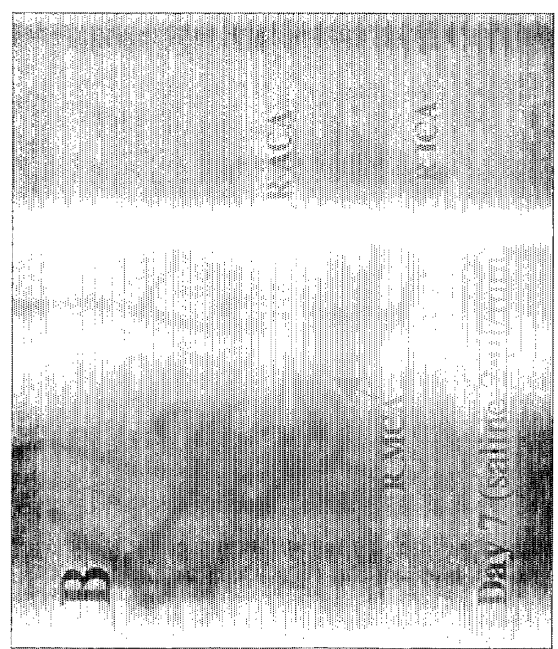


Figure 15B

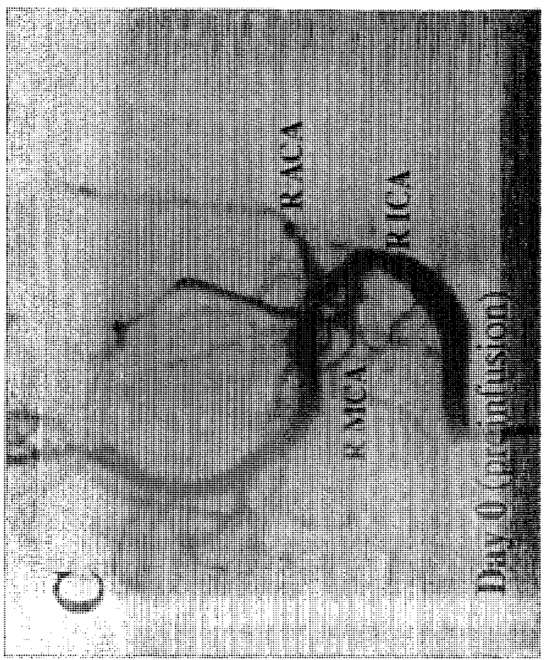


Figure 15C

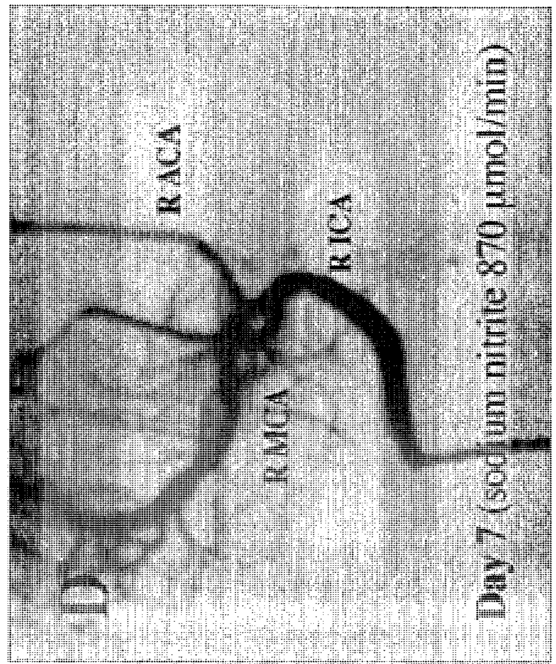
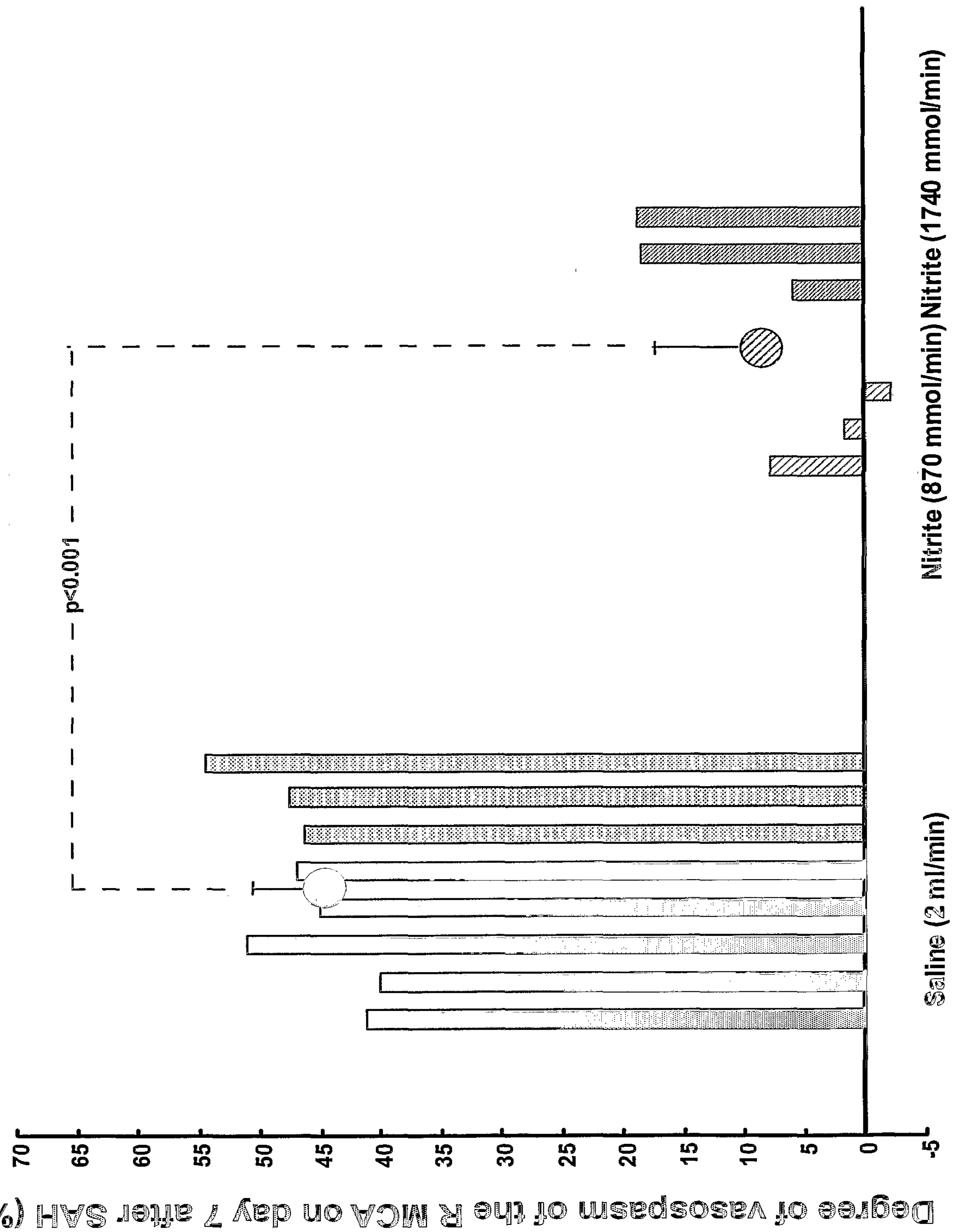


Figure 15D

Figure 16


Degree of vasospasm of the R MCA on day 7 after SAH (%)
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
p<0.001
Saline (2 ml/min)
Nitrite (870 mmol/min)
Nitrite (1740 mmol/min)

WO2006127907

Publication Title:

LOCALIZED DELIVERY OF CARDIAC INOTROPIC AGENTS

Abstract:

Abstract of WO 2006127907

(A2) Translate this text The present invention provides novel methods for the localized delivery of inotropic agents to the heart, including specific regions of the heart, such as the ventricles, for example in a subject undergoing cardiothoracic surgery, with the aim of supporting the myocardial contractile function of the heart.

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(21) International Application Number: PCT/US2006/020252

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(71) Applicant *(for all designated States except US)*: MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: EDELMAN, Elazer [US/US]; 30 Warren Street, Brookline, MA 02445 (US). LOVICH, Mark [US/US]; 30 Griggs Road, Brookline, MA 02446 (US).

(74) Agents: EISENSTEIN, Ronald, I. et al.; NIXON PEABODY LLP, 100 Summer Street, Boston, MA 02110 (US).

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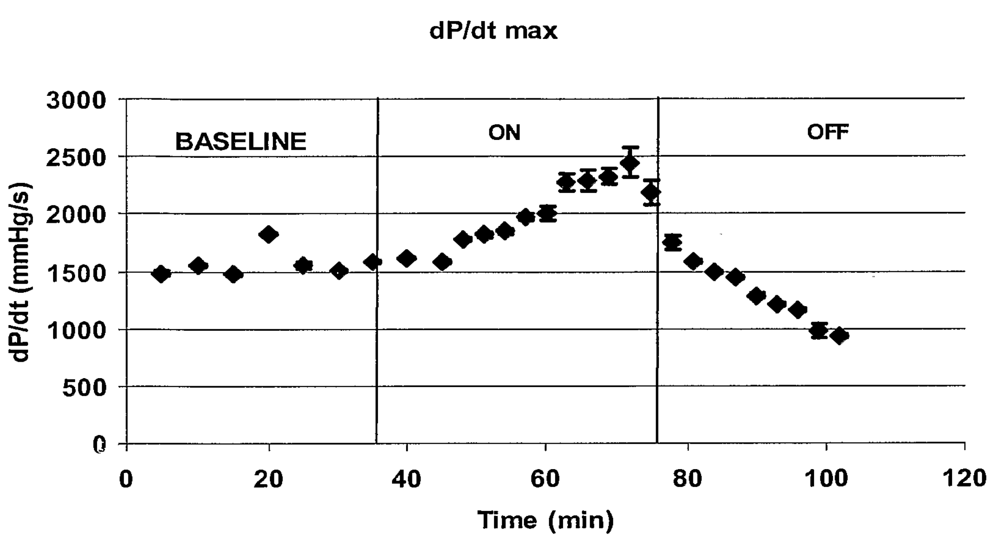
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LOCALIZED DELIVERY OF CARDIAC INOTROPIC AGENTS



(57) Abstract: The present invention provides novel methods for the localized delivery of inotropic agents to the heart, including specific regions of the heart, such as the ventricles, for example in a subject undergoing cardiothoracic surgery, with the aim of supporting the myocardial contractile function of the heart.

WO 2006/127907 A2

LOCALIZED DELIVERY OF CARDIAC INOTROPIC AGENTS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application Serial No. 60/684,594 filed May 25, 2005, the contents of which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

[002] The present invention is directed to methods for the localized delivery of inotropic agents to the heart, including specific regions of the heart such as the ventricles, in a subject in need of such contractile support.

BACKGROUND OF THE INVENTION

[003] Performance of cardiac surgery is a delicate and invasive procedure. The majority of epicardial bypass graft surgeries, and all open heart procedures, require temporary arrest of the heart to allow the surgeon to accomplish the required task without interference from heart movement. An extracorporeal machine, known as a cardiopulmonary bypass (CPB) circuit, assumes the heart and lungs' role of supplying oxygenated blood to the rest of the body while the heart is arrested. Once the surgery is completed, the heart must be re-started, and the patient weaned from the CPB.

[004] While the use of CPB makes cardiac surgery feasible, it is also associated with significant risks and difficulties. The use of a CPB machine usually requires an aortic cross-clamp to separate the heart from the rest of the circulation. Because the coronary arteries arise very close to the heart, the cross clamp must be applied distal to their ostia and therefore they receive no blood flow for prolonged periods, and thus the heart becomes ischemic. Despite numerous myocardial protection strategies, such as hypothermia and chemical cardioplegia to decrease oxygen consumption by arresting the heart, many patients' heart function is

significantly impaired by both chemical arrest and the CPB circuit itself. Chemical cadioplegia, altered coronary perfusion, embolic events and direct manual manipulation of the heart during the procedure all contribute to depression of myocardial function after it is restarted. Furthermore, the degree of post CPB dysfunction may depend on the duration of the CPB time. Patients emerge from chemical cardiac arrest with a spectrum of left ventricular dysfunction, from transient mild impairment to outright ventricular failure and inability to be separated from the CPB. Patients with preexisting ventricular dysfunction are at the greatest risk for further myocardial impairment during CPB.

[005] Moreover, because of improvements in surgical technique and intraoperative myocardial protection, as well as the increasing availability of sophisticated valvular, direct myocardial resections, repairs of septal defects, and coronary bypass procedures, more cardiac operations are being performed on patients with more advanced stages of disease and decreased ventricular function. Indeed, the number of operative risk factors, including advanced age, female gender, severity of angina, triple vessel disease, and left ventricular dysfunction, has increased among patients currently undergoing coronary artery bypass surgery [Davis PK, et al., Ann Thorac Surg 1989; 47:493-98].

[006] In addition, there are important, potentially damaging effects of CPB itself on the cardiovascular system, including increased capillary permeability with attendant transcapillary plasma loss, renal dysfunction, peripheral or central vasoconstriction, coagulopathy, platelet destruction and dysfunction, and destruction of red blood cells [Kalter RD, et al., .J Thorac Cardiovasc Surg 1979; 77:428-35; Kirklin JK, et al., J. Thorac Cardiovasc Surg 1983; 86:845-57.]. Patients with preexisting cardiomyopathies are at even greater risk for postoperative contractile dysfunction. These effects are often transient, but their timing and intensity can make it difficult to impossible in many instances to separate the patient from the CPB circuit.

[007] Weaning a patient off cardiopulmonary bypass (CPB) is a critical step of cardiac surgical procedures. Restarting the heart and returning it and the lungs to the circulation after CPB carries the potential to severely stress an already compromised heart. In the best of circumstances, weaning off CPB can be a relatively straightforward process that requires reestablishing ventilation to the lungs and slowly lowering the circulatory support from the CPB pump. In a significant number of cases however, weaning is especially difficult, and in a few situations simply impossible.

[008] Current available options to support patients who fail to wean from CPB, in order of increasing invasiveness and associated morbidity, include intravenous infusion of inotropes that enhance myocardial contractility, insertion of an intra-aortic balloon pump to augment coronary perfusion and diminish the workload on the heart, and placement of a ventricular assist device. However, each of these treatments is accompanied by significant morbidity and technical limitations, and potential toxicity. Examples of limitations associated with such treatments include proarrhythmic and systemic effects from systemic infusion of inotropes, damage from large-bore indwelling vascular access, need for patient immobility and sedation, as well as risks associated with the placement of a large mass of foreign materials with externalized connections. The pumps and devices have high rates of infection and thromboembolic complications, and require patient immobility, sedation, sometimes prolonged postoperative ventilation, and the most extreme of intensive care nursing support. Weaning of small children after prolonged, difficult and complex operations can represent a further challenge to the surgical team as assist devices may not be readily available in appropriate sizes.

[009] One of the significant challenges of supporting patients as they transition from CPB to the intensive care unit is the variability between patients regarding the timing and degree of support each patient requires. Many patients only need short-term inotropic support to help them transition from CPB to the intensive care unit, while the support required by other patients is much more extensive and

potentially associated with greater risks. Thus, it would be desirable to have less intrusive means that could be used to support these patients as they transition off CPB.

[0010] Inotropic agents are one approach used to enhance a high-risk patient's ability to wean from CPB. Pharmacologic inotropic agents enhance myocardial contractility, and fall into two broad categories: sympathomimetics such as epinephrine (adrenaline), norepinephrine (noradrenaline), dobutamine, isopreterenol, salbutamol, salmeterol, terbutaline, isoproterenol, phenylephrine, ephedrine, clonidine and dopamine, and phosphodiesterase inhibitors such as milrinone and amrinone. Each of these compounds, while increasing the inotropic state of the heart, has limitations that restrict the doses that can be given intravenously and often necessitate infusion of additional agents to counteract side effects. For example, dopamine dosing is limited by the increase in the rate and irritability of electrical excitation of the heart that accompanies the desired inotropic effect. Alternatively, phosphodiesterase inhibitors increase intracellular cyclic AMP, an intracellular signaling molecule that increases inotropy, but unfortunately dilates arterioles and causes systemic vasodilation and hypotension. As a result, vasoconstricting sympathomimetic agents often need to be co-administered and these again can lead to proarrythmogenic states and undesirable tachycardia.

[0011] One important consideration of the use of inotropic agents is that they are administered systemically and thus treat all vascular beds. Systemic side effects of sympathomimetics include potential renal and cerebral vasoconstriction, and pulmonary artery hypertension, which in turn can induce right heart failure. Other undesired effects are excess tachycardia and electrical irritability.

[0012] Accordingly, there is a need for improved methods to support patients as they transition off CPB, by improving contractile function of the heart without extraventricular effects, such as tachycardia, vasoconstriction or systemic hypotension.

SUMMARY OF THE INVENTION

[0013] The present invention provides novel methods for the localized delivery of inotropic agents to the heart, including specific regions of the heart, such as the ventricles, in a subject in need thereof.

[0014] Support of the weakened heart such as occurs while a patient is coupled to a CPB circuit, and while the patient transitions off CPB, is critical to recovery from cardiac surgery. We have discovered methods to take advantage of existing polymeric controlled release strategies to deliver inotropic agents directly or indirectly to the heart, preferably directly, including to specific regions of the heart. By locally delivering the inotropic agent directly to the heart, the systemic exposure of the inotropic agents is limited, avoiding the alterations in vascular tone, and heart rate and electrical excitability associated with systemic administration of these agents.

[0015] The methods of the present invention can be used to treat any patient in need of transient contractile support to the heart, where such support can be provided by the local delivery of inotropic agents either directly or indirectly to the heart, including specific regions of the heart, such as the ventricles. One would apply the agent through the cardiac blood stream, or preferably directly in the heart. The agent can be applied through the coronary artery or vein and onto the heart surface. The agent can also be applied through the ventricular or atrial walls and onto the heart surface. The agent can also be applied through direct and extensive surgical field exposure, minimally invasive exposure via a pericardial window or heart port, or percutaneous or endovascular catheters.

[0016] In one embodiment, the patient is in need of localized delivery of an inotropic agent to provide contractile support as a result of a surgical intervention. Surgical interventions include but are not limited to cardiac surgery, thoracic surgery, and general surgery. In another embodiment, the patient is in need of transient localized delivery of an inotropic agent to provide contractile support as a result of trauma, shock, or heart failure.

[0017] In another embodiment, the patient is in need of transient inotropic support following an intervention less invasive than a major surgical intervention, referred to herein as a minimally invasive intervention. Such minimally invasive interventions include but are not limited to a percutaneous intervention or a catheter based intervention. In such embodiments, the inotropic agent can be delivered either from inside the heart chamber or from outside the heart.

[0018] One preferred embodiment provides transient localized delivery of inotropic agents to support the heart of a patient undergoing surgery. In one embodiment, the patient requires support from a cardiopulmonary bypass (CPB) circuit. In another embodiment, the patient does not require support from a CPB circuit. In one particularly preferred embodiment, the patient is a cardiac patient.

[0019] The present invention provides the local delivery of any inotropic agent, including but not limited to sympathomimetics and phosphodiesterase inhibitors. Preferred sympathomimetics include epinephrine, norepinephrine, isoproterenol, dobutamine and dopamine, and analogues and derivatives thereof. Preferred phosphdiesterase inhibitors include milrinone and amrinone, and analogues and derivatives thereof.

[0020] Any delivery vehicle which can be loaded with an inotropic agent and directly applied to the heart can be used in the present invention. Delivery vehicles include drug-impregnated, coated or relasing sheets, patches, matrix, hydrogel, foam, gel, cream, spray, microshpere, microcapsule, composite and ointment. Certain preferred delivery vehicles are polymeric controlled release vehicles.

[0021] The delivery vehicle is loaded with the inotropic agent and locally applied to the heart using any route for application which allows its local application to the heart. In one embodiment, the delivery vehicle may be applied directly to the exposed heart during a surgical intervention, for example before the pericardium or sternum is closed. In another embodiment, the delivery vehicle may be applied through a less direct route, including but not limited to a percutaneous application or an endovascular injection.

[0022] Certain embodiments of the invention provide further localization of the delivery of the inotropic agent. In one embodiment, the delivery vehicle is placed away from the sinoatrial node or the right atrium. A preferred placement of the delivery vehicle is on the left ventricular free wall or apex of the ventricle.

[0023] One particularly preferred embodiment provides local delivery of dopamine to the ventricle without targeting the sinus node in the right atrium, limiting the excessive tachycardia observed in high dose intravenous infusion of this agent.

[0024] Another embodiment of the invention provides the use of a non-permeable barrier on the surfaces of the heart not treated with the delivery vehicle, to achieve additional localization. In another embodiment of the invention, non-permeable barriers can be used to direct drug toward the myocardium and prevent the loss of drug to ventricular blood flow or pericardial fluid.

[0025] Preferably, the delivery methods of the present invention are administered to the subject for a short time, i.e. just long enough to support the heart until it recovers from its weakened condition. Administration of the inotropic agent may last for a few hours to days, for example up to 14 days. The delivery methods of the present invention can be used to treat the heart prior to surgery, during surgery, after surgery, and any combination thereof.

DESCRIPTION OF THE FIGURES

[0026] Figure 1: Figure 1 shows contractility of the heart (max dp/dt (mmHg/s)) over time in rats administered dobutamine, a non selective beta agonist inotropic agent, which was delivered directly to the left ventricular wall. Contractility was significantly increased shortly after dobutamine was applied to the surface of the heart.

[0027] Figure 2: Figure 2 shows left ventricular systolic blood pressure over time in rats administered Dobutamine, a nonselective beta agonist inotropic agent, which was delivered directly to the left ventricular wall. Local pericardial delivery of dobutamine increased systemic blood pressure. It is known that intravenous infusion of inotropic agents reduce systemic blood pressure.

[0028] Figure 3: Figure 3 shows heart rate over time in rats administered Dobutamine, a non selective beta agonist inotropic agent, which was delivered directly to the left ventricular wall.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention provides novel methods for the localized delivery of inotropic agents to the heart, including specific regions of the heart, in a subject in need of transient contractile support. One embodiment provides localized delivery of inotropic agents to support the heart during or following cardiac surgery, including as a subject transitions off of a cardiopulmonary bypass (CPB) circuit.

[0030] The present invention provides advantages over known methods to support the weakened heart, such as while a cardiac surgery patient is coupled to a CPB circuit, and as a patient transitions off CPB. To avoid the adverse side effects associated with systemic delivery of positive inotropic agents, we have discovered methods to take advantage of existing polymeric controlled release strategies to locally deliver inotropic agents directly to the heart. By locally delivering the inotropic agent directly to the heart, the systemic exposure of the inotropic agents is limited, avoiding the peripheral arterial dilation and systemic hypotension associated with systemic administration of some of these agents, and the tachycardia and vasoconstriction associated with others. In addition, because the methods of the invention deliver the positive inotropic agent directly to localized heart surface, lower amounts, but potentially high local concentrations, can be delivered.

[0031] The inventors of the present invention have surprisingly shown that inotropic agents, when applied directly to the heart rather than systemically, increase contractility of the heart and minimize systemic side effects such as the reduction in systemic blood pressure that is seen when certain inotropic agents, such as dobutamine, isoproterenol, milrinone, or amrinone are administered systemically. Thus, the inventors have shown that local delivery of inotropic agents mimimizes systemic side effects while improving contractile function of the heart.

[0032] In one embodiment, a method of locally delivering a cardiac inotropic agent to the heart of a subject is encompassed. This method comprises locally administering to a subject in need thereof a therapeutically effective amount of at least one inotropic agent.

[0033] In one embodiment, the inotropic compound is an agent that interacts with the sympathetic nervous system and modulates calcium entry, G-proteins, ATP, or GTP, wherein the inotropic agent is selected from the group consisting of sympathomimetic compounds, phosphodiesterase inhibitors, BNP, ANP, and digitalis glycosides, and derivatives and analogues thereof.

[0034] The inotropic agent may be a sympathomimetic compound selected from the group consisting of epinephrine, norepinephrine, dobutamine, isoproterenol, salbutamol, salmeterol, terbutaline, phenylephrine, ephedrine, clonidine and dopamine, and derivatives and analogues thereof.

[0035] Alternatively, the inotropic agent may be a phosphodiesterase inhibitor selected from the group consisting of milrinone and amrinone, and derivatives and analogues thereof.

[0036] The subject to be treated may be a surgical patient. Non-limiting examples of surgical patients are a cardiac surgery patient, a thoracic surgery patient, and a general surgery patient.

[0037] In one embodiment, the cardiac surgery patient is selected from the group consisting of a cardiac surgery patient requiring support from a cardiopulmonary bypass circuit and a cardiac patient not requiring support from a cardiopulmonary bypass circuit.

[0038] In another embodiment, the subject has a condition selected from the group consisting of trauma, shock, and congestive heart failure.

[0039] In one embodiment, the inotropic agent is locally delivered to the heart by administering the inotropic agent directly to the heart via an open surgical wound. Alternatively, the local delivery comprises administering said inotropic agent directly to the heart percutaneously.

[0040] A method of reducing postoperative complications of cardiopulmonary bypass (CPB) surgery in a subject is also encompassed in the present invention. This method comprises locally administering to a subject in need thereof an effective amount of an inotropic agent in conjunction with CPB surgery of said subject. The inotropic agent may be a sympathomimetic compound or a phosphodiesterase inhibitor.

[0041] The inotropic agent may be administered to said subject during a time period consisting of 1) prior to said CPB surgery; 2) during said CPB surgery; 3) subsequent to said CPB surgery; and 4) combinations thereof.

[0042] As used herein, a "therapeutically effective amount" of the inotropic agent is an amount that is sufficient to effect myocardial contractility.

[0043] The inotropic agent may be delivered locally to the heart by its inclusion in a delivery vehicle.

Subjects for Administration

[0044] The methods of the present invention can be used to treat any patient in need of transient contractile support to the heart, where such support can be provided by the local delivery of inotropic agents directly to the heart, including specific regions of the heart, such as the ventricles.

[0045] In one embodiment, the patient is in need of localized delivery of an inotropic agent to provide transient contractile support as a result of a surgical intervention. Surgical interventions include major surgeries, including but not limited to cardiac surgery, thoracic surgery, and general surgery. In another embodiment, the patient is in need of localized delivery of an inotropic agent to provide contractile support as a result of trauma, shock, or heart failure.

[0046] In another embodiment, the patient is in need of inotropic support following an intervention less invasive than a major surgical intervention, referred to herein as a minimally invasive intervention. Such minimally invasive interventions include but are not limited to a percutaneous intervention or a catheter based intervention. In such embodiments, the inotropic agent can be delivered either from inside the heart chamber or from outside the heart, as described in detail below.

[0047] One preferred embodiment provides localized delivery of inotropic agents to support a patient undergoing surgery. In one embodiment, the surgical procedure requires the use of a cardiopulmonary bypass (CPB) circuit. In another embodiment, the procedure does not require the use of a CPB circuit. In one particularly preferred embodiment, the patient is a cardiac patient.

[0048] In order to perform many surgical procedures it is necessary to interrupt coronary blood flow. Without cardioprotective strategies such as cooling and chemical arrest, the heart would soon die. Unfortunately, no cardioprotective strategy has been shown to be optimal and some degree of post CPB contractile dysfunction is inevitable. This is not only a problem in the adult patient undergoing coronary artery bypass surgery (CABG) or other surgical procedures, it is also a significant clinical problem during surgical heart procedures to correct congenital heart defects in neonates.

[0049] Thus, local administration of the agent can begin at any time once surgery begins until twenty-four hours after surgery has ended. More typically, within 12 hours of surgery ending. Any range within these ranges can be used, such as 1, 2, 3, 4, or more hours after surgery has ended.

[0050] In certain embodiments, administration of the agent can begin before surgery, for example using a percutaneous approach for delivery of the agent.

[0051] Accordingly, the methods of the present invention can be used to treat any subject while coupled to a CPB circuit, i.e. during cardiac surgery, and/or following cardiac surgery, during their transition off of the CPB circuit. Cardiac surgery includes any surgical procedure on the heart and usually involves interruption of coronary blood flow. It can also be used to assist the heart function during and after any thoracic surgical procedure where the heart is already exposed to the surgeon.

[0052] Before turning the CPB circuit, also known as the pump, off, all clinical determinants of cardiac performance are evaluated and adjusted, in order to optimize cardiac output. All metabolic, thermal, electrolyte, acid/base, and hematologic abnormalities are corrected. Blood volume is adjusted according to central venous, left atrial or pulmonary artery pressures. Peripheral resistance is estimated and vasodilators or constrictors are instituted as required. After the drug's effectiveness is assessed, pump flow is decreased in small increments while venous return to the heart is proportionately adjusted to maintain a constant filling pressure by constricting the venous drainage to the CPB circuit.

[0053] The assessment of cardiac function by transesophageal echocardiography and hemodynamic data immediately before terminating CPB allows patients to be classified into 3 groups by decreasing risk, referred to herein as groups A, B, and C [Souza et al., Indian Journal of Extracorporeal Technology 6:2, 1998]. The methods of the present invention can be used to treat any patient in group A, B, or C, including children in need of inotropic support during cardiac surgery or during weaning from CPB.

[0054] The highest risk patients, classified herein as "Group A" patients, have severe cardiac dysfunction that makes it difficult to be removed from CPB, despite physiologic and pharmacological support. For these patients CPB is prolonged. Group A patients are by definition the hardest cases to manage. A few of these patients by the end of rewarming of the blood will have minimal or no cardiac activity, which precludes any trial of disconnection from pump. The remaining patients may be given a short trial off pump after optimization of preload, afterload and contractility by a combination of inotropes and vasoactive agents. Some of these patients will tolerate CPB removal, under maximal physiological and pharmacological support, and a few in the group may be further improved by an intra-aortic balloon pump. The patients with minimal cardiac activity and those in whom the trial off pump was unsuccessful are temporarily maintained on cardiac support with the heart-lung machine. A few hours on pump support may be a sufficient rest period to allow recovery of cardiac function and removal of CPB support in a small number of cases. For the others, a decision has to be made as to either advance to a mechanical device for prolonged support or terminate the efforts to recover cardiac action.

[0055] Children in Group A supported by full veno-arterial extracorporeal membrane oxygenation (ECMO) post cardiotomy have a poor long term survival rate [Langley et al., Eur J Cardiothorac Surg 13, 520-5, 1998] when compared with children managed with centrifugal ventricular assist devices [Thuys et al., Eur J Cardiothorac Surg 13, 130-4, 1998]. The methods of the present invention may be utilized in the treatment of children in Group A.

[0056] In certain cases, a few hours of circulatory assistance and intensive inotropic and vasodilator drug therapy may turn some Group A patients into group B. The remaining patients are candidates to a form of total circulatory mechanical support (if available) or they will not likely survive disconnection from pump [Harris C. et al., Tecnol. Extracorp. Rev. Latinoamer. 3, 13-19, 1996; El-Banayosy A., et al., Perfusion, 11, 93-102, 1996; Núñez HI., Tecnol. Extracorp. Rev. Latinoamer. 2, 33-41, 1995].

[0057] Group B patients have a mild to moderate degree of cardiac dysfunction, and require greater support and a more elaborate protocol for CPB termination than patients in Group C. Final preparations are made on partial bypass. In addition to the delivery of inotropic agents using the present invention, these patients may also be supported by physiological means such as volume resuscitation or additional pharmacological means, namely vasodilators. Some patients in this group can benefit from intra-aortic balloon pumping. Patients in this group will benefit from the methods of the present invention.

[0058] Some Group B patients may have to return to pump for better adjustment of drugs, or to have an intra-aortic balloon inserted if a marginal cardiac output is present, as demonstrated by atrial and arterial pressures, arterial and venous blood gases and pH, and spontaneous diuresis.

[0059] Group B patients include children with preoperative intracardiac shunts leading to high pulmonary blood flow, children after a heart transplant, and some adults with long standing congestive heart failure, who may present with pulmonary hypertension that precludes successful weaning. In certain instances, inhalation of nitric oxide (NO) can improve pulmonary hypertension and cardiac output and support discontinuance of CPB. Additional Group B patients include patients who received inadequate myocardial protection for any reason, including inadequate re-dosing of cardioplegia, inadequate perfusion of myocardia with cardioplegia, patients with severe ventricular hypertrophy or aortic insufficiency, surgical errors, and prolonged CPB time.

[0060] An occasional patient in group B will not tolerate CPB termination even after a few trials. These few exceptions turn into group A patients.

[0061] For lower risk "Group C" patients, inotropic support of the present invention can be provided at a lower level, and may be discontinued as the patient arrives at the intensive care area or a few hours thereafter. The methods of the present invention can be used as needed to treat Group C patients, who are anticipated to smoothly disconnect from perfusion. For these patients, after reestablishing ventilation to the lungs, pump flow can be gradually reduced while venous return to the oxygenator is decreased until bypass is minimal. Arterial pump is stopped and venous line is clamped. Final adjustment of cardiac performance is made off pump, by slowly administering residual volume from the oxygenator until ideal preload is attained. These patients maintain an adequate cardiac output, as can be confirmed by normal atrial and arterial pressures, arterial and venous blood gases and pH and adequate spontaneous diuresis.

[0062] In one particularly preferred embodiment of the invention, the methods can be used to treat any subject undergoing non cardiac thoracic surgery where the heart is exposed, to assist the heart function and/or to treat contractile dysfunction.

[0063] In some embodiments, the inotropic agent of the present invention can be co-administered with prostaglandin E1, which can act as a powerful adjunct to wean difficult transplanted children with right ventricular failure.

[0064] In some embodiments, the inotropic agent of the present invention can be co-administered with nitroprusside or other vasodilator drugs.

[0065] In some embodiments, one particularly preferred inotrope is enoximone, to provide pharmacological support during weaning of patients with severe ventricular dysfunction.

[0066] The term "subject" as used herein refers to vertebrates, particularly members of the mammalian species and includes but is not limited to, domestic animals, sports animals, primates, dogs, cats, rodents including mouse and rat, horse and humans; more preferably, the term refers to humans.

Inotropic Agents

[0067] As used herein, "inotropic agents" or "positive inotropic agents" or "inotropes" or "positive inotropes" or "inotropic antibodies" will be used interchangeably and refers to the effect such agents produce, i.e. improves cardiac output by increasing the force of myocardial muscle contraction. "Positive inotropic effect" means that the contractility of the cells is enhanced in a dose-dependent manner. A positive inotropic effect-producing amount of an inotropic agent of the invention can be administered to a subject.

[0068] Positive inotropic agents of the present invention include any agents which provide the heart with contractile support. The agent can be an inotropic agent such as a sympathomimetic or a phosphodiesterase inhibitor, as long as one obtains the desired contractile effect on the heart. Inotropic compounds include agents that interact with the sympathetic nervous system and modulate calcium entry, G-proteins, ATP and GTP. Inotropic compounds include sympathomimetic compounds, phosphodiesterase inhibitors, BNP, ANP, and digitalis glycosides. Preferably, the agent is a sympathomimetic or a phosphodiesterase inhibitor. Preferred sympathomimetics include but are not limited to epinephrine, norepinephrine, dopamine, dobutamine, dopexamine, terbutaline, and isoproterenol, and analogues and derivatives thereof. Preferred phosphdiesterase inhibitors include but are not limited to milrinone, amrinone, enoximone, and pimobendan, and analogues and derivatives thereof.

[0069] Preferably, the positive inotropic agent is administered in the form of a pharmaceutical composition. A pharmaceutical composition comprising an effective amount of the positive inotropic agent as an active ingredient can be prepared by standard procedures well known in the art, with pharmaceutically acceptable non-toxic solvents and/or sterile carriers, if necessary. For example, the inotropic agent can be embedded in a controlled-release polymer. In other embodiments the positive inotropic agent is administered without a pharmaceutical carrier.

[0070] The dose of the positive inotropic agent is a therapeutically effective dose. In particular embodiments, the positive inotropic agent can be administered at a dose which produces in the subject an effect equivalent to the systemic intravenous administration of between 2 and 20 mcg/kg/min. However, in other embodiments, higher and lower dosages can be administered to subjects. For example, a dose which produces in the subject an effect equivalent to the systemic intravenous administration of 0.5 mcg/kg/min, or 40 mcg/kg/min. Optimizing therapy to be effective across a broad population can be performed with a careful understanding of various factors to determine the appropriate therapeutic dose. Typically, the dose can be much lower than the dose administered by intravenous infusion, because the agent is being locally delivered to the heart, rather than systemic administration.

Localization of the Delivery Vehicle on the Heart

[0071] Routes for direct application of the delivery vehicle to the heart include any routes which allow the delivery vehicle to be applied locally to the heart. For example, the delivery vehicle may be applied from the blood stream, by being placed directly in the heart through the coronary arteries or veins onto the heart surface; or through the ventricular or atrial walls and onto the heart surface. The delivery vehicle may also be applied through direct application during extensive surgical field exposure, or through direct application during minimally invasive exposure, for example through a pericardial window or heart port. The delivery vehicle may also be applied through a percutaneous route, or via endovascular catheters.

[0072] In one embodiment, the delivery vehicle is loaded with the inotropic agent and placed over the heart of a surgical patient, before the sternum is closed, allowing direct release of the inotropic agent to the heart.

[0073] Placement of the delivery vehicle can be understood with reference to the different compartments of the heart. The heart is subdivided by a muscular septum into two lateral halves, which are named respectively right and left. A transverse constriction subdivides each half of the heart into two cavities, or chambers. The upper chambers consist of the left and right atria, which collect blood and help fill the lower chambers. The lower chambers consist of the left and right ventricles, which pump blood to the rest of the body. The chambers are defined by the epicardial wall of the heart. The right atrium communicates with the right ventricle by the tricuspid valve. The left atrium communicates with the left ventricle by the mitral valve. The right ventricle empties into the pulmonary artery by way of the pulmonary valve. The left ventricle empties into the aorta by way of the aortic valve.

[0074] The circulation of the heart consists of two components. First is the functional circulation of the heart, i.e., the blood flow through the heart from which blood is pumped to the lungs and the body in general. Second is the coronary circulation, i.e., the blood supply to the structures and muscles of the heart itself. The functional circulation of the heart pumps blood to the body in general, i.e., the systemic circulation, and to the lungs for oxygenation, i.e., the pulmonic and pulmonary circulation. The left side of the heart supplies the systemic circulation. The right side of the heart supplies the lungs with blood for oxygenation. Deoxygenated blood from the systematic circulation is returned to the heart and is supplied to the right atrium by the superior and inferior venae cavae. The heart pumps the deoxygenated blood into the lungs for oxygenation by way of the main pulmonary artery. The main pulmonary artery separates into the right and left pulmonary arteries, which circulate to the right and left lungs, respectively. Oxygenated blood returns to the heart at the left atrium via four pulmonary veins. The blood then flows to the left ventricle where it is pumped into the aorta, which supplies the body with oxygenated blood.

[0075] The functional circulation supplies blood to the heart by the coronary circulation. The coronary arteries arise from the proximal aorta through the left and right coronary ostia course along the epicardial surface of the heart and send of numerous branches to supply the myocardium. Blood is cleared from the muscle by cardiac veins that flow into the coronary sinus and right atria. The heart wall is surrounded by a pericardial sac, which contains it within interstitial fluid.

[0076] In one embodiment, the delivery vehicle loaded with the inotropic agent is placed over the heart, before the sternum is closed, allowing direct release of the inotropic agent to the heart. In one embodiment, the delivery vehicle is placed away from the sinoatrial node or the right atrium. A preferred placement of the delivery vehicle is on the apex of the ventricle or left ventricular free wall.

[0077] Another embodiment of the invention provides the use of a non-permeable barrier on the surfaces of the heart not treated with the delivery vehicle, to achieve additional localization. In another embodiment, the delivery vehicle itself can be coated with a non-permeable barrier, to further localize release of the agent directly to the underlying heart tissue, while minimizing release into the pericardial fluid.

[0078] One particularly preferred embodiment provides local delivery of dopamine, epinephrine, norepinephrine, isoproterenol, and dobutamine to the ventricle without targeting the sinus node in the right atrium, limiting the excessive tachycardia observed in intravenous infusion.

[0079] In one embodiment, the delivery vehicle contains an inotropic agent that must be activated or released by a second agent. That second agent can be added systemically to locally activate or release the inotropic agent. In this way, timing and/or release can be controlled at later points.

Treatment Period

[0080] Preferably, the delivery methods of the present invention are administered to the subject just long enough to support the heart until it recovers from its weakened condition. The short term or transient administration of the inotropic agent may last for a period of several minutes to several days. For example, from five minutes to 14 days. Typically, at least two hours to seven days. Preferably five hours to five days. More preferably, 2-24 hours. One can use all ranges between 5 minutes to 14 days, e.g. 12 hours to 12, 11, 10, 9, 8, 7, or fewer days.

[0081] In one embodiment of the invention, the patient is a surgical patient and the delivery methods of the present invention can be used to treat the heart prior to surgery, during surgery, after surgery, and any combination thereof.

Delivery Vehicle

[0082] The delivery vehicle of the present invention is any drug delivery means that can incorporate an inotropic agent, and is suitable for administration directly to the heart for local delivery or release of that agent. Suitability for local delivery to the heart includes the ability of a delivery vehicle to adhere to the underlying tissue. Any delivery vehicle which can be loaded with an inotropic agent and locally applied to the heart can be used in the present invention.

[0083] Examples of delivery vehicles include but are not limited to a patch, a matrix, a hydrogel, a sheet of material, a foam, a gel, a cream, a spray, and an ointment. Certain preferred delivery vehicles are polymeric controlled release vehicles. In one embodiment, the delivery vehicle is a patch, such as a transepicardial patch, that slowly releases the agents directly into the myocardium. In one embodiment, the delivery vehicle is an ointment or cream which may be placed manually on the target area of the heart. In one preferred embodiment, the delivery vehicle is a hydrogel, which may be polymerized either directly on the heart in vivo or polymerized in vitro to form a patch for administration.

[0084] In one preferred embodiment, the inotropic agent(s) of the invention are incorporated into a biocompatible delivery vehicle referred to as a matrix. The matrix can be in the form of a gel, foam, suspension, microcapsules, solid polymeric support, or fibrous structure. The matrix may also serve in a physically supporting role. There is no specific requirement as to thickness, size or shape. It is preferred that the matrix be sufficiently porous to allow the inotropic agent to diffuse out of the matrix into the surrounding tissue in roughly physiologic quantities.

[0085] Preferably, the matrix is a biodegradable material. Preferably, the hydrogel matrix degrades in a period of time minimizing tissue inflammation, for example in less than seven to ten days. Examples of a biodegradable matrices include but are not limited to synthetic polymers degrading by hydrolysis, for example, polyhydroxy acids like polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, proteins such as gelatin and collagen, or carbohydrates or polysaccharides such as cellulose and derivatized celluloses, chitosan, alginate, or combinations thereof, so that over the course of several days or weeks after implantation of the matrix material, the matrix gradually disappears.

[0086] The use of biodegradable matrices eliminates the need for surgery to remove undegraded implanted matrix. However, synthetic non-biodegradable matrices may also be used. Useful materials include but are not limited to ethylene vinyl acetate, polyvinyl alcohol, silicone, polyurethane, non-biodegradable polyesters, and polyethyleneoxide-polypropyleneoxide, and tetrafluoroethylene meshes (Teflon®).

[0087] In a preferred embodiment, the matrix is a hydrogel, defined as a matrix wherein typically approximately 900-fold by weight of the matrix is absorbed water. Hydrogels are well known in the art. Hydrogels can be formed by ionic or covalent crosslinking of a variety of water soluble polymers such as polyphosphazenes, polysaccharides such as alginate, and proteins such as gelatin. For example, one matrix material is purified gelatin-based Gelfoam™ (The Upjohn Co., Kalamazoo, Mich.) surgical sponge.

[0088] To achieve the above properties, the hydrogel is formed primarily of polymerized macromers, the macromers being themselves polymers or copolymers of one or more monomers having reactive groups providing resorbable linkages and polymerizable sites for biodegradability and polymerization. The macromers have sufficient hydrophilic character to form water-absorbent polymerized gel structures, and are at least dispersible in a substantially aqueous solution, and preferably are water-soluble, to maximize tissue adherence. The macromers are preferably made predominantly of synthetic materials. The resulting hydrogels are preferably highly compliant, so as not to impede the process of cardiac contraction. The hydrogels are preferably covalently crosslinked to ensure that they are retained at the site of application until the hydrogels degrade. In certain embodiments, the gel can be crosslinked in situ, for example by photopolymerization.

[0089] Monomers and macromers which are suitable for forming the hydrogels ("referred to here in this section collectively as "monomers") have one or more of the following properties: water soluble, partially macromeric character, containing hydrophilic groups, and being covalently reactive. When crosslinked to form gels, the resulting gels are tissue adhesive, elastic, and compliant. The monomers are preferably water soluble. Water soluble materials are soluble to at least about 0.1 gram per liter of a substantially aqueous solvent. A substantially aqueous solvent comprises at least about 50% by weight of water, and less than about 50% by weight of a non-aqueous, water-miscible solvent. If the polymers are not entirely water-soluble, they should be dispersible in water, and form micelles, typically with the aid of non-aqueous, water-miscible solvents. The non-aqueous solvent must be present in an amount that does not damage the tissue. Thus only a small amount of non-aqueous, water-miscible solvent should be present in the pre-gelled composition to minimize tissue irritation. Up to about 10% by weight of the solution can be a non-aqueous, water-miscible solvent. Examples of non-aqueous, water-miscible solvents include ethanol, isopropanol, N-methylpyrrolidone, propylene glycol, glycerol, low molecular weight polyethylene glycol, DMSO, Benzyl alcohol, and benzyl benzoate. Liquid surfactants, such as poloxamers (e.g., PLURONIC™ surfactants) and some polyethylene glycol derivatives (e.g., some TWEEN™ surfactants) can also be used as non-aqueous, water-miscible solvents.

[0090] The monomers are preferably at least partially macromeric, and are more preferably substantially to completely macromeric. Macromers tend to be innocuous to tissue because they will not readily diffuse into or penetrate cells. A macromer is a reactive monomer consisting of a polymeric material with a number-average or weight-average molecular weight of about 500 Daltons or more and at least one reactive group. To form a crosslinked gel by chain-growth polymerization, the macromers, along with any other smaller monomers, in a solution must contain on average more than one reactive group (which may be a covalently reactive group, or a group that binds non-covalently to other macromers). For polymerizations involving step-growth polymerization, the macromers must contain on average more than two reactive groups, and the solution typically contain approximately equal numbers of the two different types of reactive groups. An example of step-growth polymerization is gelation by formation of urethane linkages from the reaction of isocyanate with the hydroxyl groups. For free-radical polymerization of unsaturated materials (chain-growth polymerization), the monomers must contain on average more than one reactive group to crosslink.

[0091] The monomers are preferably covalently reactive, and thus form a covalently crosslinked gel. The crosslinked gels are elastic, and further are both elastic and compliant with soft tissue at low polymer concentrations.

[0092] Any method of covalent polymerization is potentially useful in the formation of the gels. The reactive groups may include, without limitation, ethylenically unsaturated groups, isocyanates, hydroxyls and other urethane-forming groups, epoxides or oxiranes, sulfhydryls, succinimides, maleimides, amines, thiols, carboxylic acids and activated carboxylgroups, sulfonic acids and phosphate groups. Ethylenically unsaturated groups include acrylates and other unsaturated carboxylic acids, vinylic and allylic groups, cinnamates, and styrenes. Activated carboxyl groups include anhydrides, carbonylimidazoles, succinimides, carbonyl nitrophenols, thioesters, O-acyl ureas, and other conjugated carbonyls. In general, any reactive group that will covalently bond to a second and that can maintain fluidity when exposed to water for enough time to allow deposition and reaction is of use in making a suitable reactive macromer. Due to their excellent stability and slow reactivity in aqueous solutions, ethylenically unsaturated reactive groups are preferred.

[0093] The polymerization reaction does not have to result in covalent bonds. A number of materials are known which can form gel structures by changing the ionic conditions of the medium (e.g. alginate) or by changing the temperature of the medium (e.g., agarose, certain poloxamers). Polysaccharides are typical of these materials. Gel-like structures can be formed from proteins, such as gelatin or fibrin. While it maybe more difficult to get these materials to adhere strongly to tissue, they are potentially of use in the hydrogels, particularly as depots for the drug.

[0094] Gel formation can be accelerated by inclusion of small (non-macromeric) polymerizable molecules that can assist in linking larger, polymeric macromers. These typically have molecular weights less than about 100 Da, more preferably less than 500 Da. For free radical polymerization, any of the common ethylenically unsaturated molecules can be used. These include derivatives of acrylic and methacrylic acid, such as acrylamide, hydroxyethyl methacrylate (HEMA), and diacrylated or polyacrylated glycols and oligoglycols. Allyl groups (e.g., allyl glycidyl ether) and vinyl groups (e.g., N-vinyl caprolactam and N-vinyl pyrrolidone) are also of use. Other unsaturated compounds include cinnamic acid and its esters, and maleic, fumaric and itaconic acids and their derivatives.

[0095] Polymerization is initiated by any convenient reaction, including photopolymerization, chemical or thermal free-radical polymerization, redox reactions, cationic polymerization, and chemical reaction of active groups (such as isocyanates, for example.) Polymerization is preferably initiated using photoinitiators. Photoinitiators that generate a free radical or a cation on exposure to UV light are well known to those of skill in the art. Free-radicals can also be formed in a relatively mild manner from photon absorption of certain dyes and chemical compounds. The polymerizable groups are preferably polymerizable by free radical polymerization. The preferred polymerizable groups are acrylates, diacrylates, oligoacrylates, methacrylates, dimethacrylates, oligomethacrylates, cinnamates, dicinnamates, oligocinnamates, and other biologically acceptable photopolymerizable groups.

[0096] These groups can be polymerized using photoinitiators that generate free radicals upon exposure to light, including UV (ultraviolet) and IR (infrared) light, preferably long-wavelength ultraviolet light (LWUV) or visible light. LWUV and visible light are preferred because they cause less damage to tissue and other biological materials than short-wave UV light. Useful photoinitiators are those which can be used to initiate polymerization of the macromers without cytotoxicity and within a short time frame, minutes at most and most preferably seconds. Exposure of dyes, preferably in combination with co-catalysts such as amine, to light, preferably visible or LWUV light, can generate free radicals. Light absorption by the dye causes the dye to assume a triplet state, and the triplet state subsequently reacts with the amine to form a free radical which initiates polymerization, either directly or via a suitable electron transfer reagent or co-catalyst, such as an amine. Polymerization can be initiated by irradiation with light at a wavelength of between about 200-1200 nm, most preferably in the long wavelength ultraviolet range or visible range, 320 nm or higher, and most preferably between about 365 and 550 nm. Numerous dyes can be used for photopolymerization. Suitable dyes are well known to those of skill in the art. Alternatively, suitable chemical, thermal and redox systems may initiate the polymerization of unsaturated groups by generation of free radicals in the initiator molecules, followed by transfer of these free radicals to the unsaturated groups to initiate a chain reaction. Examples include but are not limited to peroxides, other peroxygen compounds, and azobisbutyronitrile.

[0097] As used herein, a "biodegradable" material is one that decomposes under normal in vivo physiological conditions into components that can be metabolized or excreted. Functional groups having degradable or resorbable linkages are incorporated into the structure of the hydrogel matrix to provide for its resorption over time. These functional groups may be incorporated within the macromers to form part of the backbone of the polymer strands of the hydrogel or as crosslinks between the polymer strands. Examples of degradable units may include, but are not limited to, esters, carbonates, carbamates and the like. The length of time it takes for the hydrogel to biodegrade may be tailored to provide a hydrogel that persists long enough to generate the required tissue level of the drug through the treatment period, which can last up to the seventh postoperative day, or preferably up to the tenth or fourteenth day. Given the achievement of this objective, shorter degradation or resorption times such as less than about three months are generally preferred. Degradation or resorption times less than about fifteen days are particularly preferred.

[0098] As used herein, a "biocompatible" material is one that stimulates only a mild, often transient, implantation response, as opposed to a severe or escalating response. Biocompatibility may be determined by histological examination of the implant site at various times after implantation. One sign of poor biocompatibility can be a severe, chronic, unresolved phagocytic response at the site. Another sign of poor biocompatibility can be necrosis or regression of tissue at the site. In the preferred embodiment, a biocompatible material elicits a minimal or no fibrosis or inflammation. This can be achieved through selection of hydrogel composition, and particularly through the use of hydrogel components resulting in degradation of the hydrogel in vivo in less than about two weeks, more preferably within seven to ten days.

[0099] In a preferred embodiment, the hydrogel composition is selected to provide acceptable levels of fibrosis or tissue reaction. This can be achieved through the selection of the reactive formulation, and other techniques known to those skilled in the art in drug delivery utilizing polymeric delivery devices.

[00100] Preferably, the inotropic agents are poorly soluble in water (i.e. hydrophobic). In terms of the solubility classification of the United States Pharmacopoeia (USP 24/NF 19, effective Jan. 1, 2000; p. 2254), the preferred solubility classes are: "slightly soluble", requiring 100 to 1000 parts of solvent to dissolve; "very slightly soluble", requiring 1000 to 10,000 parts of solvent; and "practically insoluble, or insoluble", requiring over 10,000 parts of solvent. Collectively, these classes are defined herein as "poorly soluble".

[00101] An inotropic agent applied in a single application directly to the heart is expected to be similarly or more effective to intravenous administration, with a potential reduction in side effects because a lower required dose and limited spread is anticipated.

[00102] The slow dissolution rate for poorly soluble inotropic agents controls their rate of efflux from the gel. The rate of efflux for such inotropic agents can also be controlled by selecting the particle size of the drug particles that are suspended in the macromer solution before its polymerization. Particles of a particular size can be made by any known method, including grinding, milling, cryofracture, precipitation, spraying, spray drying, and/or classification. Dispersion and stabilization of the particles within the macromer solution may be achieved with the use of surfactants.

[00103] When more soluble inotropic agents are used, their efflux rate from the gel can be altered to achieve the necessary delivery rate. Such soluble inotropic agents include those falling in United States Pharmacopoeia classes "very soluble", "freely soluble", "soluble", and "sparingly soluble". Typical means of altering release rates include encapsulating the agents in micro particles or liposomes and conjugating

the agents to macromolecules. They can be made less soluble by altering the salt or using the free acid/base form of the agents.

[00104] In one embodiment, pre-encapsulation is used for the small, water-soluble drugs (typically of molecular weights less than 1000 Da) that are incorporated into hydrogels, to decrease the rate of release of these drugs. The encapsulation may be by any conventional means. One means is entrapment in micro particles of a degradable, water-insoluble polymer. Typical materials are polymers and copolymers of lactic acid, glycolic acid, and copolymers thereof (e.g., PLGA). Other materials used to form suitable micro particles are copolymers of ethylene and vinyl acetate (EVAC) and polymers of anhydrides, such as poly sebacic anhydride. Particles of drug may also be pre-encapsulated with polymers such as EVAC and PLGA, or with thin layers of materials that dissolve in vivo, for example, the enteric coatings or other coatings typically used for oral delivery, such as gelatin.

[00105] Release of more soluble inotropic agents can be slowed by conjugating small molecules to polymers by degradable or reversible linkages. Many such systems are described in the art. In one embodiment, such systems are generated by immobilizing a binding or targeting molecule for the drug, such as an antibody or lectin, which is saturated with the drug, in the gel. In another typical embodiment, drug is attached to a polymer bearing reactive groups, such as to the hydroxyl of polyvinyl alcohol, to a carboxyl, sulfonate or amine group of a polysaccharide or the hydroxyl or carboxyl of an alpha-hydroxy acid (e.g., lactic or glycolic acid), or to a carboxylic group on a polymer (e.g., alginate, polyacrylic acid) via an anhydride, an ester, a carbonate, or carbamate linkage. Many similar methods are described in the art.

[00106] The solubility of some agents can be decreased by preparing them in their neutral ("free base") form. Such agents often can also be administered as suspensions in oil, which in turn is dispersed in water, usually with surfactant stabilizers.

[00107] The level of loading of the inotropic agent in the delivery vehicle will normally be as high as practical, while leaving a margin of loading to prevent premature precipitation or aggregation, or inhibition of gel formation. The concentration of the inotropic agent can be between 0.5 and 1% by weight, but this will depend in part upon the source and form of the inotropic agent. Gel polymerization rate and final gel may be significantly affected by drug concentration. Use of other macromers affects the optimal level. Fortunately, acceptable loading ranges are easily determined for a particular system by varying the loading and determining the properties of the formed gel.

[00108] In one method, the inotropic agent is provided in a formulation that forms a hydrogel in vivo, i.e. after its components are administered to the heart.

[00109] In a second method, the inotropic agent is provided to the patient in a preformed hydrogel "patch", i.e. formed before administration to the heart.

[00110] The hydrogels of the present invention are formed by a polymerization reaction, which may be any reaction that can be carried out in a substantially aqueous environment and is not damaging to tissue. The gels may be polymerized in vivo or in vitro.

[00111] The adherence of gels to tissue can be optimized by techniques that employ functional primers, as described in U.S. Pat. No. 5,800,373 to Melanson et al., U.S. Pat. Nos. 5,844,016, or 5,900,245 to Sawhney et al. for gels formed by polymerization of ethylenically unsaturated precursors. Suitable gel compositions form strong bonds to tissue. These techniques are also applicable to creating strong adherence of the materials to tissue, including tissue to which it is difficult to obtain adherence by conventional methods, for example, cartilage.

[00112] A general procedure for applying materials to the tissue involves brushing or dabbing primer over a larger area than that over which the material is applied. Thereafter, material is brushed or dabbed over the deposited primer. Then

bulk material is applied by dripping (if liquid) or spreading (if paste) over yet a smaller area of the treated zone. Then light (at appropriate wavelength, intensity, distance and for an appropriate time) is applied at each zone, or other means of polymerizing the material are used.

[00113] Methods for in vivo and in vitro hydrogel polymerization are known in the art, for example as described in published patent applications 20020150622 and 20050004428, which are hereby incorporated by reference.

[00114] For in vivo polymerization, the inotropic agent is formulated in appropriate excipients (if any) in a vial, and is taken up in a known amount of hydrogel forming material. This solution is applied to the tissue, and polymerization is effected to form a gel adherent to the tissue. Preferably, the solution is polymerized by illumination of a photoinitiator or photosensitizer in the solution. In this case, the mixing of two solutions at the time of application will not necessarily form a gel; however once the solutions are illuminated by light of an appropriate frequency, a gel will form, as described in U.S. Pat. No. 5,410,016 to Hubbell et al. incorporated herein by reference in its entirety.

[00115] In vivo polymerization has the advantage of being able to produce "good" to "excellent" adherence when polymerized on the tissue surface. This is particularly true when the tissue is first primed or otherwise pretreated with an agent (primer) stimulating polymerization (as known to those skilled in the art, for example, as described in U.S. Pat. No. 5,844,016 to Sawhney et al. and U.S. Pat. No. 5,834,274 to Hubbell et al. incorporated herein by reference in their entirety) prior to the application of the macromer composition containing the inotropic drug. See also U.S. Pat. Nos. 5,567,435; 5,844,016; 5,986,043; 6,060,582; and 6,306,922 incorporated herein by reference in their entirety. In these methods, an aqueous solution containing a photoinitiation system, including one or more photoinitiators, photosensitizers and co-initiators, amine or amide electron transfer agent, redox accelerant system for the photoinitiation system (such as a metal ion and a peroxide); and a photopolymerizable

macromer solution, are applied to the tissue, and the solution is polymerized by exposure to UV or visible light at room or body temperature.

[00116] For in vitro polymerization, hydrogel patches containing the inotropic agent are polymerized in vitro and then adhered to the surface of the heart. The inotropic agent in any suitable formulation can be entrapped in a hydrogel in vitro, which is optionally preserved by freezing or drying, and is subsequently transferred to the cardiac tissue. The preformed gel patch, or more than one preformed gel patch, is then adhered to the cardiac tissue. Adhesion of the patch may be achieved by the polymerization of a hydrogel-forming material, which may be the same as or different from the material used to form the gel patch, placed between the preformed gel patch and the tissue, or optionally encapsulates the entire pre-formed gel. Adhesion may also be achieved by completing polymerization of a partially polymerized gel patch onto the tissue. A partially polymerized gel patch is prepared by reducing time exposure to polymerization conditions or by quenching polymerization.

[00117] In vitro polymerization has the advantage of providing a reliable means of delivering a precisely defined dose of the inotropic agent. The preformed gels should have the same properties as gels formed in vivo. This method of application may be regarded as another form of application of an encapsulated drug to the tissue, since the adhesion to the tissue is provided by a hydrogel that is formed in situ on the tissue. The preferred method of attaching the gels to the tissue surface is to use macromer solutions to adhere the preformed gel to the tissue. Adherence is also preferably in the "good" to "excellent" range.

[00118] A material is tissue adherent if it requires a force to remove the material from the tissue. Thus, the general and practically useful measurement of adherence is that the gel, when applied to the tissue, remains attached to the tissue for at least as long as is required to obtain the therapeutic effect of the drug. Typically, this time period will be sufficiently long to observe at least about 10% elution of the

drug, and preferably 20% elution or more, before detachment or degradation of the gel.

[00119] Ex vivo tests can be used to determine a material's potential adherence. In evaluating potential adherence of materials, it is useful to have an in vitro test to determine formulations that are likely to have the desired degree of adherence to the tissue surface. One method of judging adherence is to require that upon a gradual increase in a detaching force, the force required to remove the gel from the tissue is greater than or approximately equal to the force required to cause cohesive failure of the gel (or the tissue, if lesser). Thus on attempting to remove the material, either the material or the tissue experiences cohesive failure at a lesser force than, or at approximately the same force as, the force at which the bond between the material and the tissue experiences adhesive failure. Materials that require a force of about 20 dynes/cm^2 to remove them from the tissue are sufficiently adhesive for delivery of inotropic agents.

[00120] Adherence can be described qualitatively as "excellent", when cohesive failure is required for removal from the surface, "good" when failure is partially cohesive and partially adhesive, "fair" when removal requires only adhesive failure (i.e., detachment of the gel from the surface) and more than 20 dynes/cm^2 of force is required to produce adhesive failure, and "poor" if none of these criteria are satisfied. Force can be measured using a mechanical properties tester, such as an Instron™ tester or other device.

[00121] The delivery vehicles of the invention are preferably highly compliant with the tissue to which they adhere. Thus, the delivery vehicles stretch and bend along with the tissue. Cardiac tissue is in continual motion, and the delivery vehicle should not significantly disturb this motion. It is preferable that the response to stress within these limits be substantially elastic, i.e., reversible. Thus the delivery vehicle should remain as a coherent material for at least the period required for delivery of the inotropic agent.

[00122] Techniques for producing strong adherence of a preformed hydrogel, a patch, or other delivery vehicle to the cardiac tissue include applying an initiator or promoter of polymerization to the tissue at the site; applying a thin layer of gelling solution having a high concentration of a polymerizable reagent at the site; applying materials bearing one half of a reactive pair to the site, optionally a member of a reactive pair which is also reactive with tissue; and applying mechanical action to a layer of polymerizable material on the tissue (before polymerization) to ensure that no layer of fluid, such as mucus or the like, separates the polymerizable material from the tissue.

[00123] As described herein, the delivery vehicles of the invention, including hydrogels, patches, ointments and creams, can be applied at the time of surgery and the drug delivered directly to the affected cardiac tissue. For a hydrogel polymerized in situ, the gel can be applied in open surgery by any method. In one embodiment, the delivery vehicle such as an ointment, cream, or gel is preferably brushed or sprayed onto the tissue surface for example by using a device designed for percutaneous use, but may be dripped from a mixing apparatus.

[00124] The therapeutic compositions of this invention are administered by local administration to the heart, as by application of a patch, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

[00125] It is important to provide a way for the physician to deliver a well-defined amount of the inotropic agent, so that the therapeutic effect can be obtained.

[00126] The dosage of the inotropic agents for use in a human or animal and the minimum duration can be determined with only routine experimentation in view of animal studies and the known drug kinetics, including half-life, solubility and other

readily ascertainable properties. The effective dosage can be determined from tissue concentrations and physiological effects over time in cardiac tissue of animals, after application of a known concentration of the drug in the delivery vehicle. Such animal studies are routine in determining dosage for any drug. The dosage of the inotropic agent will also be optimized based on the period of time over which delivery is to be obtained and the release rate from the delivery vehicle, as well as the degradation characteristics of the delivery vehicle, to deliver a therapeutically effective dose to the heart tissue.

[00127] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's myocardium to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

[00128] Any formulation containing the active ingredients, which is suitable for the intended use, as are generally known to those of skill in the art, can be used. Suitable pharmaceutically acceptable carriers are known to those of skill in the art. The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[00129] As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal without the production of undesirable physiological effects.

[00130] In one embodiment, the inotropic agent may be administered in liposomes or microspheres or microparticles. Methods for preparing liposomes and microspheres for administration to a patient are well known to those of skill in the art. U.S. Pat. No. 4,789,734, the contents of which are hereby incorporated by reference,

describes methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is provided by G. Gregoriadis, Chapter 14, "Liposomes," Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979).

[00131] Microspheres formed of polymers or proteins are well known to those skilled in the art, and can be tailored for direct administration to the heart using the delivery vehicles of the present invention. Suitable liposomes for targeting ischemic tissue are generally less than about 200 nanometers and are also typically unilamellar vesicles, as disclosed, for example, in U.S. Pat. No. 5,593,688 to Baldeschweiler, entitled "Liposomal targeting of ischemic tissue," the contents of which are hereby incorporated by reference.

[00132] Preferred microparticles are those prepared from biodegradable polymers, such as polyglycolide, polylactide and copolymers thereof. Those of skill in the art can readily determine an appropriate carrier system depending on various factors, including the desired rate of drug release and the desired dosage.

[00133] The formulations may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like. The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation.

[00134] The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of

auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

[00135] The compositions of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

[00136] Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

[00137] As with the use of other pharmaceutical compositions, the individual patient can be monitored by various ways, including but not limited to invasive hemodynamic monitors, including arterial and central venous pressure monitoring; pulmonary artery catheters, which can include hemodilution cardiac output monitors, and/or continuous mixed venous oxygen saturation monitoring, in addition to pulmonary artery and pulmonary capillary wedge pressures; transesophageal or transthoracic echocardiography; and continuous electrocardiographic monitoring.

EXAMPLES

<u>Methods</u>

[00138] Sprague Dawley rats (900g – 1100g) were anesthetized with an intraperitoneal injection of ketamine and xylazine. The rat was laid on a heating pad and maintained euthermic with warming lights. A 24 gauge intravenous catheter was placed in the tail vein and Ringer's lactate solution (LR) was infused at 10 cc/hr. The trachea was exposed through a vertical incision and cannulated with a 16 gauge blunt cannula that served as an endotracheal tube. The tube was connected to a ventilator for control of ventilation and respiration. The respiratory rate was set to 60 breaths per minute, inspiratory to expiratory time was set to 1:2 and inspiratory flow was 2 liters per minute. A midline sternotomy was performed, the heart exposed and the pericardium resected. The right carotid artery was dissected clean of fascia and care was taken to preserve the adjacent vagus nerve. A polyethelene (PE-50) cannula was inserted via an arteriotomy into the carotid artery and advanced through the ascending aorta into the left ventricle. This ventricular cannula was connected to a high fidelity pressure transducer and digital data acquisition system to record hemodynamic measures. Heart rate (HR), left ventricular systolic blood pressure (SBP), and the maximum rate of change of blood pressure in the left ventricle during isovolemic contraction (dp/dt max) was all recorded. The dp/dt max is the gold standard index of myocardial contractility.

[00139] Following cannulation the rat was stabilized for 30 minutes. Five-second recordings of HR, SBP and dp/dt max were captured every 3 to 5 minutes . SBP and dp/dt max were averaged over all the beats captured in the 5-second interval. Dobutamine (313 mcg/ml), a potent beta agonist inotropic agent, was delivered to the left ventricular free wall through the sternotomy using an infusion pump connected to a 24 guage IV cannula that was suspended directly over the heart (4 mcg/min, 0.8 ml/hr). In this fashion, drug was administered directly to the heart and only in the area exposed by resected pericardium. After 30 minutes the pericardial application of dobutamine was terminated and hemodynamic measurements were recorded for an additional 30 minutes.

Results

[00140] The HR, SBP and contractile response (dp/dt max) to the pericardial application of dobutamine are shown in Figures 1-3.

[00141] This experiment demonstrates that dobutamine can be applied directly to the myocardial surface and exert positive inotropic effects without the systemic effects seen with systemic infusion. Contractility, as expressed by the maximum dp/dt of the left ventricular pressure during isovolemic contraction increased significantly and shortly after dobutamine was applied to the free surface of the heart (Figure 1). Dobutamine given in an intravenous infusion, in addition to increasing myocardial contractility and cardiac output, dilates smooth muscle through peripheral beta-receptors and leads to vasodilatation and reduction in systemic blood pressure. In this experiment, local pericardial dobutamine increased systemic blood pressure, likely from increased force of contraction and cardiac output in the presence of constant vascular tone (Figure 2). This suggests that the usual peripheral vasodilatory side effects of dobutamine infusion were eliminated by local application to the heart. Dobutamine is also a potent chronotrope and topical application with possible diffusion to the sino-atrial node, which normally functions as the pacemaker for the heart, increased heart rate (Figure 3). These data show that potent inotropic agents such as sympathomimetics and phosphodiesterase inhibitors can be locally applied to the heart and improve contractile function, while minimizing systemic side effects.

[00142] All references described herein are incorporated by reference in their entirety.

We claim:

1. A use of a cardiac inotropic agent in the treatment of a subject in need therof, comprising locally administering to the subject a therapeutically effective amount of at least one inotropic agent.

2. The use of claim 1, wherein the inotropic agent is an agent that interacts with the sympathetic nervous system and modulates calcium entry, G-proteins, ATP, or GTP, wherein the inotropic agent is selected from the group consisting of sympathomimetic compounds, phosphodiesterase inhibitors, BNP, ANP, and digitalis glycosides, and derivatives and analogues thereof.

3. The use of claim 1, wherein the inotropic agent is a sympathomimetic compound selected from the group consisting of epinephrine, norepinephrine, dobutamine, isoproterenol, salbutamol, salmeterol, terbutaline, phenylephrine, ephedrine, clonidine and dopamine, and derivatives and analogues thereof.

4. The use of claim 1, wherein the inotropic agent is a phosphodiesterase inhibitor selected from the group consisting of milrinone, enoximone and amrinone, and derivatives and analogues thereof.

5. The use of claim 1, wherein the subject is a surgical patient and is selected from the group consisting of a cardiac surgery patient, a thoracic surgery patient, and a general surgery patient.

6. The use of claim 1, wherein the subject is a cardiac surgery patient, and wherein the cardiac surgery patient is selected from the group consisting of a cardiac surgery patient requiring support from a cardiopulmonary bypass circuit and a cardiac patient not requiring support from a cardiopulmonary bypass circuit.

7. The use of claim 1, wherein the subject has a condition selected from the group consisting of trauma, shock, acute congestive heart failure and chronic congestive heart failure.

8. The use of claim 1, wherein the therapeutically effective amount of the inotropic agent is sufficient to effect myocardial contractility.

9. The use of claim 1, wherein the inotropic agent is delivered locally to the heart by its inclusion in a delivery vehicle.

10. The use of claim 1, wherein the inotropic agent is delivered locally to the heart by its inclusion in a delivery vehicle, and wherein the delivery vehicle is selected from the group consisting of a drug-impregnated, coated or relasing sheet, patch, matrix, hydrogel, foam, gel, cream, spray, microshpere, microcapsule, composite and an ointment.

11. The use of claim 1, wherein the local administration comprises administering said inotropic agent directly to the heart via an open surgical wound.

12. The use of claim 1, wherein the local administration comprises administering said inotropic agent directly to the heart percutaneously.

13. A use of an inotropic agent in a method of reducing postoperative complications of cardiopulmonary bypass (CPB) surgery in a subject comprising: locally administering to a subject in need thereof an effective amount of an inotropic agent in conjunction with said CPB surgery of said subject.

14. The use of claim 13, wherein the inotropic agent is administered to said subject during a time period selected from the group consisting of prior to said CPB surgery, during said CPB surgery, subsequent to said CPB surgery and combinations thereof.

15. The use of claim 13, wherein the inotropic agent is selected from the group consisting a sympathomimetic compound or a phosphodiesterase inhibitor.

16. The use of claim 13, wherein the therapeutically effective amount of the inotropic agent is sufficient to effect myocardial contractility.

17. The use of claim 13, wherein the inotropic agent is delivered locally to the heart by its inclusion in a delivery vehicle.

18. The use of claim 13, wherein the inotropic agent is delivered locally to the heart by its inclusion in a delivery vehicle, and wherein the delivery vehicle is selected from the group consisting of a drug-impregnated, coated or relasing sheet, patch, matrix, hydrogel, foam, gel, cream, spray, microshpere, microcapsule, composite and an ointment.

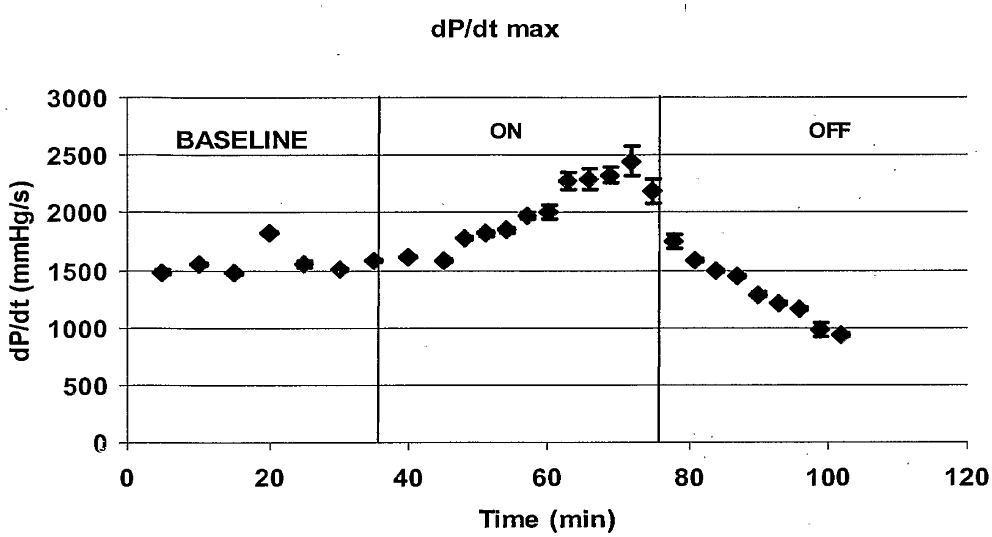

dP/dt max
BASELINE
ON
OFF
dP/dt (mmHg/s)
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
Time (min)

FIGURE 1

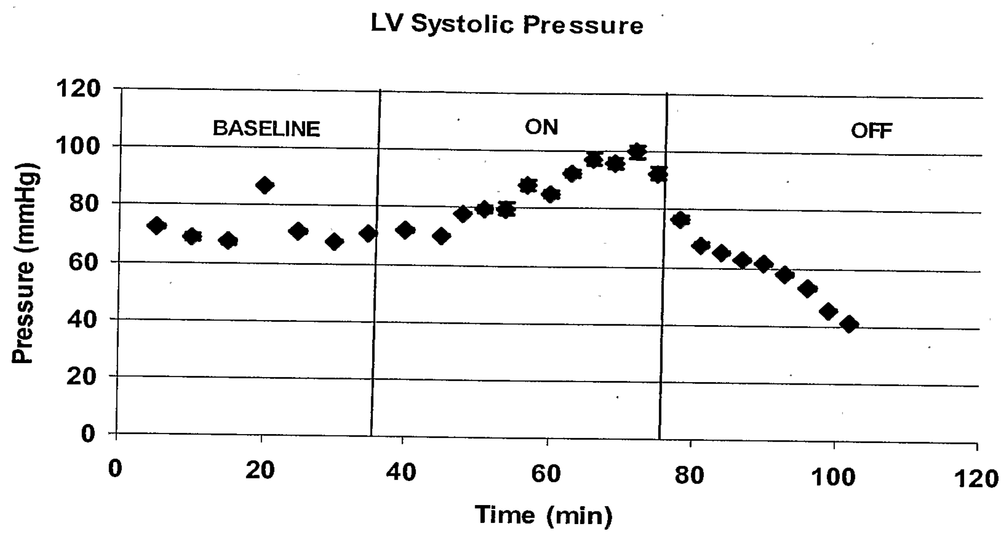

LV Systolic Pressure
BASELINE
ON
OFF
Pressure (mmHg)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)

FIGURE 2

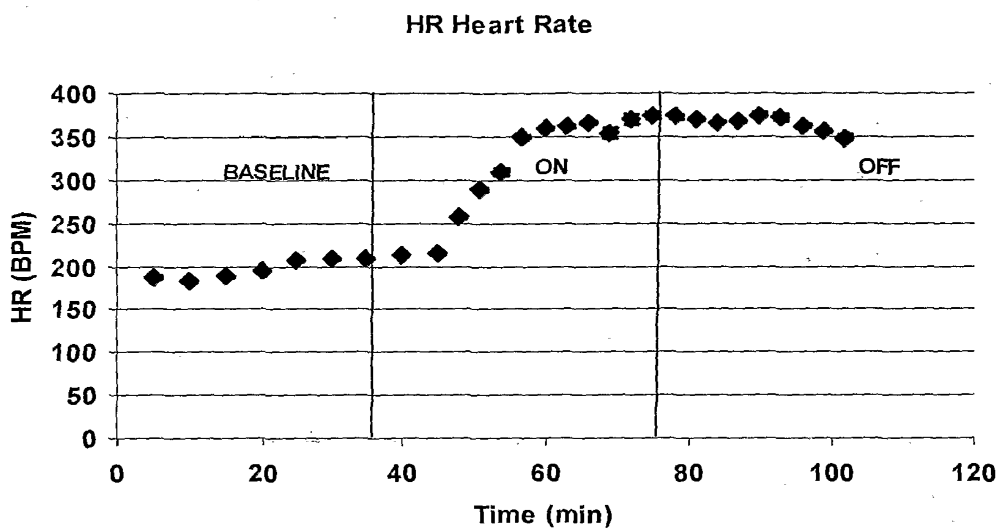

HR Heart Rate
400
350
300
250
200
150
100
50
0
HR (BPM)
BASELINE
ON
OFF
0
20
40
60
80
100
120
Time (min)

FIGURE 3

WO2010019540

Publication Title:

TREATMENT OF PULMONARY ARTERIAL HYPERTENSION

Abstract:

Abstract of WO 2010019540

(A1) Translate this text The present invention pertains to the use of

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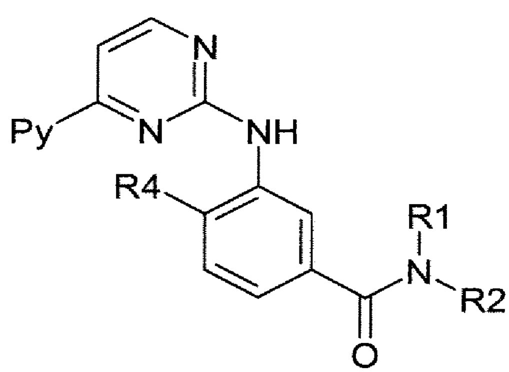
(54) **Title**: TREATMENT OF PULMONARY ARTERIAL HYPERTENSION



(I)

(57) **Abstract**: The present invention pertains to the use of 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I wherein the radicals and symbols are as defined herein, or a pharmaceutically acceptable salt thereof, for the manufacture of medicament for treating pulmonary arterial hypertension (PAH), especially in patients who failed prior PAH therapy.

WO 2010/019540 A1

TREATMENT OF PULMONARY ARTERIAL HYPERTENSION

The invention relates to the use of 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide (also known as "Imatinib" [International Non-proprietary Name]; hereinafter: "COMPOUND I") or a pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I as defined below or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of pulmonary arterial hypertension, to COMPOUND I or a pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I as defined below or a pharmaceutically acceptable salt thereof for the treatment of pulmonary arterial hypertension, and to a method of treating warm-blooded animals including humans suffering from pulmonary arterial hypertension, by administering to a said animal in need of such treatment an effective dose of COMPOUND I or a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof.

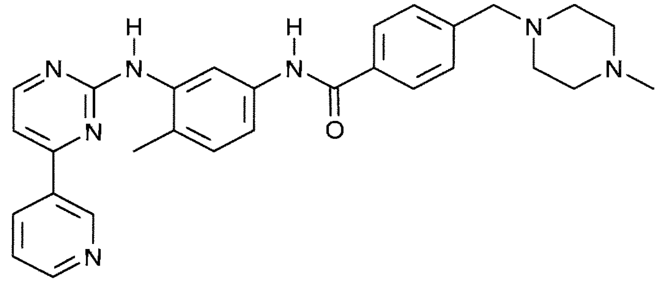
Pulmonary arterial hypertension is a life-threatening disease characterized by a marked and sustained elevation of pulmonary artery pressure. The disease results in right ventricular (RV) failure and death. Current therapeutic approaches for the treatment of chronic pulmonary arterial hypertension mainly provide symptomatic relief, as well as some improvement of prognosis. Although postulated for all treatments, evidence for direct anti-proliferative effects of most approaches is missing. In addition, the use of most of the currently applied agents is hampered by either undesired side effects or inconvenient drug administration routes. Pathological changes of hypertensive pulmonary arteries include endothelial injury, proliferation and hyper-contraction of vascular smooth muscle cells (SMCs).

The instant invention is a response to the need for an alternative therapy in the treatment of pulmonary hypertension, especially pulmonary arterial hypertension.

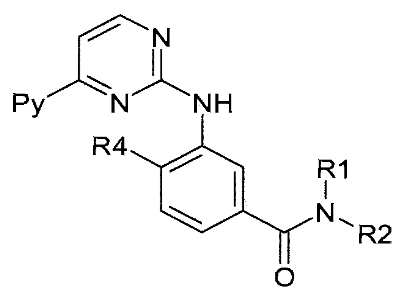
United States patent specification US 2006/0154936 disclosed the use of COMPOUND I alone or in combination with other medication as an alternative to existing therapies for the treatment of pulmonary hypertension.

It has now surprisingly been demonstrated that pulmonary arterial hypertension can be successfully treated with COMPOUND I, or pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof, in particular in patients who failed prior therapy.

In a first aspect the present invention concerns the use of COMPOUND I having the formula



or a pharmaceutically acceptable salt thereof, or a pyrimidylaminobenzamide of formula I



(I)

wherein

Py denotes 3-pyridyl,

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for treating pulmonary arterial hypertension, especially in patients who failed prior PAH therapy.

In a second aspect the present invention concerns 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof, or a pyrimidylaminobenzamide of formula I as defined above or a pharmaceutically acceptable salt thereof, for use in treating pulmonary arterial hypertension (PAH) in patients who failed prior PAH therapy.

In a third aspect the present invention concerns a method of treating warm-blooded animals including humans suffering from pulmonary arterial hypertension, by administering to a said animal in need of such treatment an effective dose of 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof or a pyrimidylamino-benzamide of formula I as defined above or a pharmaceutically acceptable salt thereof.

In a fourth aspect the present invention concerns a method of treating a human suffering from

(a) idiopathic or primary pulmonary hypertension,

(b) familial hypertension,

(c) pulmonary hypertension secondary to, but not limited to, connective tissue disease, congenital heart defects (shunts), pulmonary fibrosis, portal hypertension, HIV infection, sickle cell disease, drugs and toxins (e.g., anorexigens, cocaine), chronic hypoxia, chronic pulmonary obstructive disease, sleep apnea, and schistosomiasis,

(d) pulmonary hypertension associated with significant venous or capillary involvement (pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis),

(e) secondary pulmonary hypertension that is out of proportion to the degree of left ventricular dysfunction,

(f) persistent pulmonary hypertension in newborn babies,

especially in patients who failed prior PAH therapy, which comprises administering to said human in need of such treatment a dose effective against the respective disorder of 4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pyrimidylaminobenzamide of formula I as defined above or a pharmaceutically acceptable salt thereof.

The preparation of COMPOUND I and the use thereof, especially as an anti-tumor agent, are described in Example 21 of European patent application EP-A-0 564 409, the contents of which is hereby incorporated by reference, and in corresponding applications and patents in numerous other countries, e.g. in US patent 5,521,184 and in Japanese patent 2706682.

Pharmaceutically acceptable salts of COMPOUND I are pharmaceutically acceptable acid addition salts, like for example with inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxy-benzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid.

The monomethanesulfonic acid addition salt of COMPOUND I (hereinafter "COMPOUND I mesylate" or "imatinib mesylate" or "COMPOUND I monomethanesulfonate") and a preferred crystal form thereof, e.g. the β-crystal form, are described in PCT patent application WO99/03854 published on January 28, 1999.

Possible pharmaceutical preparations, containing an effective amount of COMPOUND I or a pharmaceutically acceptable salt thereof are also described in WO99/03854 , the contents of which is incorporated herein by reference.

According to formula I, the following suitable, preferred, more preferred or most preferred aspects of the invention may be incorporated independently, collectively or in any combination.

Preference is also given to pyrimidylaminobenzamides of formula I, wherein py is 3-pyridyl and wherein the radicals mutually independently of each other have the following meanings:

- R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl; more preferably hydrogen;
- R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;
- R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and
- R_4 represents lower alkyl, especially methyl.

A preferred pyrimidylaminobenzamide of formula I is 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl] benzamide, also known as “nilotinib”.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated:

The prefix "lower" denotes a radical having up to and including a maximum of 7, especially up to and including a maximum of 4 carbon atoms, the radicals in question being either linear or branched with single or multiple branching.

Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

Lower alkyl is preferably alkyl with from and including 1 up to and including 7, preferably from and including 1 to and including 4, and is linear or branched; preferably, lower alkyl is butyl, such as n-butyl, sec-butyl, isobutyl, tert-butyl, propyl, such as n-propyl or isopropyl, ethyl or methyl. Preferably lower alkyl is methyl, propyl or tert-butyl.

Lower acyl is preferably formyl or lower alkylcarbonyl, in particular acetyl.

An aryl group is an aromatic radical which is bound to the molecule via a bond located at an aromatic ring carbon atom of the radical. In a preferred embodiment, aryl is an aromatic radical having 6 to 14 carbon atoms, especially phenyl, naphthyl, tetrahydronaphthyl, fluorenyl or phenanthrenyl, and is unsubstituted or substituted by one or more, preferably up to three, especially one or two substituents, especially selected from amino, mono- or disubstituted amino, halogen, lower alkyl, substituted lower alkyl, lower alkenyl, lower alkynyl, phenyl, hydroxy, etherified or esterified hydroxy, nitro, cyano, carboxy, esterified carboxy, alkanoyl, benzoyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amidino, guanidino, ureido, mercapto, sulfo, lower alkylthio, phenylthio, phenyl-lower alkylthio, lower alkylphenylthio, lower alkylsulfinyl, phenylsulfinyl, phenyl-lower alkylsulfinyl, lower alkylphenylsulfinyl, lower alkylsulfonyl, phenylsulfonyl, phenyl-lower alkylsulfonyl, lower alkylphenylsulfonyl, halogen-lower alkylmercapto, halogen-lower alkylsulfonyl, such as especially trifluoromethanesulfonyl, dihydroxybora (-B(OH)$_2$), heterocyclyl, a mono- or bicyclic heteroaryl group and lower alkylene dioxy bound at adjacent C-atoms of the ring, such as methylene dioxy. Aryl is more preferably phenyl, naphthyl or tetrahydronaphthyl, which in each case is either unsubstituted or independently substituted by one or two substituents selected from the group comprising halogen, especially fluorine, chlorine, or bromine; hydroxy; hydroxy etherified by lower alkyl, e.g. by methyl, by halogen-lower alkyl, e.g. trifluoromethyl, or by phenyl; lower alkylene dioxy bound to two adjacent C-atoms, e.g. methylenedioxy, lower alkyl, e.g. methyl or propyl; halogen-lower alkyl, e.g. trifluoromethyl; hydroxy-lower alkyl, e.g. hydroxymethyl or 2-hydroxy-2-propyl; lower alkoxy-lower alkyl; e.g. methoxymethyl or 2-methoxyethyl; lower alkoxycarbonyl-lower alkyl, e.g. methoxy-carbonylmethyl; lower alkynyl, such as 1-propynyl; esterified carboxy, especially lower alkoxycarbonyl, e.g. methoxycarbonyl, n-propoxy carbonyl or iso-propoxy carbonyl; N-mono-substituted carbamoyl, in particular carbamoyl monosubstituted by lower alkyl, e.g. methyl, n-propyl or iso-propyl; amino; lower alkylamino, e.g. methylamino; di-lower alkylamino, e.g. dimethylamino or diethylamino; lower alkylene-amino, e.g. pyrrolidino or piperidino; lower oxaalkylene-amino, e.g. morpholino, lower azaalkylene-amino, e.g. piperazino, acylamino, e.g. acetylamino or benzoylamino; lower alkylsulfonyl, e.g. methylsulfonyl; sulfamoyl; or phenylsulfonyl.

A cycloalkyl group is preferably cyclopropyl, cyclopentyl, cyclohexyl or cycloheptyl, and may be unsubstituted or substituted by one or more, especially one or two, substituents selected from the group defined above as substituents for aryl, most preferably by lower alkyl, such as methyl, lower alkoxy, such as methoxy or ethoxy, or hydroxy, and further by oxo or fused to a benzo ring, such as in benzcyclopentyl or benzcyclohexyl.

Substituted alkyl is alkyl as last defined, especially lower alkyl, preferably methyl; where one or more, especially up to three, substituents may be present, primarily from the group selected from halogen, especially fluorine, amino, N-lower alkylamino, N,N-di-lower alkylamino, N-lower alkanoylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, and phenyl-lower alkoxycarbonyl. Trifluoromethyl is especially preferred.

Mono- or disubstituted amino is especially amino substituted by one or two radicals selected independently of one another from lower alkyl, such as methyl; hydroxy-lower alkyl, such as 2-hydroxyethyl; lower alkoxy lower alkyl, such as methoxy ethyl; phenyl-lower alkyl, such as benzyl or 2-phenylethyl; lower alkanoyl, such as acetyl; benzoyl; substituted benzoyl, wherein the phenyl radical is especially substituted by one or more, preferably one or two, substituents selected from nitro, amino, halogen, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, and carbamoyl; and phenyl-lower alkoxycarbonyl, wherein the phenyl radical is unsubstituted or especially substituted by one or more, preferably one or two, substituents selected from nitro, amino, halogen, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, and carbamoyl; and is preferably N-lower alkylamino, such as N-methylamino, hydroxy-lower alkylamino, such as 2-hydroxyethylamino or 2-hydroxypropyl, lower alkoxy lower alkyl, such as methoxy ethyl, phenyl-lower alkylamino, such as benzylamino, N,N-di-lower alkylamino, N-phenyl-lower alkyl-N-lower alkylamino, N,N-di-lower alkylphenylamino, lower alkanoylamino, such as acetylamino, or a substituent selected from the group comprising benzoylamino and phenyl-lower alkoxycarbonylamino, wherein the phenyl radical in each case is unsubstituted or especially substituted by nitro or amino, or also by halogen, amino, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, carbamoyl or aminocarbonylamino. Disubstituted amino is also lower alkylene-amino, e.g. pyrrolidino, 2-oxopyrrolidino or piperidino; lower oxaalkylene-amino, e.g. morpholino, or lower azaalkylene-amino, e.g. piperazino or N-substituted piperazino, such as N-methylpiperazino or N-methoxycarbonylpiperazino.

Halogen is especially fluorine, chlorine, bromine, or iodine, especially fluorine, chlorine, or bromine.

Etherified hydroxy is especially C_8-C_{20}alkyloxy, such as n-decyloxy, lower alkoxy (preferred), such as methoxy, ethoxy, isopropyloxy, or tert-butyloxy, phenyl-lower alkoxy, such as benzyloxy, phenyloxy, halogen-lower alkoxy, such as trifluoromethoxy, 2,2,2-trifluoroethoxy or 1,1,2,2-tetrafluoroethoxy, or lower alkoxy which is substituted by mono- or bicyclic hetero-aryl comprising one or two nitrogen atoms, preferably lower alkoxy which is substituted by imidazolyl, such as 1H-imidazol-1-yl, pyrrolyl, benzimidazolyl, such as 1-benzimidazolyl, pyridyl, especially 2-, 3- or 4-pyridyl, pyrimidinyl, especially 2-pyrimidinyl, pyrazinyl, isoquinolinyl, especially 3-isoquinolinyl, quinolinyl, indolyl or thiazolyl.

Esterified hydroxy is especially lower alkanoyloxy, benzoyloxy, lower alkoxycarbonyloxy, such as tert-butoxycarbonyloxy, or phenyl-lower alkoxycarbonyloxy, such as benzyloxycarbonyloxy.

Esterified carboxy is especially lower alkoxycarbonyl, such as tert-butoxycarbonyl, iso-propoxycarbonyl, methoxycarbonyl or ethoxycarbonyl, phenyl-lower alkoxycarbonyl, or phenyloxycarbonyl.

Alkanoyl is primarily alkylcarbonyl, especially lower alkanoyl, e.g. acetyl.

N-Mono- or N,N-disubstituted carbamoyl is especially substituted by one or two substituents independently selected from lower alkyl, phenyl-lower alkyl and hydroxy-lower alkyl, or lower alkylene, oxa-lower alkylene or aza-lower alkylene optionally substituted at the terminal nitrogen atom.

A mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted, refers to a heterocyclic moiety that is unsaturated in the ring binding the heteroaryl radical to the rest of the molecule in formula I and is preferably a ring, where in the binding ring, but optionally also in any annealed ring, at least one carbon atom is replaced by a heteroatom selected from the group consisting of nitrogen, oxygen and sulfur; where the binding ring preferably has 5 to 12, more preferably 5 or 6 ring atoms; and which may be unsubstituted or substituted by one or more, especially one or two, substituents selected from the group defined above as substituents for aryl, most preferably by lower alkyl, such as methyl, lower alkoxy, such as methoxy or ethoxy, or hydroxy. Preferably the mono- or bicyclic heteroaryl group is selected from 2H-pyrrolyl, pyrrolyl, imidazolyl, benzimidazolyl, pyrazolyl, indazolyl, purinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalyl, quinazolinyl, quinnolinyl, pteridinyl, indolizinyl, 3H-indolyl, indolyl, isoindolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, furazanyl, benzo[d]pyrazolyl, thienyl and furanyl. More preferably the mono- or bicyclic heteroaryl group is selected from the group consisting of pyrrolyl, imidazolyl, such as 1H-imidazol-1-yl, benzimidazolyl, such as 1-benzimidazolyl, indazolyl, especially 5-indazolyl, pyridyl, especially 2-, 3- or 4-pyridyl, pyrimidinyl, especially 2-pyrimidinyl, pyrazinyl, isoquinolinyl, especially 3-isoquinolinyl,

quinolinyl, especially 4- or 8-quinolinyl, indolyl, especially 3-indolyl, thiazolyl, benzo[d]pyrazolyl, thienyl, and furanyl. In one preferred embodiment of the invention the pyridyl radical is substituted by hydroxy in ortho position to the nitrogen atom and hence exists at least partially in the form of the corresponding tautomer which is pyridin-(1H)2-one. In another preferred embodiment, the pyrimidinyl radical is substituted by hydroxy both in position 2 and 4 and hence exists in several tautomeric forms, e.g. as pyrimidine-(1H, 3H)2,4-dione.

Heterocyclyl is especially a five, six or seven-membered heterocyclic system with one or two heteroatoms selected from the group comprising nitrogen, oxygen, and sulfur, which may be unsaturated or wholly or partly saturated, and is unsubstituted or substituted especially by lower alkyl, such as methyl, phenyl-lower alkyl, such as benzyl, oxo, or heteroaryl, such as 2-piperazinyl; heterocyclyl is especially 2- or 3-pyrrolidinyl, 2-oxo-5-pyrrolidinyl, piperidinyl, N-benzyl-4-piperidinyl, N-lower alkyl-4-piperidinyl, N-lower alkyl-piperazinyl, morpholinyl, e.g. 2- or 3-morpholinyl, 2-oxo-1H-azepin-3-yl, 2-tetrahydrofuranyl, or 2-methyl-1,3-dioxolan-2-yl.

Pyrimidylaminobenzamides within the scope of formula I, wherein Py is 3-pyridyl and the process for their manufacture are disclosed in WO 04/005281, the contents of which is incorporated herein by reference.

Pharmaceutically acceptable salts of pyrimidylaminobenzamides of formula I, wherein Py is 3-pyridyl, are especially those disclosed in WO2007/015871. In one preferred embodiment nilotinib is employed in the form of its hydrochloride monohydrate. WO2007/015870 discloses certain polymorphs of nilotinib and pharmaceutically acceptable salts thereof useful for the present invention.

The pyrimidylaminobenzamides of formula I, wherein Py is 3-pyridyl, can be administered by any route including orally, parenterally, e.g., intraperitoneally, intravenously, intramuscularly, subcutaneously, intratumorally, or rectally, or enterally. Preferably, the pyrimidyl-aminobenzamides of formula I, wherein py is 3-pyridyl, is administered orally, preferably at a daily dosage of 50-2000 mg. A preferred oral daily dosage of nilotinib is 200 - 1200 mg, e.g. 800 mg, administered as a single dose or divided into multiple doses, such as twice daily dosing.

The term “treatment” as used herein means curative treatment and prophylactic treatment.

The term “curative” as used herein means efficacy in treating ongoing episodes of pulmonary hypertension, especially pulmonary arterial hypertension,.

The term “prophylactic” means the prevention of the onset or recurrence of pulmonary hypertension, especially pulmonary arterial hypertension,.

Throughout this specification and in the claims that follow, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The invention also pertains to a pharmaceutical preparation for the treatment of pulmonary arterial hypertension comprising COMPOUND I.

Short Description of the Figures

Fig. 1 depicts the change in pulmonary vascular resistance (PVR) in patients obtaining Imatinib mesylate.

Fig. 2 depicts the change in pulmonary vascular resistance (PVR) in patients obtaining placebo.

Fig. 3 depicts the change in cardiac output (CO) in patients obtaining Imatinib mesylate.

Fig. 4 depicts the change in cardiac output (CO) in patients obtaining placebo.

Fig. 5 depicts the change in pulmonary artery pressure (PAP) in patients obtaining Imatinib mesylate.

Fig. 6 depicts the change in pulmonary artery pressure (PAP) in patients obtaining placebo.

Fig. 7 depicts the patient disposition of the intention to treat (ITT) population.

Fig. 8 depicts the mean change from baseline in pulmonary hemodynamics after 6 months of treatment with imatinib or placebo. (a) mean pulmonary artery pressure (PAPm); (b) cardiac output (CO); (c) pulmonary vascular resistance (PVR); (d) 6-minute walking distance (6MWD).

Fig. 9 depicts the mean change from baseline to study end in pulmonary hemodynamics in patients randomized to imatinib or placebo, stratified by baseline PVR ≥1,000 dynes.sec.cm^{-5} (imatinib N=8; placebo N=12) or <1,000 dynes.sec.cm^{5} (imatinib N=12; placebo N=9). (a) mean pulmonary artery pressure (PAPm); (b) cardiac output (CO); (c) pulmonary vascular resistance (PVR); (d) 6-minute walking distance (6MWD).

World Health Organization Classification of Functional Status of Patients With Pulmonary Hypertension

The status of their pulmonary hypertension can be assessed in patients according to the World Health Organization (WHO) classification (modified after the New York Association Functional Classification) as detailed below:

Class I – Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope.

Class II – Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dispend or fatigue, chest pain or near syncope.

Class III – Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnea or fatigue, chest pain or near syncope.

Class IV – Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

In a preferred embodiment of the present invention the medicament is designated for treating pulmonary arterial hypertension in patients who failed prior therapy, especially after receiving at least one prostanoid, endothelin antagonist or PDE V inhibitor.

In a further preferred embodiment of the present invention the medicament is designated for treating pulmonary arterial hypertension in patients who are more severely affected, in particular in patients with Class II to Class IV functional status, more preferably Class III or IV functional status.

In a further preferred embodiment of the present invention the medicament is designated for treating pulmonary arterial hypertension in patients who are harboring BMPR2 mutations.

In a more general aspect, the present invention provides a method of treating humans suffering from

(a) idiopathic or primary pulmonary hypertension,
(b) familial hypertension,
(c) pulmonary hypertension secondary to, but not limited to, connective tissue disease, congenital heart defects (shunts), pulmonary fibrosis, portal hypertension, HIV infection, sickle cell disease, drugs and toxins (e.g., anorexigens, cocaine), chronic hypoxia, chronic pulmonary obstructive disease, sleep apnea, and schistosomiasis,
(d) pulmonary hypertension associated with significant venous or capillary involvement (pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis),
(e) secondary pulmonary hypertension that is out of proportion to the degree of left ventricular dysfunction,
(f) persistent pulmonary hypertension in newborn babies,

especially in patients who failed prior PAH therapy, which comprises administering to said human in need of such treatment a dose effective against the respective disorder of 4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof, respectively, preferably a dose effective against the respective disorder of a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof.

Depending on species, age, individual condition, mode of administration, and the clinical picture in question, effective doses, for example daily doses of about 100-1000 mg, preferably 200-600 mg, especially 400 mg of COMPOUND I, are administered to warm-blooded animals of about 70 kg bodyweight. For adult patients a starting dose corresponding to 400 mg of COMPOUND I free base daily can be recommended. For patients with an inadequate response after an assessment of response to therapy with a dose corresponding to 400 mg of COMPOUND I free base daily, dose escalation can be safely considered and patients may be treated as long as they benefit from treatment and in the absence of limiting toxicities.

The invention relates also to a method for administering to a human subject having pulmonary arterial hypertension a pharmaceutically effective amount of COMPOUND I or a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof to the human subject. Preferably, COMPOUND I or a pyrimidylaminobenzamide of formula I or a pharmaceutically acceptable salt thereof is administered once daily for a period exceeding 3 months. The invention relates especially to such method wherein a daily dose of COMPOUND I mesylate corresponding to 100 to 1000 mg, e.g. 200 to 800 mg, especially 400-600 mg, preferably 400 mg, of COMPOUND I free base is administered.

According to the present invention, COMPOUND I is preferably in the form of the monomethanesulfonate salt, e.g. in the β-crystal form of the monomethanesulfonate salt.

The invention relates to a method of treating a warm-blooded animal, especially a human, suffering from pulmonary hypertension, especially pulmonary arterial hypertension, comprising administering to the animal a combination which comprises (a) COMPOUND I or a pyrimidylaminobenzamide of formula I and (b) at least one compound selected from compounds indicated for the treatment of pulmonary arterial hypertension, such as calcium channel antagonists, e.g. nifedipine, e.g. 120 to 240 mg/d, or diltiazem, e.g. 540 to 900 mg/d, prostacyclin, the prostacyclin analogues iloprost, flolan and treprostinil, adenosine, inhaled nitric oxide, anticoagulants, e.g. warfarin, digoxin, endothelin receptor blockers, e.g. bosentan, phosphodiesterease inhibitors, e.g. sildenafil, norepinephrine, angiotensin-converting enzyme inhibitors e.g. enalapril or diuretics; a combination comprising (a) and (b) as defined above and optionally at least one pharmaceutically acceptable carrier for simultaneous, separate or sequential use, in particular for the treatment of pulmonary arterial hypertension; a pharmaceutical composition comprising such a combination; the use of such a combination for the preparation of a medicament for the delay of progression or treatment of pulmonary arterial hypertension; and to a commercial package or product comprising such a combination.

The structure of the active agents identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium “The Merck Index” or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

When the combination partners employed in the combinations as disclosed herein are applied in the form as marketed as single drugs, their dosage and mode of administration can take place in accordance with the information provided on the package insert of the respective marketed drug in order to result in the beneficial effect described herein, if not mentioned herein otherwise.

It can be shown by established test models that the COMPOUND I or a pyrimidylamino-benzamide of formula I or a pharmaceutically acceptable salt thereof, results in a more effective prevention or preferably treatment of pulmonary arterial hypertension. COMPOUND I or a pharmaceutically acceptable salt thereof has significant fewer side effects as a current therapy. Furthermore, COMPOUND I or a pharmaceutically acceptable salt thereof, results in beneficial effects in different aspects, such as, e.g. incremental benefit with time or to reverse the disease process. COMPOUND I, or a pharmaceutically acceptable salt thereof, shows an unexpected high potency to prevent or eliminate pulmonary arterial hypertension, because of its unexpected multifunctional activity, and its activity on different aspects of pulmonary arterial hypertension.

The person skilled in the pertinent art is fully enabled to select a relevant test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects (i.e. good therapeutic margin, and other advantages mentioned herein). The pharmacological activity is, for example, demonstrated by *in vitro* and *in vivo* test procedures such as rodent models of pulmonary arterial hypertension, or in a clinical study as essentially described hereinafter. The following Examples illustrate the invention described above, but are not, however, intended to limit the scope of the invention in any way.

Example 1: A randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of six months treatment with the tyrosine kinase inhibitor Imatinib Mesylate for the treatment of pulmonary arterial hypertension

Primary objectives

• To assess the safety and tolerability of oral Imatinib Mesylate compared with placebo in patients with pulmonary arterial hypertension (PAH).

• To evaluate efficacy of oral Imatinib Mesylate as measured by an improvement in 6-minute walk test.

Secondary objective(s)

• To evaluate the efficacy of oral Imatinib Mesylate as measured by improvement in clinical status (assessment of WHO class and Borg Score), and changes in pulmonary homodynamic parameters (including mean pulmonary arterial pressure, mean Pulmonary Artery Wedge pressure, Systolic Arterial Pressure, Heart Rate, and Cardiac Output, Pulmonary Vascular Resistance, Systemic Vascular Resistance), time to clinical worsening, changes in plasma biomarker levels.

Design:

In the study a total of 60 patients with PAH was enrolled who have been shown to be deteriorating on, or not tolerating, standard therapy (prostanoids (i.v., s.c., inhaled), endothelin-1 antagonists, or PDE-5 inhibitors), but may still be continuing with the standard therapy. Eligible patients were randomized to receive oral Imatinib Mesylate 200mg daily rising to 400mg after 2 weeks, or matching placebo. Treatment continued for 6 months with weekly visits for the first four weeks followed by monthly visits up to six months (Week 24). Safety and efficacy assessments were performed at pre-specified time points up to Week 24. Male or female patients aged 18 years or older with pulmonary arterial hypertension according to the Venice Classification (2003) of either primary (idiopathic), familial or secondary to systemic sclerosis (excluding those with marked pulmonary fibrosis) and a WHO classification of II to IV (maximum of 50% of patients will be class IV) were included. Patients harboring a mutation in BMPR2 gene were identified. Patients had been receiving therapy with prostanoids (i.v., s.c., inhaled), endothelin-1 antagonists, or PDE-5 inhibitors, but have shown to be deteriorating (not improving on), or not tolerating this standard therapy. PAH medication had been stable for at least 3 months prior to inclusion in the study (Baseline visit). Imatinib Mesylate was applied as 100 mg clinical trial formulation capsules for oral administration and matching placebo capsules. The 200 mg dose consisted of 2 x 100mg capsules or 2 x matching placebo. The 400 mg dose consisted of 4 x 100 mg capsules or matching placebo. Patients were instructed to take the study drug once daily with a meal and a large glass (8oz/200 mL) of water and not to chew the medication, but to swallow it whole.

Efficacy assessments

• Six minute walk test and Borg Score: Screening, Baseline, Week 4, Week 8, Week 12, Week 16, Week 20, Week 24/Study Completion.

• WHO Assessment: Screening, Baseline, Week 4, Week 8, Week 12, Week 16, Week 20, Week 24/Study Completion

• Hemodynamic parameters (PAP, PAWP, SAP, HR, CO, PVR and SVR) from right sided heart catheritization: Baseline and Week 24/Study Completion.

Results

Table 1 - Change in Key Variables Baseline to Study End (mean [percent])

	mPAP (mmHg)	CO (l/min)	PVR (dyne/s · cm)–5	PCWP (mmHg)	6MW
IM N=19	-6.42 (-11%)	0.83 (20%)	-300 (-29%)	-0.4 (-4%)	18.1 (5%)
Placebo N=21	-2.66 (-4%)	0.11 (3%)	-81 (-8%)	1.4 (19%)	-12 (-3%)
IM - Placebo	-3.75 (7%)	0.71 (17%)	218 (-21%)	1.8 (23%)	30 (8%)
P Value	0.27	0.017	0.029	0.07	0.06

Table 2 - Change by Baseline PVR / PVR<1000

	mPAP	PVR	CO	6MW
IM (N=7)	-4.61538	-173.769	0.291538	3.2
PL (N=12)	-3.25	-74.375	0.57375	14.4

Table 3 - Change by Baseline PVR / PVR>1000

	mPAP	PVR	CO	6MW
IM (N=12)	-8.57143	-596.571	1.271429	70
PL(N=9)	-6.33333	-121.75	0.229167	-32

6MW: 6-minute walk test; CO: cardiac output; IM: Imatinib mesylate;; PAP: pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; PL: placebo; PVR: pulmonary vascular resistance

The study demonstrates a clear beneficial change in pulmonary vascular resistance (PVR), cardiac output (CO) and six minute walk in response to Imatinib mesylate compared to placebo. A trend in reduction in pulmonary artery pressure (PAP) was also seen. There was a difference in the number of deaths (5 versus 3) in favor of Imatinib mesylate.

Example 2: A randomized, double-blind, placebo-controlled trial to evaluate imatinib treatment for patients with severe pulmonary arterial hypertension with inadequate response to established therapy

Introduction

Pulmonary arterial hypertension (PAH) (defined as a mean pulmonary artery pressure [PAPm] of ≥25 mmHg at rest or 30 mmHg with exercise, mean pulmonary capillary wedge pressure [PCWPm] ≤15 mmHg and pulmonary vascular resistance [PVR] > 240 dynes.sec.cm^{-5}) leads to progressive increases in pulmonary vascular resistance (PVR), right ventricular failure and death if untreated. Estimated 1 and 3 year survival rates in idiopathic PAH (IPAH) without targeted therapy are 68% and 48%, respectively.

Current drug therapy recommendations for PAH vary depending on the patient's functional class (FC, World Health Organization's [WHO] Modification for Pulmonary Hypertension of the New York Heart Association Functional Class). The phosphodiesterase type 5 (PDE5) inhibitor sildenafil, oral endothelin receptor antagonists (ERAs) bosentan, ambrisentan and sitaxsentan, and prostacyclin analogues epoprostenol (intravenous), iloprost (inhaled) and treprostinil (subcutaneous or intravenous) are approved for patients in FC II-IV. Patients in FC III or IV who fail to improve or deteriorate with monotherapy can be treated with combination therapy, atrial septostomy and/or transplantation (lung or heart/lung). However, to date, none of these therapeutic options cure PAH despite improvement in survival; PAH remains a progressive and frequently fatal condition. Two recent meta-analyses highlighted the beneficial effects of prostacyclin analogues, ERAs and PDE5 inhibitors on exercise capacity and some other clinical endpoints in PAH patients, while only the most recent report by Galie et al. provided evidence of improved survival by the aforementioned treatments.

Pathological changes in the pulmonary arteries of patients with PAH include the formation of plexiform lesions, and smooth muscle and fibroblast proliferation leading to vascular obstruction. Platelet-derived growth factor (PDGF) is a vascular smooth muscle cell mitogen activating signal transduction pathways associated with smooth muscle hyperplasia in pulmonary hypertension. PDGF and its receptor (PDGFR) have been implicated in the pathobiology of pulmonary hypertension in animal studies and in patients with PAH thereby offering a potential new target for treatment.

Imatinib, a tyrosine kinase inhibitor that inhibits PDGFR α and β kinases, Abl, DDR and c-KIT, may therefore prove efficacious in the treatment of PAH. Several case reports have provided promising results thus warranting further study of imatinib in PAH.

In the present study the effects of imatinib versus placebo were compared in a randomized, double-blind, placebo-controlled pilot study in PAH patients who had not adequately improved with prostacyclin analogues, ERAs, PDE5 inhibitors and/or combinations of these therapies.

Methods

1. Study objectives and design

The primary objectives were to assess the safety and tolerability of imatinib compared with placebo in PAH patients and to evaluate its efficacy using the 6-minute walk test (6MW test). Secondary objectives included changes in hemodynamic variables, and in FC.

Patients ($\geq$18 years) in FC II-IV with idiopathic or familial PAH, or PAH associated with systemic sclerosis or congenital heart disease (WHO group I) and PVR > 300 dynes.sec.cm^{-5} were eligible. Patients were on stable PAH medication(s) for > 3 months before enrolment. Females of child-bearing potential used double-barrier contraception.

Patients with other causes of PAH were excluded. Patients were not allowed to use nonspecific PDE inhibitors, chronic inhaled nitric oxide therapy or catecholamines during the study. Additional exclusion criteria included: participation in another clinical trial within 3 months, donation or loss of blood (>400 mL) within 8 weeks or a history of another significant illness within 4 weeks. Patients were also excluded if they had pre-existing lung disease, coagulation disorders, thrombocytopenia, major bleeding or intracranial haemorrhage, history of latent bleeding risk, elevated liver transaminases (>4 times upper limit of normal [ULN]), elevated bilirubin (>2 times ULN), elevated serum creatinine (>200 µmol/L), history of elevated intracranial pressure, pregnancy, breast feeding, sickle cell anaemia, history of clinically significant drug allergy or atopic allergy, history of immunodeficiency, hepatitis B or C, or history of drug or alcohol abuse. Patients were excluded if they had known hypersensitivity to the study drug, any condition that could alter the study drug pharmacokinetics or put them at risk, if their underlying disease was likely to result in failure to survive the study, or if they were unable to perform the 6MW test due to a condition other than PAH. Eligible patients were enrolled at 7 centres in Germany, the United Kingdom, Austria, and the United States and randomized 1:1 to treatment with either imatinib or placebo.

The study was designed, implemented and reported in accordance with International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice and all applicable local regulations (including European Directive 2001/83/EC and US Code of Federal Regulations Title 21) and with the ethical principles laid down in the Declaration of Helsinki. This study was approved by institutional review boards at all centres and all patients signed informed consent before enrolment. All deaths and safety data were reviewed throughout the study by an external data safety monitoring board.

2. Interventions

Treatment with imatinib (or placebo) was initiated at a dose of 200 mg orally once daily for the first two weeks of treatment. If treatment was well tolerated, the dose was increased to 400 mg/day. If the 400 mg dose was not well tolerated, down-titration to 200 mg was permitted. Patients and investigators were blind to the treatment allocation. The blinding could be broken in an emergency.

3. Efficacy assessments

The primary efficacy outcome was the between-group difference in the 6MW distance (6MWD) at baseline and at 6 months. Complete hemodynamic parameters were assessed with standard techniques. FC was classified according to the WHO modification of the NYHA criteria for pulmonary hypertension.

4. Exploratory Analysis

To generate new hypotheses and to identify patient subgroups that may respond better than other subgroups to imatinib, additional subgroup analyses were conducted in patients with PVR values of ≥1,000 vs. <1,000 dynes.sec.cm^{-5} (the median of the data).

5. Safety assessments

Monitoring of blood cell counts, hepatic and renal function parameters, echocardiography and cardiac magnetic resonance imaging (in selected centres) was conducted during the study. Patients were also interviewed via regular telephone calls between scheduled study visits.

6. Statistical analysis

The planned sample size of 60 subjects was selected to address both safety and the primary efficacy outcome (6MWD). For the primary efficacy outcome it was estimated that the study had 80% power to detect a 55 m increase in the 6MWD with 95% confidence (two-sided $p<0.05$), based on a standard deviation (SD) of 75 m.

Analyses were conducted within the intention-to-treat (ITT) population, which consisted of all patients who received at least one dose of study medication. Dropouts were excluded from the analysis The primary efficacy analysis (6MWD) was performed using analysis of covariance (ANCOVA) with baseline value as a covariate. ANCOVAs were also used to assess between-group differences in pulmonary hemodynamics and blood gases. Missing data were not imputed so only subjects with assessment both at baseline and post-treatment were included in the ANCOVA analysis. FC was compared using Fisher's test.

In addition, exploratory analyses (post-hoc) were performed in subgroups classified according to baseline PVR values ≥or < 1,000 dynes.sec.cm^{-5} at baseline (i.e. the median PVR in the study).

Results

1. Disposition and baseline characteristics:

Fifty-nine patients (40 female; 19 male) were enrolled with 42 (71.2%) completing the 6 month study (Figure 7). The majority of dropouts not related to death were to worsening of PAH. Baseline characteristics were similar between the two treatment groups (Table 4). Overall, patients had a mean age of 44.3 years, mean weight of 68.7 kg and mean body mass index of 24.6 kg/m^2. Fifty five of the 59 patients were Caucasian and 78% had idiopathic PAH (Table 4). At baseline, 79% of the imatinib- and 81% of the placebo-group patients were receiving combination therapy (Table 4).

Table 4. Baseline characteristics of the intention to treat (ITT) population

	Imatinib (N=28)	Placebo (N=31)
Age (years), mean (SD)	44.4 (15.3)	44.2 (15.7)
Gender, male/female, n (%)	10 (36)/ 8 (64)	9 (29)/22 (71)
Ethnicity, n (%)		
Caucasian	26 (92)	29 (94)
Asian	0	1 (3)
Black	1 (4)	0
Pacific Islander	0	1 (3)
Hispanic	1 (4)	0
Weight (kg), mean (SD)	70.1 (14.7)	67.4 (23.4)
Height (cm), mean (SD)	168.6 (8.8)	164.3 (8.6)
Diagnosis, n (%)		
Idiopathic pulmonary hypertension	21 (75)	25 (81)
Familial pulmonary hypertension	2 (7)	0
Pulmonary hypertension secondary to systemic sclerosis	1 (4)	5 (16)
Other	4 (14)	1 (3)
WHO classification, n (%)*		
Class II	13 (48)	7 (23)
Class III	12 (44)	23 (74)
Class IV	2 (7)	1 (3)
PAH specific treatments, n (%)		
ERA alone	2 (7)	4 (13)
Sildenafil alone	2 (7)	0 (0)
Prostacylin analog alone	2 (7)	1 (3)
ERA + prostacylin analog	1 (4)	3 (10)
ERA + sildenafil	12 (43)	9 (29)
Sildenafil + prostacyclin analog	5 (18)	3 (10)
ERA + sildenafil + prostacyclin	4 (14)	10 (32)
Calcium channel blocker	0	1 (3)

SD: standard deviation; PH: pulmonary hypertension; prostacyclin analogues (iloprost, epoprostenol, trepostinil and beraprost); ERA: endothelin receptor antagonists (bosentan and ambrisentan)
*WHO assessment was not available for one patient receiving imatinib

2. Efficacy outcomes:

The mean (±SD) 6MWD did not significantly change in the imatinib group vs. placebo (+22±63 vs. –1.0±53 m; mean treatment difference 21.7 m ; 95% CI (-13.0, 56.5); p=0.21) (Table 5; Figure 8). There was, however, a significant decrease in PVR (mean treatment difference -230.7 dynes ; 95% CI (-383.7, -77.8; p=0.004) and increase in cardiac output (CO; mean treatment difference 0.68 L/min ; 95% CI (0.10, 1.26; p=0.02) in imatinib recipients compared with placebo (Figure 8). There was no significant difference in PAPm (Figure 8) or change in FC between imatinib and placebo treated patients (data not shown).

There was an increase in arterial and mixed venous oxygen saturation ($p<0.05$) with imatinib. Systemic arterial oxygen saturation increased from 88±9% to 93±5% with imatinib treatment compared with no change with placebo (92±4% at baseline vs. 92±3% at end of study) (mean treatment difference 2.4%; 95% CI (0.5, 4.3)); mixed venous oxygen saturation increased from 58±10% to 65±7% with imatinib treatment (consistent with the increase in CO) compared with a decrease with placebo (61±6% at baseline vs. 57±9% at end of study) (mean treatment difference 7.0%; 95% CI (2.1, 11.9)).

Table 5. Six-minute walking distance (6MWD) observed at baseline and end of study, and changes from baseline following imatinib and placebo therapy in patients with PAH. The change is expressed as the average alteration in 6MWD from baseline.

	Imatinib		**Placebo**		**Treatment difference (m)** [b]	**p-value** [b]
	Distance walked (m), mean (SD)	**Change vs. baseline (m)** [a] **mean (SD)**	**Distance walked (m), mean (SD)**	**Change vs. baseline (m)** [a] **mean (SD)**		
Baseline	392 (89) N=28	—	369 (118) N=29	—	—	—
Study end	419 (85) N=21	22 (63) N=21	399 (86) N=22	–1 (53) N=21	21.7	0.21

[a] Patients with both a baseline and end of study assessment.

[b] ANCOVA of ITT population

3. Exploratory subgroup analyses:

In patients with a baseline PVR ≥1,000 dynes.sec.cm^{-5}, there was a substantial improvement between baseline and study end for PAPm, CO, PVR and 6MWD in the imatinib group compared with placebo (Figure 9). However, among patients with a baseline PVR < 1,000 dynes.sec.cm^{-5}, no major differences between baseline and study end for PAPm, CO, PVR or 6MWD were observed (Figure 9).

4. Safety and tolerability:

The most common adverse events (AEs) observed in this clinical study were as expected for this population and this drug. The most common AEs reported in the imatinib group were nausea (N=14; 50%), headache (N=10; 35.7%) and peripheral edema (N=7; 25.0%). These

AEs did not lead to discontinuation of study drug. Nausea was controlled by taking the medication with food. A total of 21 (75%) patients in the imatinib group and 24 (77%) patients in the placebo group reported AEs of mild intensity, 20 (71%) in the imatinib group and 19 (61%) in the placebo group patients reported AEs of moderate intensity, and 9 (32%) patients in the imatinib group and 5 (16%) patients in the placebo group reported AEs of severe intensity. Serious AEs (SAEs) were reported for 11 imatinib recipients (39%) and 7 placebo recipients (23%). SAEs in the imatinib group included cardiac arrest (N=2), vertigo (n=1), pancreatitis (N=1), catheter related complication (N=1), liver dysfunction (N=2), dizziness (N=1), presyncope (N=1), syncope (N=1), haemoptysis (N=1), worsening pulmonary hypertension (N=3), and arterial rupture (N=1). SAEs in the placebo group included atrial flutter (N=1), cardiac arrest (N=2), right ventricular failure (N=2), general physical health deterioration (N=1), fluid retention (N=1), dizziness (N=1), and worsening pulmonary hypertension (N=3).

Overall there was a fall in the haemoglobin levels with imatinib (151±14 to 128±16 g/L, SD) and a rise in hemoglobin levels with placebo (143±25 to 152±25 g/L). There were no relevant changes over time on the following variables: white blood cell count, platelet count albumin, alkaline phosphatase, total bilirubin, calcium, cholesterol, creatinine, g-GT, glucose, lactate dehydrogenase, inorganic phosphorus, lipase, amylase, potassium, total protein, C-reactive protein, glutamate oxalacetate transaminase, glutamate pyruvate transaminase, sodium, triglycerides, urea, and uric acid.

There were three deaths in each group. Two additional patients died in the placebo group within 2 months of completing the study. One patient in the imatinib group and one patient in the placebo group had rupture of the pulmonary artery (fatal in both cases).

Discussion

This is the first randomized, double-blind, placebo controlled trial to assess the safety, tolerability and efficacy of the tyrosine kinase inhibitor imatinib in patients with PAH. Although imatinib appeared safe and well tolerated over a 6 month period, the primary efficacy parameter (6MWD) did not improve in patients randomized to imatinib compared with placebo, despite significant improvement in secondary endpoints.

Treatment efficacy

Overall, 59 patients were enrolled. As per study protocol, only patients on background treatment with at least one PAH specific drug (i.e. prostacyclin analogues, ERAs, PDE5 inhibitors) who had not adequately improved were enrolled (56% of patients were receiving two drugs and 24% receiving three drugs at baseline). This may have contributed to the reduced improvement in 6MWD observed in this study compared with previous studies in which only treatment naïve patients were included. In clinical trials in which background specific medications have been allowed, the overall improvement in 6MWD has been less than in the treatment naïve trials.

Safety aspects

It has been suggested that inhibition of the ABL tyrosine kinase pathway may infrequently induce myocardial damage in patients receiving long-term treatment with imatinib for chronic myelogenous leukemia (CML). However, a long-term, multicenter study in a large population of patients with CML showed an acceptable safety profile for imatinib. A review of all patients receiving imatinib shows that 0.5% of patients per year developed incident congestive cardiac failure (no risk factors present). In patients with CML receiving imatinib, 0.4% of patients per year develop congestive cardiac failure compared with 0.75% per year for patients receiving interferon gamma plus Ara-C. Considering the potential for cardiotoxicity which could be even more problematic for patients with PAH, regular assessments of cardiac function by echocardiography and measurements of serum cardiac troponin levels were performed in this trial. Overall, there were no signals indicating a potential detrimental effect of imatinib on myocardial function when compared to the overall safety profile of the placebo group. In contrast, some of the beneficial effect of imatinib on PVR reduction appeared to be due to improvements in CO, suggestive of improved right ventricular contractility in patients with PAH. Nonetheless, cardiac safety remains a key concern with other kinase inhibitors, such as sunitinib.

Exploratory subgroup analysis

Although no significant increases in 6MWD were observed with imatinib compared with placebo, significant improvements in CO and PVR were observed. These observations led us to undertake a post-hoc analysis stratifying patients by baseline PVR. In patients with baseline PVR ≥1,000 dynes.sec.cm^{-5}, there was a substantial improvement from baseline to study end for 6MWD, PVR, and CO in the imatinib group, when compared with placebo (Figure 9). This was not observed in the patients with PVR levels <1,000 dynes.sec.cm^{-5}.

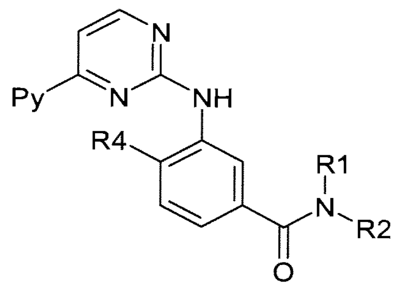
However, these results have to be interpreted with caution as this was an unplanned analysis. In addition, tyrosine kinase inhibitors are not recognized to have any significant vasodilator or inotropic effects, with their effects considered anti-proliferative and pro-apoptotic. One hypothesis that could explain the current study results is that for treatment with imatinib to be effective, a certain degree of disease severity (i.e. vascular proliferation) may be needed. However, as these data are hypothesis generating, it cannot be excluded that less severe patients with PAH may also benefit from long-term imatinib therapy via a preventive mechanism.

Conclusion and perspective

The results of this pilot study suggest that imatinib is safe and well tolerated in patients with PAH. In addition, the efficacy analyses provide proof of concept supporting the use of agents targeting proliferative growth factor pathways in PAH.

__Claims:__

1. Use of 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I



(I)

wherein

Py denotes 3-pyridyl,

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for treating pulmonary arterial hypertension (PAH) in patients who failed prior PAH therapy.

2. The use according to claim 1, wherein 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof is used.

3. The use according to claim 2 wherein 4-(4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide is used in the form of the mono-methanesulfonate salt.

4. The use according to claim 1, wherein a pyrimidylaminobenzamide of formula I, wherein the radicals and symbols have the meaning as defined in claim 1 or a pharmaceutically acceptable salt thereof, is used.

5. The use according to claim 4, wherein the pyrimidylaminobenzamide is 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl] benzamide.

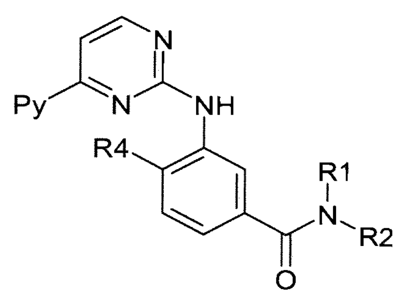
6. The use according to claim 5, wherein 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl] benzamide is used in the form of its hydrochloride monohydrate.

7. The use according to any one of claims 1 to 6, wherein prior PAH therapy included receiving at least one prostanoid, endothelin antagonist or PDE V inhibitor.

8. The use according to any one of claims 1 to 6, wherein the medicament is designated for treating PAH in patients who are more severely affected.

9. The use according to any one of claims 1 to 6, wherein the medicament is designated for treating PAH in patients who are harboring BMPR2 mutations.

10. A method of treating humans suffering from pulmonary arterial hypertension (PAH) in patients who failed prior PAH therapy, which comprises administering to a said human in need of such treatment a dose effective against PAH of 4-methylpiperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl]-benzamide or a pharmaceutically acceptable salt thereof or a pyrimidylaminobenzamide of formula I



(I)

wherein

Py denotes 3-pyridyl,

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl;

benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

or a pharmaceutically acceptable salt thereof.

11. A method of treating humans suffering from

(a) idiopathic or primary pulmonary hypertension,

(b) familial hypertension,

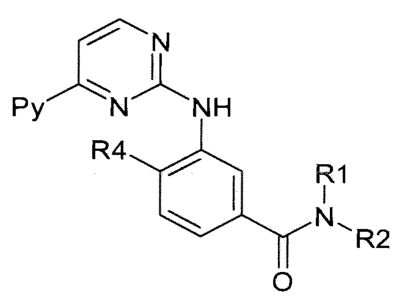
(c) pulmonary hypertension secondary to, but not limited to, connective tissue disease, congenital heart defects (shunts), pulmonary fibrosis, portal hypertension, HIV infection, sickle cell disease, drugs and toxins (e.g., anorexigens, cocaine), chronic hypoxia, chronic pulmonary obstructive disease, sleep apnea, and schistosomiasis,

(d) pulmonary hypertension associated with significant venous or capillary involvement (pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis),

(e) secondary pulmonary hypertension that is out of proportion to the degree of left ventricular dysfunction,

(f) persistent pulmonary hypertension in newborn babies,

which comprises administering to said human in need of such treatment a dose effective against the respective disorder a pyrimidylaminobenzamide of formula I



(I)

wherein

Py denotes 3-pyridyl,

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

or a pharmaceutically acceptable salt thereof.

Fig. 1

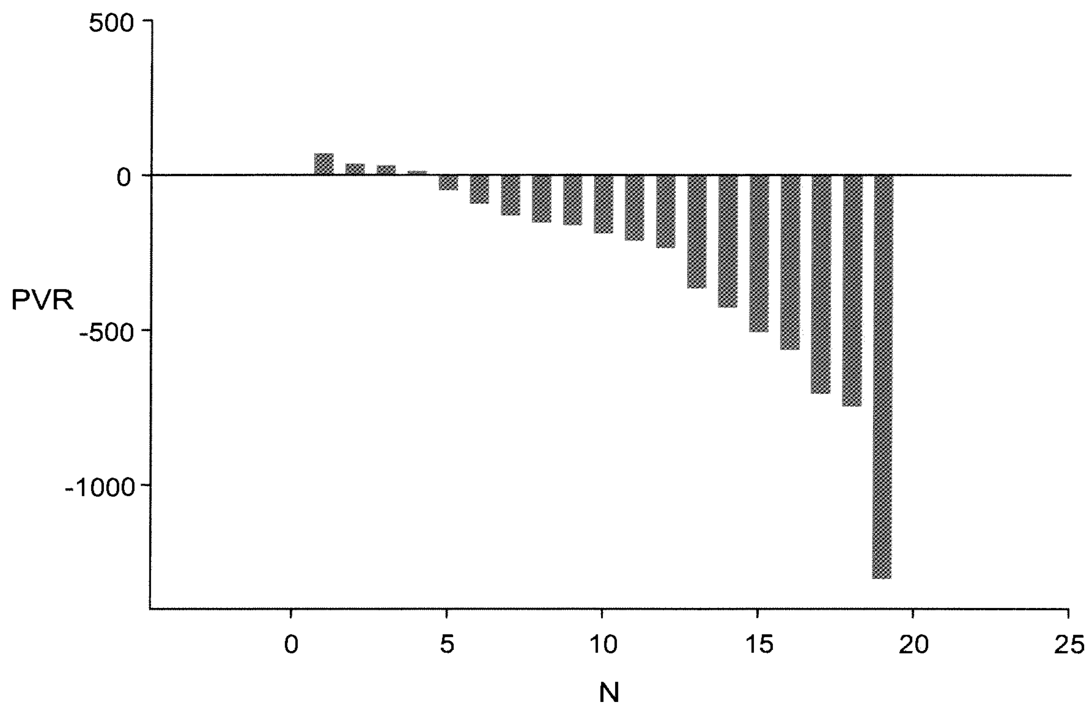


Fig. 2

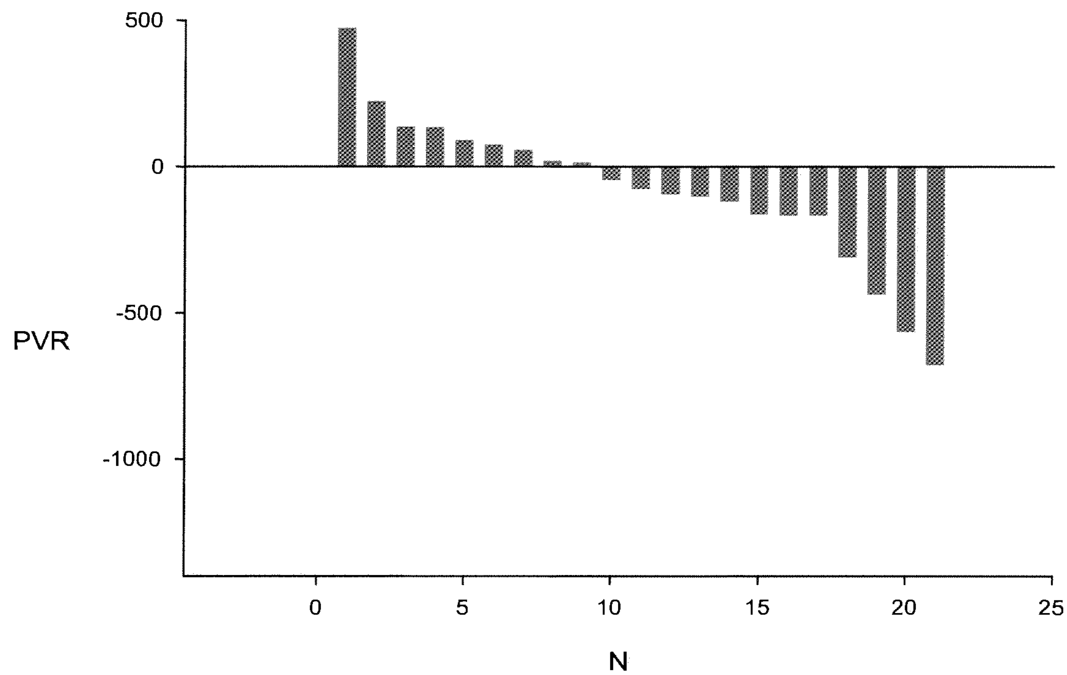


Fig. 3

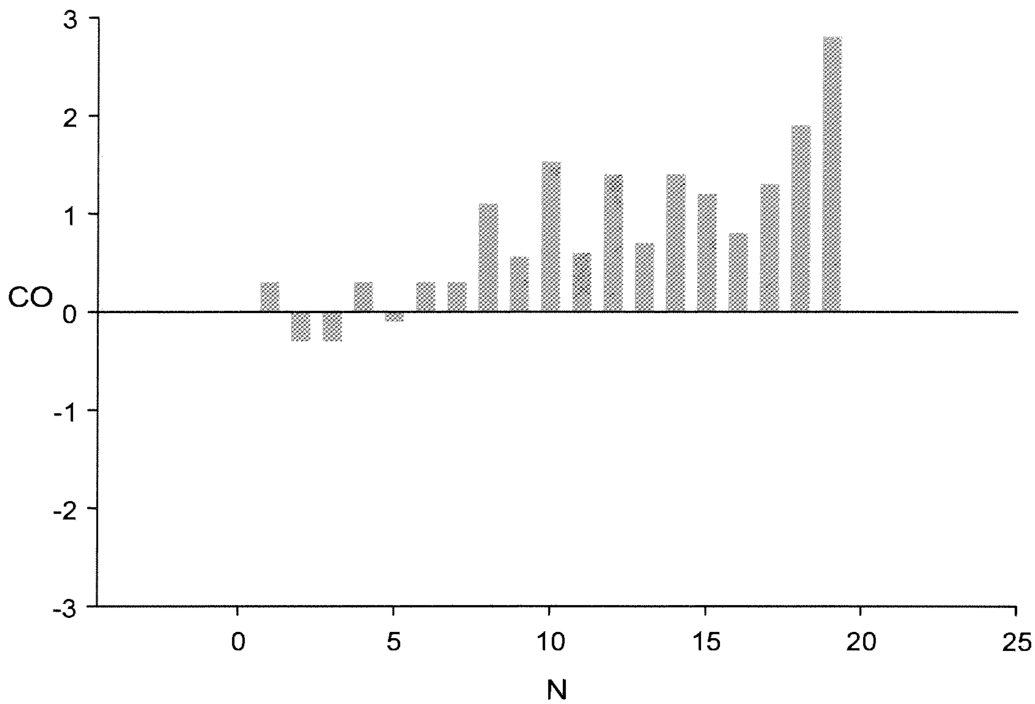


Fig. 4

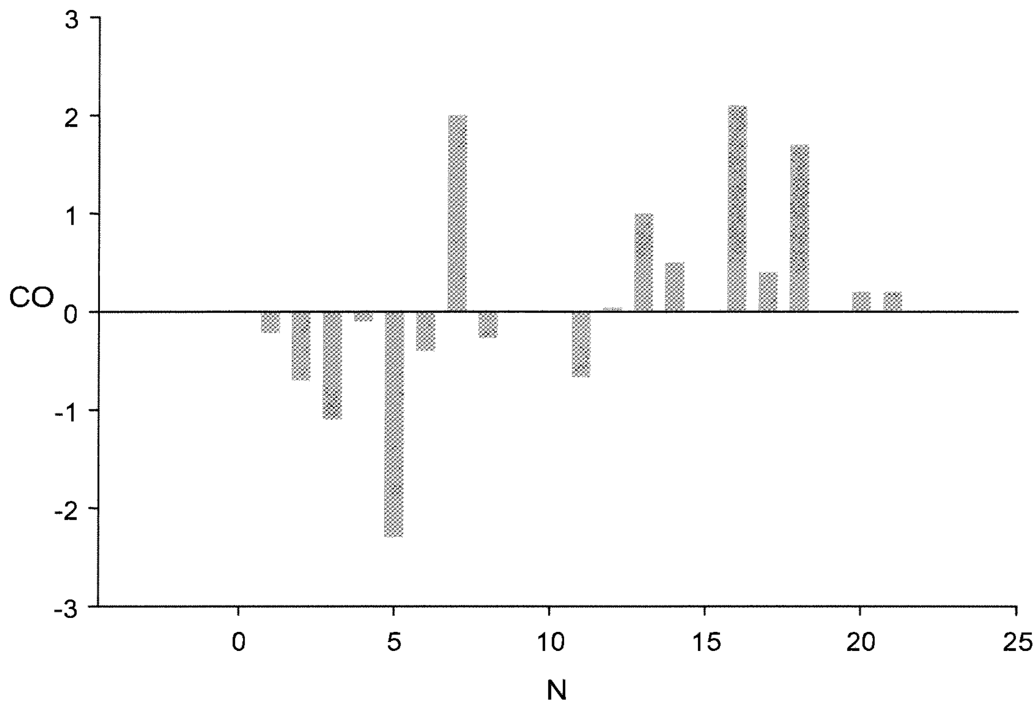


Fig. 5

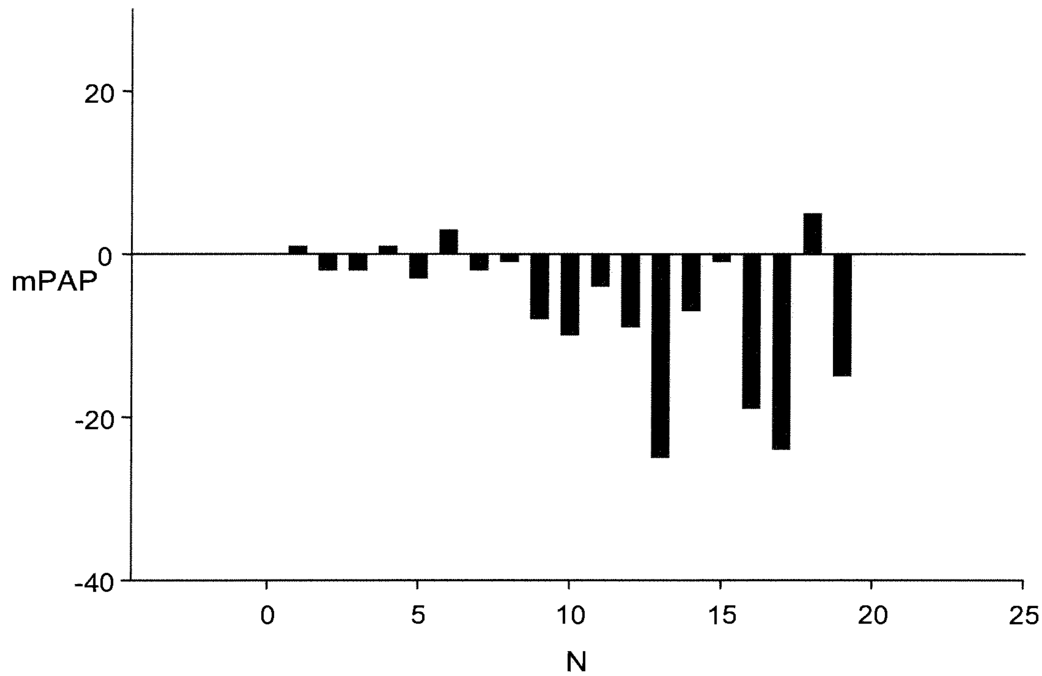


Fig. 6

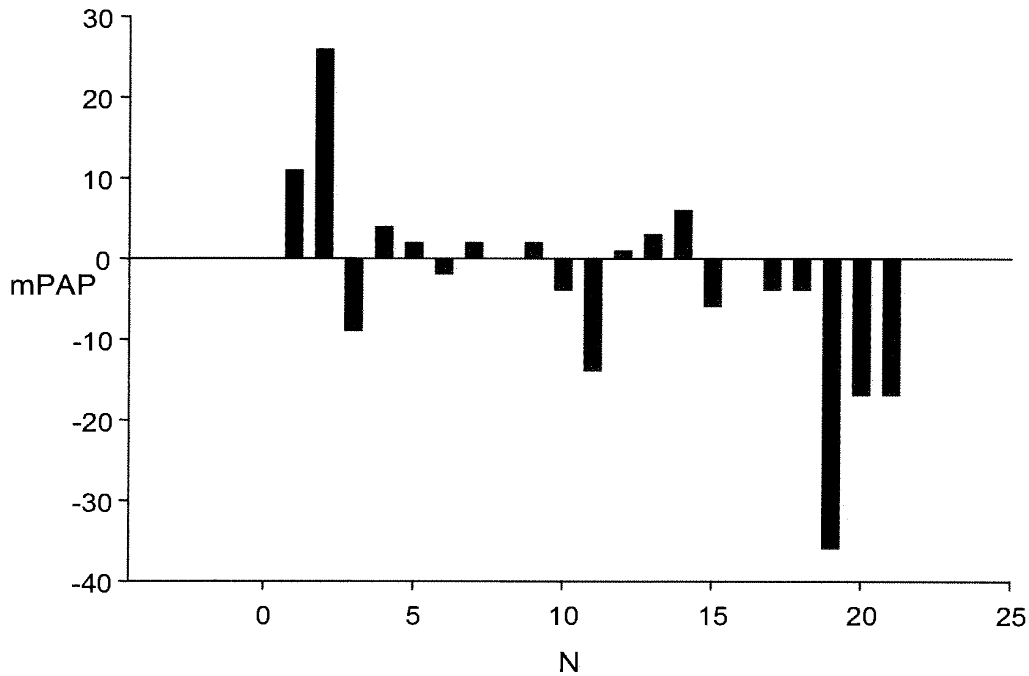
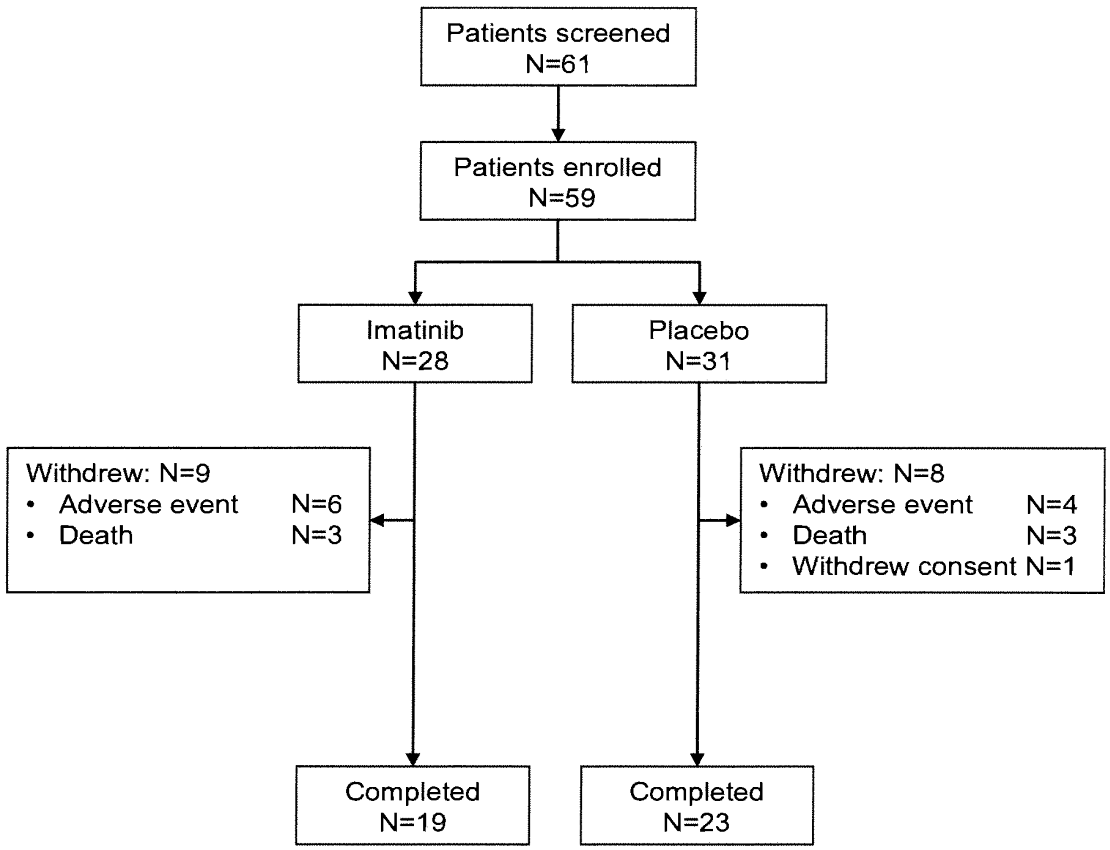


Fig. 7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Patients enrolled
N=59
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N=28
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• Adverse event N=6
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Fig. 8

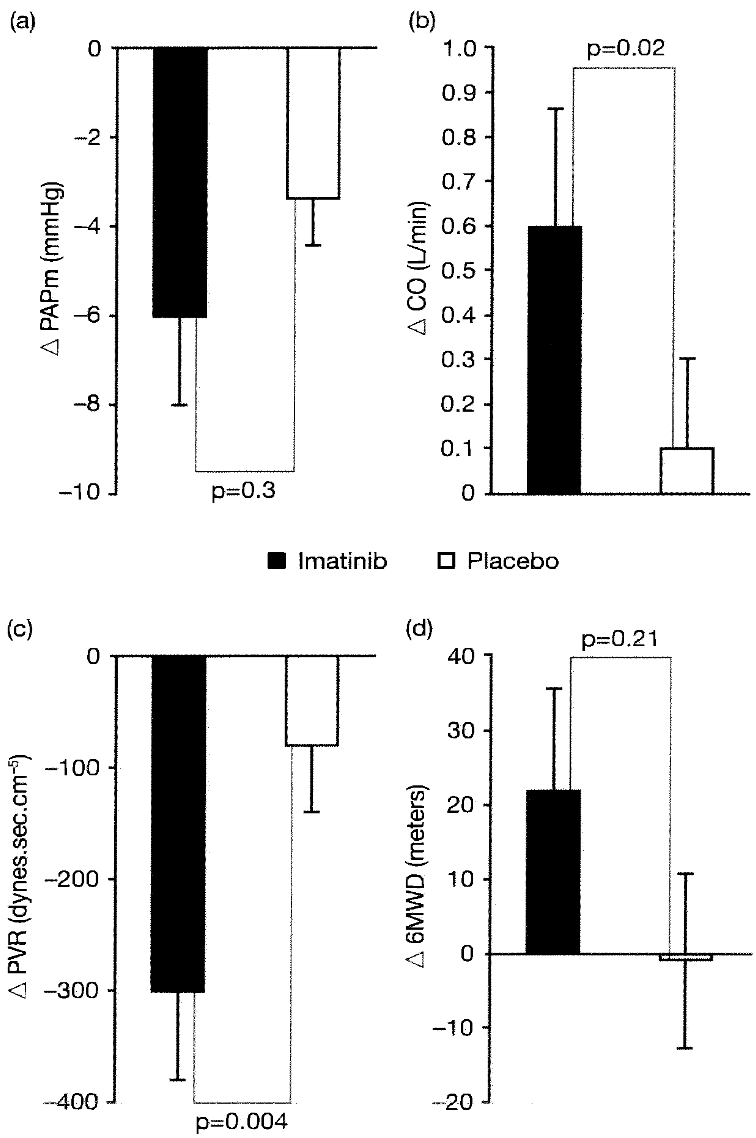
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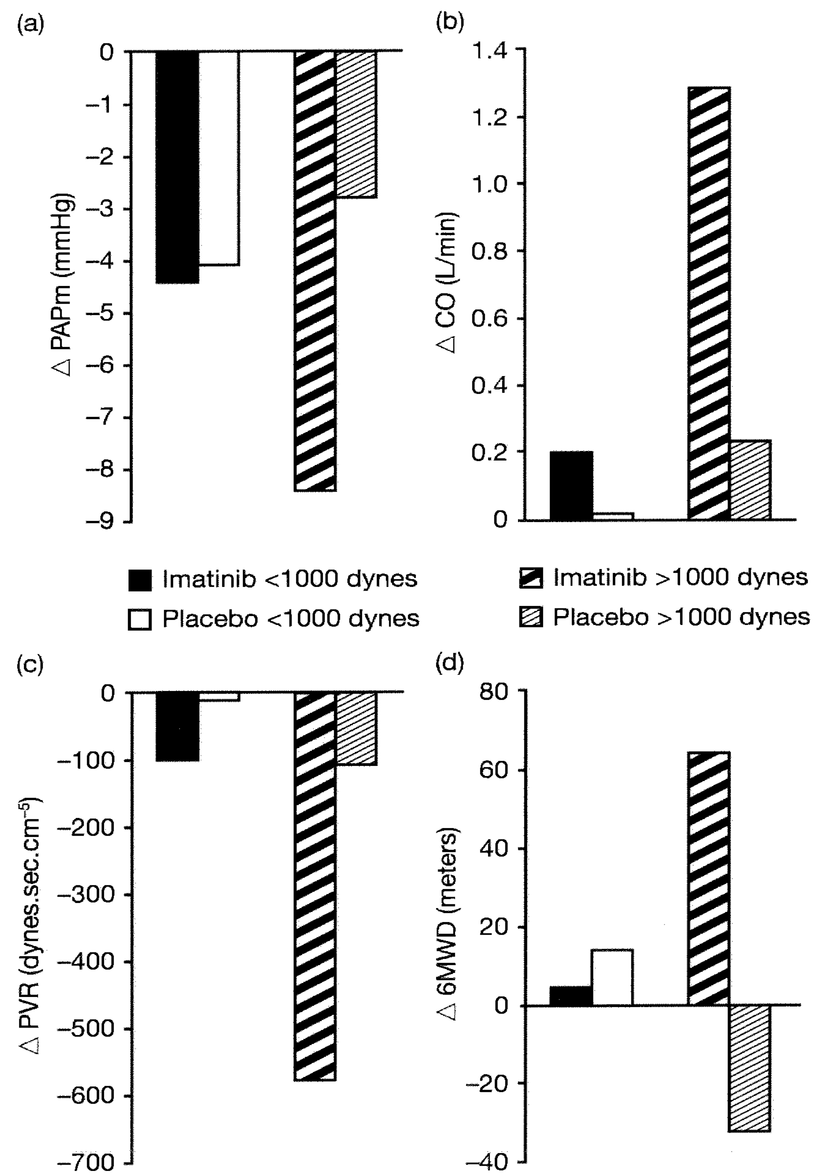
Fig. 9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Imatinib >1000 dynes
Placebo >1000 dynes
(c)
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−700
△ PVR (dynes.sec.cm⁻⁵)
(d)
80
60
40
20
0
−20
−40
△ 6MWD (meters)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/053358

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/506 A61P9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GHOFRANI, H. A. ET AL.: "Imatinib for the Treatment of Pulmonary Arterial Hypertension" THE NEW ENGLAND JOURNAL OF MEDICINE, vol. 353, no. 13, 2005, pages 1412-1413, XP002550196 the whole document	1-3,7-11

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2009	27/10/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Sahagún Krause, H

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHERMULY R T ET AL: "REVERSAL OF EXPERIMENTAL PULMONARY HYPERTENSION BY PDGF INHIBITION" JOURNAL OF CLINICAL INVESTIGATION, AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, US, vol. 115, no. 10, 1 October 2005 (2005-10-01), pages 2811-2821, XP008056354 ISSN: 0021-9738 abstract	1-3,7,11
Y	WO 2006/079539 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; MANLEY PAUL W [CH]; MARTI) 3 August 2006 (2006-08-03) claim 8	1-11
A	ZIMMERMANN J ET AL: "Potent and selective inhibitors of the Abl-kinase: phenylamino-pyrimidine (PAP) derivatives" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB, vol. 7, no. 2, 21 January 1997 (1997-01-21), pages 187-192, XP004135990 ISSN: 0960-894X compound 13	1-11
Y	PERROS, F ET AL.: "Platelet-derived Growth Factor Expression and Function in Idiopathic Pulmonary Arterial Hypertension" AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, vol. 178, 2008, pages 81-88, XP002550197 the whole document	1-11
Y	WO 2004/005281 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; BREITENSTEIN WERNER [CH];) 15 January 2004 (2004-01-15) cited in the application claim 1, page 2, lines 1-2, table in pages 69-70	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/053358

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 2006079539	A	03-08-2006	AU	2006208638	A1	03-08-2006
			BR	PI0606872	A2	21-07-2009
			CA	2594687	A1	03-08-2006
			EP	1843771	A2	17-10-2007
			JP	2008528531	T	31-07-2008
			KR	20070104567	A	26-10-2007
			US	2008114001	A1	15-05-2008
WO 2004005281	A	15-01-2004	AT	414699	T	15-12-2008
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			CA	2491632	A1	15-01-2004
			CN	1675195	A	28-09-2005
			DK	1532138	T3	23-03-2009
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			EP	1532138	A1	25-05-2005
			EP	2100891	A1	16-09-2009
			ES	2318164	T3	01-05-2009
			HK	1077811	A1	24-07-2009
			JP	4110140	B2	02-07-2008
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			US	2008188451	A1	07-08-2008
			US	2006167015	A1	27-07-2006
			US	2007093506	A1	26-04-2007

Form PCT/ISA/210 (patent family annex) (April 2005)

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulminary Hypertens
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Daniel Leo Hayes/Anna Goforth
Attorney Docket Number:	I001-0002USC4

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	82	82
Utility Search Fee	2111	1	270	270
Utility Examination Fee	2311	1	110	110
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Petition fee- 37 CFR 1.17(h) (Group III)	1464	1	130	130

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**592**

Electronic Acknowledgement Receipt

EFS ID:	7870664
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulminary Hypertens
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Daniel Leo Hayes/Anna Goforth
Filer Authorized By:	Daniel Leo Hayes
Attorney Docket Number:	I001-0002USC4
Receipt Date:	22-JUN-2010
Filing Date:	
Time Stamp:	19:31:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$592
RAM confirmation Number	6199
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		MV9460.PDF	236005 33d50d32dc3fbd1158bdee4cc920925ba0b81dfe	yes	27

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	22
Claims	23	26
Abstract	27	27

Warnings:

Information:

2	Oath or Declaration filed	MV9708.PDF	242838 520d458d06cd4ae56f29fe2ae3791fb96812b53c	no	3

Warnings:

Information:

3	Application Data Sheet	MW2778.PDF	49042 cae3d6505573f2936d9a8d96cf11d29e2c7153f8	no	4

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5	Statement of preexamination search	MV7407.PDF	680378 4246a463ac8707681c6773b495b7982e884c1cb6	no	18

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6	Examination support document	MV7410.PDF	1014232 fa12b49dc3dd3de11fc09b267652962c21551e77	no	21

Warnings:

Information:

7	Miscellaneous Incoming Letter	MV7421.PDF	56771 779c70d9005bc854ac22390e182899c247ab7c10	no	1

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8	Information Disclosure Statement (IDS) Filed (SB/08)	MV7419.PDF	690067 38ddc238c857552d51b451474ec10715397dd414	no	10
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13	NPL Documents	Dickstein.pdf	1164317 b68f0ae57fd156332846c0019da45b6ae2f2870f	no	7
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14	Foreign Reference	EP1682672complete.pdf	1400970 738c8464cb7592f1ef420f485d6c139d9f0a057a	no	34
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17	NPL Documents	Findlay.pdf	1893043 81532a5cb90c341f74678bbc27570b953dffc322	no	6
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18	NPL Documents	Greenough.pdf	1514355 cc1d63f4b66c23a47027ce740844d182d1c92c0a	no	10
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19	NPL Documents	Henrichsen.pdf	113722 306fa13203b9eaaf5bbb176599b02d1292d3c682	no	1
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22	NPL Documents	Murray.pdf	181184 81d18e921668524c8444efdd72ed6d6e7c38c4e0	no	13
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23	NPL Documents	Ovodov.pdf	1106949 097f67216a6d0fa01657885de7eae021c8bd8a05	no	10
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24	NPL Documents	Roberts.pdf	125664 72d7b695f9f735d59fa15a67f388a29956756a67	no	6
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26	NPL Documents	Steinhorn.pdf	1410301 8d7fe85135307fc77abd49827b2001bd70dbd1db	no	6
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27	NPL Documents	UseOfInhaledNitricOxide.pdf	43761 312c680628f980dc28a0244d3670e572e2a976dc	no	2
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28	NPL Documents	Watson.pdf	336709 7dbe96512560171b59f13aa529668b469413b68f	no	13
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29	NPL Documents	Weinberger.pdf	142277 5892581a366750e3587cd30581d2d7beaada4b06	no	11
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Information:					
30	Foreign Reference	WO2005004884.pdf	6242109 aa65b10d87c6ca4e7d6fe966d8c9cdf78e1ca50a	no	79
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32	Foreign Reference	WO2010019540.pdf	2841627 9b0cf949c0e30bd57a3ff13d8f93515ce219043e	no	46
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33	Fee Worksheet (PTO-875)	fee-info.pdf	36754 c9ecda4482d29d7458256d49d39543553bcb9758	no	2
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METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of and claims priority to U.S. Patent Application Serial No. 12/494,598, entitled "Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension", filed on June 30, 2009, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] INOmax®, (nitric oxide) for inhalation is an approved drug product for the treatment of term and near-term (>34 weeks gestation) neonates having hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension.

[0003] The use of inhaled NO (iNO) has been studied and reported in the literature. (Kieler-Jensen M et al., 1994, Inhaled Nitric Oxide in the Evaluation of Heart Transplant Candidates with Elevated Pulmonary Vascular Resistance, *J Heart Lung Transplantation* 13:366-375; Pearl RG et al., 1983, Acute Hemodynamic Effects of Nitroglycerin in Pulmonary Hypertension, *American College of Physicians* 99:9-13; Ajami GH et al., 2007, Comparison of the Effectiveness of Oral Sildenafil Versus Oxygen Administration as a Test for Feasibility of Operation for Patients with Secondary Pulmonary Arterial Hypertension, *Pediatr Cardiol*; Schulze-Neick I et al., 2003, Intravenous Sildenafil Is a Potent Pulmonary Vasodilator in Children With Congenital Heart Disease, *Circulation* 108(Suppl II):II-167-II-173; Lepore JJ et al., 2002, Effect of Sildenafil on the Acute Pulmonary Vasodilator Response to Inhaled Nitric Oxide in Adults with Primary Pulmonary Hypertension, *The American Journal of Cardiology* 90:677-680; and Ziegler JW et al., 1998, Effects of Dipyridamole and Inhaled Nitric Oxide in Pediatric Patients with Pulmonary Hypertension, *American Journal of Respiratory and Critical Care Medicine* 158:1388-95).

SUMMARY OF THE INVENTION

[0004] One aspect of the invention relates to a pre-screening methodology or protocol having exclusionary criteria to be evaluated by a medical provider prior to treatment of a patient with iNO. One objective of the invention is to evaluate and possibly exclude from treatment patients eligible for treatment with iNO, who have pre-existing left ventricular dysfunction (LVD). Patients who have pre-existing LVD may experience, and are at risk of, an increased rate of adverse events or serious adverse events (e.g., pulmonary edema) when treated with iNO. Such patients may be characterized as having a pulmonary capillary wedge pressure (PCWP) greater than 20 mm Hg, and should be evaluated on a case-by-case basis with respect to the benefit versus risk of using iNO as a treatment option.

[0005] Accordingly, one aspect of the invention includes a method of reducing the risk or preventing the occurrence, in a human patient, of an adverse event (AE) or a serious adverse event (SAE) associated with a medical treatment comprising inhalation of nitric oxide, said method comprising the steps or acts of (a) providing pharmaceutically acceptable nitric oxide gas to a medical provider; and, (b) informing the medical provider that excluding human patients who have pre-existing left ventricular dysfunction from said treatment reduces the risk or prevents the occurrence of the adverse event or the serious adverse event associated with said medical treatment.

[0006] Further provided herein is a method of reducing the risk or preventing the occurrence, in a human patient, of an adverse event or a serious adverse event associated with a medical treatment comprising inhalation of nitric oxide, said method comprising the steps or acts of (a.) providing pharmaceutically acceptable nitric oxide gas to a medical provider; and, (b.) informing the medical provider that human patients having pre-existing left ventricular dysfunction experience an increased risk of serious adverse events associated with said medical treatment.

[0007] Another aspect of the invention is a method of reducing one or more of an AE or a SAE in an intended patient population in need of being treated with iNO comprising the steps or acts of (a.) identifying a patient eligible for iNO treatment; (b) evaluating and screening the patient to identify if the patient has pre-existing LVD, and (c) excluding from iNO treatment a patient identified as having pre-existing LVD.

[0008] Another aspect of the invention is a method of reducing the risk or preventing the occurrence, in a patient, of one or more of an AE or a SAE associated with a medical treatment comprising iNO, the method comprising the steps or acts of (a.) identifying a patient in need of receiving iNO treatment; (b.) evaluating and screening the patient to identify if the patient has pre-existing LVD; and (c.)administering iNO if the patient does not have pre-existing LVD, thereby reducing the risk or preventing the occurrence of the AE or the SAE associated with the iNO treatment. Alternatively, step (c) may comprise further evaluating the risk versus benefit of utilizing iNO in a patient where the patients has clinically significant LVD before administering iNO to the patient.

[0009] In an exemplary embodiment of the method, the method further comprises informing the medical provider that there is a risk associated with using inhaled nitric oxides in human patients who have preexisting or clinically significant left ventricular dysfunction and that such risk should be evaluated on a case by case basis.

[0010] In another exemplary embodiment of the method, the method further comprises informing the medical provider that there is a risk associated with using inhaled nitric oxide in human patients who have left ventricular dysfunction.

[0011] In an exemplary embodiment of the methods described herein, a patient having pre-existing LVD is characterized as having PCWP greater than 20 mm Hg.

[0012] In an exemplary embodiment of the method, the patients having pre-existing LVD demonstrate a PCWP $\geq$ 20 mm Hg.

[0013] In another exemplary embodiment of the method, the iNO treatment further comprises inhalation of oxygen (O_2) or concurrent ventilation.

[0014] In another exemplary embodiment of the method, the patients having pre-existing LVD have one or more of diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease, congenital heart disease, or, associations thereof.

[0015] In another exemplary embodiment of the method, the patient population comprises children.

[0016] In another exemplary embodiment of the method, the patient population comprises adults.

[0017] In another exemplary embodiment of the method, the patients who have pre-existing LVD are at risk of experiencing and increased rate of one or more AEs or SAEs selected from pulmonary edema, hypotension, cardiac arrest, electrocardiogram changes, hypoxemia, hypoxia, bradycardia or associations thereof.

[0018] In another exemplary embodiment of the method, the intended patient population in need of being treated with inhalation of nitric oxide has one or more of idiopathic pulmonary arterial hypertension characterized by a mean pulmonary artery pressure (PAPm) > 25 mm Hg at rest, PCWP ≤ 15 mm Hg, and, a pulmonary vascular resistance index (PVRI) > 3 $u \cdot m^2$; congenital heart disease with pulmonary hypertension repaired and unrepaired characterized by PAPm > 25 mm Hg at rest and PVRI > 3 $u \cdot m^2$; cardiomyopathy characterized by PAPm > 25 mm Hg at rest and PVRI > 3 $u \cdot m^2$; or, the patient is scheduled to undergo right heart catheterization to assess pulmonary vasoreactivity by acute pulmonary vasodilatation testing.

[0019] In another exemplary embodiment of any of the above methods, the method further comprises reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient. The left ventricular afterload may be minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient. The left ventricular afterload may also be minimized or reduced using an intra-aortic balloon pump.

DETAILED DESCRIPTION OF THE EXEMPLARY EMBODIMENTS

[0020] INOmax® (nitric oxide) for inhalation was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999. Nitric oxide, the active substance in INOmax®, is a selective pulmonary vasodilator that increases the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from the lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios. INOmax® significantly improves oxygenation, reduces the need for extracorporeal oxygenation and is indicated to be used in conjunction with ventilatory support and other appropriate agents. The current FDA-approved prescribing information for INOmax® is incorporated herein by reference in its entirety.

[0021] INOmax® is a gaseous blend of NO and nitrogen (0.08% and 99.92% respectively for 800 ppm; and 0.01% and 99.99% respectively for 100 ppm) and is supplied in aluminium cylinders as a compressed gas under high pressure. In general, INOmax® is administered to a patient in conjunction with ventilatory support and O_2. Delivery devices suitable for the safe and effective delivery of gaseous NO for inhalation include the INOvent®, INOmax DS®, INOpulse®, INOblender®, or other suitable drug delivery and regulation devices or components incorporated therein, or other related processes, which are described in various patent documents including USPNs 5,558,083; 5,732,693; 5,752,504; 5,732,694; 6,089,229; 6,109,260; 6,125,846; 6,164,276; 6,581,592; 5,918,596; 5,839,433; 7,114,510; 5,417;950; 5,670,125; 5,670,127; 5,692,495; 5,514,204; 7,523,752; 5,699,790; 5,885,621; US Patent Application Serial Nos. 11/355,670 (US 2007/0190184); 10/520,270 (US 2006/0093681); 11/401,722 (US 2007/0202083); 10/053,535 (US 2002/0155166); 10/367,277 (US 2003/0219496); 10/439,632 (US 2004/0052866); 10/371,666 (US 2003/0219497); 10/413,817 (US 2004/0005367); 12/050,826 (US 2008/0167609); and PCT/US2009/045266, all of which are incorporated herein by reference in their entirety.

[0022] Such devices deliver INOmax® into the inspiratory limb of the patient breathing circuit in a way that provides a constant concentration of NO to the patient throughout the inspired breath. Importantly, suitable delivery devices provide continuous integrated monitoring of inspired O_2, NO_2 and NO, a comprehensive alarm system, a suitable power source for uninterrupted NO delivery and a backup NO delivery capability.

[0023] As used herein, the term "children" (and variations thereof) includes those being around 4 weeks to 18 years of age.

[0024] As used herein, the term "adult" (and variations thereof) includes those being over 18 years of age.

[0025] As used herein, the terms "adverse event" or "AE" (and variations thereof) mean any untoward occurrence in a subject, or clinical investigation subject administered a pharmaceutical product (such as nitric oxide) and which does not necessarily have a causal relationship with such treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal/investigational product, whether or not related to the investigational product. A relationship to the investigational product is not necessarily proven or implied. However, abnormal values are not reported as adverse events unless considered clinically significant by the investigator.

[0026] As used herein, the terms "adverse drug reaction" or "ADR" (and variations thereof) mean any noxious and unintended response to a medicinal product related to any dose.

[0027] As used herein, the terms "serious adverse event" or "SAE" (or "serious adverse drug reaction" or "serious ADR") (and variations thereof) mean a significant hazard or side effect, regardless of the investigator's opinion on the relationship to the investigational product. A serious adverse event or reaction is any untoward medical occurrence that at any dose: results in death; is life-threatening (which refers to an event/reaction where the patient was at risk of death at the time of the event/reaction, however this does not refer to an event/reaction that hypothetically may have caused death if it were more severe); requires inpatient hospitalization or results in prolongation of existing hospitalization; results in persistent or significant disability/incapacity; is a congenital anomaly/birth defect; or, is a medically important event or reaction. Medical and scientific judgment is exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed above--these are also considered serious. Examples of such medical events include cancer, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalizations, or the development of drug dependency or drug abuse. Serious clinical laboratory abnormalities directly associated with relevant clinical signs or symptoms are also reported.

[0028] Left Ventricular Dysfunction. Patients having pre-existing LVD may be described in general as those with elevated pulmonary capillary wedge pressure, including those with diastolic dysfunction (including hypertensive cardiomyopathy), those with systolic dysfunction, including those with cardiomyopathies (including ischemic or viral cardiomyopathy, or idiopathic cardiomyopathy, or autoimmune disease related cardiomyopathy, and side effects due to drug related or toxic-related cardiomyopathy), or structural heart disease, valvular heart disease, congenital heart disease, idiopathic pulmonary arterial hypertension, pulmonary hypertension and cardiomyopathy, or associations thereof. Identifying patients with pre-existing LVD is known to those skilled in the medicinal arts, and such techniques for example may include assessment of clinical signs and symptoms of heart failure, or echocardiography diagnostic screening.

[0029] Pulmonary Capillary Wedge Pressure. Pulmonary capillary wedge pressure, or "PCWP", provides an estimate of left atrial pressure. Identifying patients with pre-existing PCWP is known to those skilled in the medicinal arts, and such techniques for example may include measure by inserting balloon-tipped, multi-lumen catheter (also known as a Swan-Ganz catheter). Measure of PCWP may be used as a means to diagnose the severity of LVD (sometimes also referred to as left ventricular failure). PCWP is also a desired measure when evaluating pulmonary hypertension. Pulmonary hypertension is often caused by an increase in pulmonary vascular resistance (PVR), but may also arise from increases in pulmonary venous pressure and pulmonary blood volume secondary to left ventricular failure or mitral or aortic valve disease.

[0030] In cardiac physiology, afterload is used to mean the tension produced by a chamber of the heart in order to contract. If the chamber is not mentioned, it is usually assumed to be the left ventricle. However, the strict definition of the term relates to the properties of a single cardiac myocyte. It is therefore only of direct relevance in the laboratory; in the clinic, the term end-systolic pressure is usually more appropriate, although not equivalent.

[0031] The terms "left ventricular afterload" (and variations thereof) refer to the pressure that the chamber of the heart has to generate in order to eject blood out of the chamber. Thus, it is a consequence of the aortic pressure since the pressure in the ventricle must be greater than the systemic pressure in order to open the aortic valve. Everything else held equal, as afterload increases, cardiac output decreases. Disease processes that increase the left ventricular afterload include increased blood pressure and aortic valve disease. Hypertension (Increased blood pressure) increases the left ventricular afterload because the left ventricle has to work harder to eject blood into the aorta. This is because the aortic valve won't open until the pressure generated in the left ventricle is higher than the elevated blood pressure. Aortic stenosis increases the afterload because the left ventricle has to overcome the pressure gradient caused by the stenotic aortic valve in addition to the blood pressure in order to eject blood into the aorta. For instance, if the blood pressure is 120/80, and the aortic valve stenosis creates a trans-valvular gradient of 30 mmHg, the left ventricle has to generate a pressure of 110 mmHg in order to open the aortic valve and eject blood into the aorta. Aortic insufficiency increases afterload because a percentage of the blood that is ejected forward regurgitates back through the diseased aortic valve. This leads to elevated systolic blood pressure. The diastolic blood pressure would fall, due to regurgitation. This would result in an increase pulse pressure. Mitral regurgitation decreases the afterload. During ventricular systole, the blood can regurgitate through the diseased mitral valve as well as be ejected through the aortic valve. This means that the left ventricle has to work less to eject blood, causing a decreased afterload. Afterload is largely dependent upon aortic pressure.

[0032] An intra-aortic balloon pump (IABP) is a mechanical device that is used to decrease myocardial oxygen demand while at the same time increasing cardiac output. By increasing cardiac output it also increases coronary blood flow and therefore myocardial oxygen delivery. It consists of a cylindrical balloon that sits in the aorta and counterpulsates. That is, it actively deflates in systole increasing forward blood flow by reducing afterload thus, and actively inflates in diastole increasing blood flow to the coronary arteries. These actions have the combined result of decreasing myocardial oxygen demand and increasing myocardial oxygen supply. The balloon is inflated during diastole by a computer controlled mechanism, usually linked to either an ECG or a pressure transducer at the distal tip of the catheter; some IABPs, such as the Datascope System 98XT, allow for asynchronous counterpulsation at a set rate, though this setting is rarely used. The computer controls the flow of helium from a cylinder into and out of the balloon. Helium is used because its low viscosity allows it to travel quickly through the long connecting tubes, and has a lower risk of causing a harmful embolism should the balloon rupture while in use. Intraaortic balloon counterpulsation is used in situations when the heart's own cardiac output is insufficient to meet the oxygenation demands of the body. These situations could include cardiogenic shock, severe septic shock, post cardiac surgery and numerous other situations.

[0033] Patients eligible for treatment with iNO. In general, patients approved for treatment of iNO are term and near-term (>34 weeks gestation) neonates having hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, a condition also known as persistent pulmonary hypertension in the newborn (PPHN). Due to the selective, non-systemic nature of iNO to reduce pulmonary hypertension, physicians skilled in the art further employ INOmax® to treat or prevent pulmonary hypertension and improve blood O_2 levels in a variety of other clinical settings, including in both pediatric and adult patients suffering from acute respiratory distress syndrome (ARDS), pediatric and adult patients undergoing cardiac or transplant surgeries, pediatric and adult patients for testing to diagnose reversible pulmonary hypertension, and in pediatric patients with congenital diaphragmatic hernia. In most, if not all, of these applications, INOmax® acts by preventing or treating reversible pulmonary vasoconstriction, reducing pulmonary arterial pressure and improving pulmonary gas exchange.

[0034] A small proportion of INOmax® sales stem from its use by clinicians in a premature infant population. In these patients, INOmax® is generally utilized by physicians as a rescue therapy primarily to vasodilate the lungs and improve pulmonary gas exchange. Some physicians speculate that INOmax® therapy may promote lung development and/or reduce or prevent the future development of lung disease in a subset of these patients. Although the precise mechanism(s) responsible for the benefits of INOmax® therapy in these patients is not completely understood, it appears that the benefits achieved in at least a majority of these patients are due to the ability of INOmax® to treat or prevent reversible pulmonary vasoconstriction.

[0035] In clinical practice, the use of INOmax® has reduced or eliminated the use of high risk systemic vasodilators for the treatment of PPHN. INOmax®, in contrast to systemic vasodilators, specifically dilates the pulmonary vasculature without dilating systemic blood vessels. Further, iNO preferentially vasodilates vessels of aveoli that are aerated, thus improving V/Q matching. In contrast, systemic vasodilators may increase blood flow to atelectatic (deflated or collapsed) alveoli, thereby increasing V/Q mismatch and worsening arterial oxygenation. (*See* Rubin LJ, Kerr KM, Pulmonary Hypertension, in *Critical Care Medicine: Principles of Diagnosis and Management in the Adult*, *2d Ed.*, Parillo JE, Dellinger RP (eds.), Mosby, Inc. 2001, pp. 900-09 at 906; Kinsella JP, Abman SH, The Role of Inhaled Nitric Oxide in Persistent Pulmonary Hypertension of the Newborn, in *Acute Respiratory Care* *of the Neonate: A Self-Study Course*, *2d Ed.*, Askin DF (ed.), NICU Ink Book Publishers, 1997, pp. 369-378 at 372-73).

[0036] INOmax® also possesses highly desirable pharmacokinetic properties as a lung-specific vasodilator when compared to other ostensibly "pulmonary-specific vasodilators." For example, the short half-life of INOmax® allows INOmax® to exhibit rapid "on" and "off" responses relative to INOmax® dosing, in contrast to non-gaseous alternatives. In this way, INOmax® can provide physicians with a useful therapeutic tool to easily control the magnitude and duration of the pulmonary vasodilatation desired. Also, the nearly instantaneous inactivation of INOmax® in the blood significantly reduces or prevents vasodilatation of non-pulmonary vessels.

[0037] The pivotal trials leading to the approval of INOmax® were the CINRGI and NINOS study.

[0038] <u>CINRGI study</u>. (See Davidson et al., March 1998, Inhaled Nitric Oxide for the Early Treatment of Persistent Pulmonary Hypertension of the term Newborn; A Randomized, Double-Masked, Placebo-Controlled, Dose-Response, Multicenter Study; *PEDIATRICS* Vol. 101, No. 3, p. 325).

[0039] This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax® would reduce the receipt of extracorporeal membrane oxygenation (ECMO) in these patients. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS) (35%), idiopathic persistent pulmonary hypertension of the newborn (PPHN) (30%), pneumonia/sepsis (24%), or respiratory distress syndrome (RDS) (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean oxygenation index (OI) of 44 cm H_2O/mm Hg were randomly assigned to receive either 20 ppm INOmax® (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients that exhibited a $PaO_2 > 60$ mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax® or placebo. The primary results from the CINRGI study are presented in Table 4. ECMO was the primary endpoint of the study.

Table 1: Summary of Clinical Results from CINRGI Study

	Placebo	**INOmax®**	**P value**
Death or ECMO	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

[0040] Significantly fewer neonates in the ECMO group required ECMO, and INOmax® significantly improved oxygenation, as measured by PaO_2, OI, and alveolar-arterial gradient.

[0041] NINOS study. (See Inhaled Nitric Oxide in Full-Term and Nearly Full-Term Infants with Hypoxic Respiratory Failure; NEJM, Vol. 336, No. 9, 597).

[0042] The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether iNO would reduce the occurrence of death and/or initiation of ECMO in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤ 14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O/mmHg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm NO for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = > 20 mmHg, partial = 10–20 mm Hg, no response = < 10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm NO or control gas. The primary results from the NINOS study are presented in Table 2.

Table 2: Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*, †	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

[0043] Adverse Events from CINRGI & NINOS. Controlled studies have included 325 patients on INOmax® doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax®, a result adequate to exclude INOmax® mortality being more than 40% worse than placebo.

[0044] In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax® and placebo-treated groups.

[0045] From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax® and 212 patients who received placebo. Among these patients, there was no evidence of an AE of treatment on the need for re-hospitalization, special medical services, pulmonary disease, or neurological squeal.

[0046] In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, per ventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

[0047] The table below shows adverse reactions that occurred in at least 5% of patients receiving INOmax® in the CINRGI study. None of the differences in these adverse reactions were statistically significant when iNO patients were compared to patients receiving placebo.

Table 3: ADVERSE REACTIONS ON THE CINRGI TRIAL

Adverse Reaction	Placebo (n=89)	Inhaled NO (n=97)
Atelectasis	5 (4.8%)	7 (6.5%)
Bilirubinemia	6 (5.8%)	7 (6.5%)
Hypokalemia	5 (4.8%)	9 (8.3%)
Hypotension	3 (2.9%)	6 (5.6%)
Thrombocytopenia	20 (19.2%)	16 (14.8%)

[0048] Post-Marketing Experience. The following AEs have been reported as part of the post-marketing surveillance. These events have not been reported above. Given the nature of spontaneously reported post-marketing surveillance data, it is impossible to determine the actual incidence of the events or definitively establish their causal relationship to the drug. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax® in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

[0049] An analysis of AEs and SAEs from both the CINRGI and NINOS studies, in addition to post-marketing surveillance, did not suggest that patients who have pre-existing LVD could experience an increased risk of AEs or SAEs. Nor was it predictable to physicians skilled in the art that patients having pre-existing LVD (possibly identified as those patients having a PCWP greater than 20 mmHg) should be evaluated in view of the benefit versus risk of using iNO in patients with clinically significant LVD, and that these patients should be evaluated on a case by case basis.

EXAMPLE 1: INOT22 STUDY

[0050] The INOT22, entitled "Comparison of supplemental oxygen and nitric oxide for inhalation plus oxygen in the evaluation of the reactivity of the pulmonary vasculature during acute pulmonary vasodilatory testing" was conducted both to access the safety and effectiveness of INOmax® as a diagnostic agent in patients undergoing assessment of pulmonary hypertension (primary endpoint), and to confirm the hypothesis that iNO is selective for the pulmonary vasculature (secondary endpoint).

[0051] During, and upon final analysis of the INOT22 study results, applicants discovered that rapidly decreasing the pulmonary vascular resistance, via the administration of iNO to a patient in need of such treatment, may be detrimental to patients with concomitant, pre-existing LVD. Therefore, a precaution for patients with LVD was proposed to be included in amended prescribing information for INOmax®. Physicians were further informed to consider reducing left ventricular afterload to minimize the occurrence of pulmonary edema in patients with pre-existing LVD.

[0052] In particular, the INOT22 protocol studied consecutive children undergoing cardiac catheterization that were prospectively enrolled at 16 centers in the US and Europe. Inclusion criteria: 4 weeks to 18 years of age, pulmonary hypertension diagnosis, i.e. either idiopathic pulmonary hypertension (IPAH) or related to congenital heart disease (CHD) (repaired or unrepaired) or cardiomyopathy, with pulmonary vascular resistance index (PVRI) > 3 u-m^2. Later amendments, as discussed herein, added an additional inclusionary criteria of a PCWP less than 20 gmm Hg. Patients were studied under general anaesthesia, or with conscious sedation, according to the practice of the investigator. Exclusion criteria: focal infiltrates on chest X-ray, history of intrinsic lung disease, and/or currently taking PDE-5 inhibitors, prostacyclin analogues or sodium nitroprusside. The study involved supplemental O_2 and NO for inhalation plus O_2 in the evaluation of the reactivity of the pulmonary vasculature during acute pulmonary vasodilator testing. Consecutive children undergoing cardiac catheterization were prospectively enrolled at 16 centers in the US and Europe. As hypotension is expected in these neonatal populations, the comparison between iNO and placebo groups is difficult to assess. A specific secondary endpoint was evaluated in study INOT22 to provide a more definitive evaluation.

[0053] The primary objective was to compare the response frequency with iNO and O_2 vs. O_2 alone; in addition, all subjects were studied with iNO alone. Patients were studied during five periods: Baseline 1, Treatment Period 1, Treatment Period 2, Baseline 2 and Treatment Period 3. All patients received all three treatments; treatment sequence was randomized by center in blocks of 4; in Period 1, patients received either NO alone or O_2 alone, and the alternate treatment in Period 3. All patients received the iNO and O_2 combination treatment in Period 2. Once the sequence was assigned, treatment was unblinded. Each treatment was given for 10 minutes prior to obtaining hemodynamic measurements, and the Baseline Period 2 was at least 10 minutes.

[0054] Results for the intent-to-treat (ITT) population, defined as all patients who were randomized to receive drug, indicated that treatment with NO plus O_2 and O_2 alone significantly increased systemic vascular resistance index (SVRI) (Table 4). The change from baseline for NO plus O_2 was 1.4 Woods Units per meter^2 (WU·m^2) ($p = 0.007$) and that for O_2 was 1.3 WU·m^2 ($p = 0.004$). While the change from baseline in SVRI with NO alone was -0.2 WU·m^2 ($p = 0.899$) which demonstrates a lack of systemic effect.

Table 4: SVRI Change From Baseline by Treatment (Intent-to-Treat)

	Treatment		
SVRI ($WU \cdot m^2$)	**NO Plus O_2 (n=109)**	**O_2 (n=106)**	**NO (n=106)**
Baseline (room air)			
Mean	17.2	17.6	18.0
Standard Deviation (SD)	8.86	9.22	8.44
Median	15.9	16.1	16.2
Minimum, maximum	-7.6, 55.6	-7.6, 55.6	1.9, 44.8
Post-treatment			
Mean	18.7	18.9	17.8
SD	9.04	8.78	9.40
Median	17.1	17.1	15.4
Minimum, maximum	3.0, 47.4	3.9, 43.6	3.3, 50.7
Change From Baseline			
Mean	1.4	1.3	-0.2
SD	5.94	5.16	4.65
Median	1.2	1.0	0.2
Minimum, maximum	-20.5, 19.1	-18.1, 17.7	-12.5, 12.7
p-value[a]	0.007	0.004	0.899
Pairwise comparisons NO plus O_2 versus O_2, p=0.952 NO plus O_2 versus NO, p=0.014 O_2 versus NO, p=0.017			

[a] p-value from a Wilcoxon Signed Rank Test. Only patients with data to determine response at both treatments are included in this analysis.
Source: INOT22 CSR Table 6.4.1 and Appendix 16.2.6 (ATTACHMENT 1)

[0055] The ideal pulmonary vasodilator should reduce PVRI and/or PAPm while having no appreciable effect on systemic blood pressure or SVRI. In this case, the ratio of PVRI to SVRI would decrease, given some measure of the selectivity of the agent for the pulmonary vascular bed. The change in the ratio of PVRI to SVRI by treatment is shown in Table 5.

Table 5: Change in Ratio of PVRI to SVRI by Treatment (Intent-to-Treat)

	Treatment		
Ratio PVRI/SVRI	**NO Plus O_2 (n=108)**	**O_2 (n=105)**	**NO (n=106)**
Baseline			
Mean	0.6	0.5	0.6
SD	0.60	0.45	0.56
Median	0.5	0.5	0.4
Minimum, Maximum	-1.6, 4.7	-1.6, 1.8	0.0, 4.7
Post Treatment			
Mean	0.4	0.4	0.5
SD	0.31	0.31	0.46
Median	0.3	0.4	0.3
Minimum, Maximum	0.0, 1.3	0.0, 1.4	-1.2, 2.2
Change from Baseline			
Mean	-0.2	-0.1	-0.1
SD	0.52	0.31	0.54
Median	-0.1	-0.1	0.0
Minimum, Maximum	-4.4, 2.0	-1.6, 2.0	-4.4, 1.6
P Value[1]	< 0.001	< 0.001	0.002

[1] Wilcoxon Signed Rank Test
Source: INOT22 CSR Table 6.5.1 (ATTACHMENT 2)

[0056] All three treatments have a preferential effect on the pulmonary vascular bed, suggesting that all three are selective pulmonary vasodilators. The greatest reduction in the ratio was during treatment with NO plus O_2, possibly due to the decrease in SVRI effects seen with O_2 and NO plus O_2. These results are displayed as percent change in the ratio (See Table 6).

Table 6: Percent Change in Ratio of PVRI to SVRI by Treatment (Intent-to-Treat)

	Treatment		
Ratio PVRI/SVRI	**NO Plus O_2 (n=108)**	**O_2 (n=105)**	**NO (n=106)**
Baseline			
Mean	0.6	0.5	0.6
SD	0.60	0.45	0.56
Median	0.5	0.5	0.4
Minimum, Maximum	-1.6, 4.7	-1.6, 1.8	0.0, 4.7
Post Treatment			
Mean	0.4	0.4	0.5
SD	0.31	0.31	0.46
Median	0.3	0.4	0.3
Minimum, Maximum	0.0, 1.3	0.0, 1.4	-1.2, 2.2
Percent Change from Baseline			
Mean	-33.5	-19.3	-6.2
SD	36.11	34.59	64.04
Median	-34.0	-21.3	-13.8
Minimum, Maximum	-122.2, 140.1	-122.7, 93.3	-256.1, 294.1
P Value[1]	< 0.001	< 0.001	0.006

[1] Wilcoxon Signed Rank Test
Source: INOT22 CSR Table 6.5.2 (ATTACHMENT 3)

[0057] NO plus O_2 appeared to provide the greatest reduction in the ratio, suggesting that NO plus O_2 was more selective for the pulmonary vasculature than either agent alone.

[0058] Overview of Cardiovascular Safety. In the INOT22 diagnostic study, all treatments (NO plus O_2, O_2, and NO) were well-tolerated. Seven patients of 134 treated experienced an AE during the study. These included cardiac arrest, bradycardia, low cardiac output (CO) syndrome, elevated ST segment (the portion of an electrocardiogram between the end of the QRS complex and the beginning of the T wave) on the electrocardiography (ECG) decreased O_2 saturation, hypotension, mouth hemorrhage and pulmonary hypertension (PH). The numbers of patients and events were too small to determine whether risk for AEs differed by treatment, diagnosis, age, gender or race. Eight patients are shown in Table 5 due to the time period in which events are reported. AEs were reported for 12 hours or until hospital discharge (which limits the period in which such events can be reported). There is technically no time limit in which SAEs are to be reported. So, there were 7 AEs during the study and at least one SAE after the study.

[0059] A total of 4 patients had AEs assessed as being related to study drug. These events included bradycardia, low CO syndrome, ST segment elevation on the ECG, low O_2 saturation, PH and hypotension. All but 2 AEs were mild or moderate in intensity and were resolved. Study treatments had slight and non-clinically significant effects on vital signs including heart rate, systolic arterial pressure and diastolic arterial pressure. When an investigator records an AE, they are required to say if (in their opinion) the event is related to the treatment or not. In this case, 4 of 7 were considered by the investigator to be related to treatment.

[0060] The upper limit of normal PCWP in children is 10-12 mm Hg and 15 mm Hg in adults. In INOT22, a baseline PCWP value was not included as exclusion criteria. However, after the surprising and unexpected identification of SAEs in the early tested patients, it was determined that patients with pre-existing LVD had an increased risk of experiencing an AE or SAE upon administration (e.g., worsening of left ventricular function due to the increased flow of blood through the lungs). Accordingly, the protocol for INOT22 was thereafter amended to exclude patients with a baseline PCWP greater than 20 mm Hg after one patient experienced acute circulatory collapse and died during the study. The value "20 mm Hg" was selected to avoid enrollment of a pediatric population with LVD such that they would be most likely at-risk for these SAEs.

[0061] SAEs were collected from the start of study treatment until hospital discharge or 12 hours, whichever occurred sooner. Three SAEs were reported during the study period, and a total of 7 SAEs were reported. Three of these were fatal SAEs and 4 were nonfatal (one of which led to study discontinuation). In addition, one non-serious AE also lead to discontinuation. A list of subjects who died, discontinued or experienced an SAE is provided in Table 5 below.

Table 5: Subjects that died, discontinued or experienced SAEs

Patient number	AE	Serious?	Fatal?	Discontinued treatment?
01020	Desaturation (hypoxia)	No	No	Yes
02002	Pulmonary edema	Yes	No	No
04001	Hypotension and cardiac arrest	Yes	Yes	No
04003	Hypotension and ECG changes	Yes	No	Yes
04008	Hypotension and hypoxemia	Yes	Yes	No
05002	Hypoxia and bradycardia (also pulmonary edema)	Yes	Yes	No
07003	Cardiac arrest	Yes	No	No
17001	Hypoxia	Yes	No	No

[0062] Two of the 3 fatal SAEs were deemed related to therapy. All 4 non-fatal SAEs were also considered related to therapy. The numbers of patients and events were too small to determine whether risk for SAEs differed by treatment, diagnosis, age, gender or race. At least two patients developed signs of pulmonary edema (subjects 05002 and 02002). This is of interest because pulmonary edema has previously been reported with the use of iNO in patients with LVD, and may be related to decreasing PVRI and overfilling of the left atrium. (Hayward CS et al., 1996, Inhaled Nitric Oxide in Cardiac Failure: Vascular Versus Ventricular Effects, *J Cardiovascular Pharmacology* 27:80-85; Bocchi EA et al., 1994, Inhaled Nitric Oxide Leading to Pulmonary Edema in Stable Severe Heart Failure, *Am J Cardiology* 74:70-72; and, Semigran MJ et al., 1994, Hemodynamic Effects of Inhaled Nitric Oxide in Heart Failure, *J Am Coll Cardiology* 24:982-988).

[0063] Although the SAE rate is within range for this population, it appears that patients with the most elevated PCWP at baseline had a disproportionately high number of these events. (Bocchi EA et al., 1994; Semigran MJ et al., 1994).

[0064] In the INOT22 study, 10 of the total 134 patients had a baseline PCWP ≥ 18 mm Hg (7.5%), of which, 3 subjects (04001, 02002 and 04003) had a SAE or were prematurely discontinued from the study (30%) compared to 6.5% for the entire cohort.

[0065] Although there were very few significant AEs in the INOT22 study, these events are consistent with the expected physiologic changes in patients with severe LVD. The events also corroborate prior observations that iNO is rapidly acting, selective for the pulmonary vasculature, and well-tolerated in most patients. The actual incidence of acute LVD during acute ventricular failure (AVT) is unknown. However, it is reasonable to expect that a significant number of patients are at-risk for an increased incidence of SAEs upon iNO treatment based upon the nature of the underlying nature of the illness, i.e., pulmonary hypertension and cardiovascular disease more generally. Thus, it would be advantageous to have physicians identify these patients prior to beginning iNO treatment, so that the physicians are alerted to this possible outcome.

[0066] Benefits and Risks Conclusions. The INOT22 study was designed to demonstrate the physiologic effects of iNO in a well defined cohort of children (i.e., intended patient population) with pulmonary hypertension using a high concentration, 80 ppm, of iNO, i.e., one that would be expected to have the maximal pharmacodynamic effect. INOT22 was the largest and most rigorous pharmacodynamic study of iNO conducted to date, and it confirms a number of prior observations, such as iNO being rapidly acting, selective for the pulmonary vasculature, and well-tolerated in most patients.

[0067] It is also acknowledged that rapidly decreasing the PVR may be undesirable and even dangerous in patients with concomitant LVD. In the INOT22 study, the overall numbers of SAEs and fatal SAEs are within the expected range for patients with this degree of cardiopulmonary disease. The overall rate is 7/124 (5.6%), which is closely comparable to the rate of 6% recently reported in a very similar cohort of patients. (Taylor CJ et al., 2007, Risk of cardiac catheterization under anaesthesia in children with pulmonary hypertension, *Br J Anaesth* 98(5):657-61). Thus, the overall rate of SAEs would seem to be more closely related to the underlying severity of illness of the patients rather than to the treatments given during this study.

[0068] The INOT22 study results demonstrate that patients who had pre-existing LVD may experience an increased rate of SAEs (e.g., pulmonary edema). During the course of the study, the protocol was amended to exclude patients with a PCWP > 20 mmHg. The benefit/risk of using iNO in patients with clinically significant LVD should be evaluated on a case by case basis. A reduction in left ventricular afterload may perhaps be applied to minimize the occurrence of pulmonary edema.

CLAIMS

We Claim:

1. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, said method comprising:

a. providing pharmaceutically acceptable nitric oxide gas to a medical provider; and,

b. informing the medical provider that excluding said patients who have pre-existing left ventricular dysfunction from said treatment reduces the risk or prevents the occurrence of the adverse event or serious adverse event associated with said medical treatment.

2. The method of claim 1, wherein the adverse event or serious adverse event is one or more of pulmonary edema, hypotension, cardiac arrest, electrocardiogram changes, hypoxemia, hypoxia and bradycardia, or, associations thereof.

3. The method of claim 1, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

4. The method of claim 3, wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient.

5. The method of claim 3, wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

6. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, said method comprising:

a. providing pharmaceutically acceptable nitric oxide gas to a medical provider; and,

b. informing the medical provider that such patients that have pre-existing left ventricular dysfunction experience an increased rate of adverse events or serious adverse events associated with said medical treatment.

7. The method of claim 6, further comprising informing the medical provider of a risk of an adverse event or a serious adverse event in such patients who have a pulmonary capillary wedge pressure greater than 20 mm Hg.

8. The method of claim 6, further comprising informing the medical provider that there is a risk associated with using inhaled nitric oxides in such patients who have pre-existing or clinically significant left ventricular dysfunction and that such risk should be evaluated on a case by case basis.

9. The method of claim 6, further comprising informing the medical provider that there is a risk associated with using inhaled nitric oxide in such patients who have left ventricular dysfunction.

10. The method of claim 6, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

11. The method of claim 10, wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient.

12. The method of claim 10, wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

13. A method of reducing one or more adverse events or serious adverse events in an intended patient population comprising children in need of being treated with inhaled nitric oxide comprising:

a. identifying a patient eligible for inhaled nitric oxide treatment;

b. evaluating and screening the patient to identify if the patient has pre-existing left ventricular dysfunction; and

c. excluding from inhaled nitric oxide treatment any patient having pre-existing left ventricular dysfunction.

14. The method of claim 13, wherein the patient having pre-existing left ventricular dysfunction also exhibits a pulmonary capillary wedge pressure greater than 20 mm Hg.

15. The method of claim 13, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

16. The method of claim 15,

wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient, or,

wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

17. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, the method comprising:

a. identifying said patient in need of receiving inhalation of nitric oxide treatment;

b. evaluating and screening the patient to identify if the patient has pre-existing left ventricular dysfunction; and

c. administering the inhalation of nitric oxide if the patient has not been diagnosed as having pre-existing left ventricular dysfunction, thereby reducing the risk or preventing the occurrence of the adverse event or significant adverse event associated with the inhalation of nitric oxide treatment.

18. The method of claim 17, wherein the patient diagnosed as having pre-existing left ventricular dysfunction also exhibits a pulmonary capillary wedge pressure greater than 20 mm Hg.

19. The method of claim 17, further comprising reducing left ventricular afterload to minimize or reduce the risk of the occurrence of an adverse event or serious adverse event being pulmonary edema in the patient.

20. The method of claim 19,

wherein the left ventricular afterload is minimized or reduced by administering a pharmaceutical dosage form comprising nitroglycerin or calcium channel blocker to the patient, or,

wherein the left ventricular afterload is minimized or reduced using an intra-aortic balloon pump.

ABSTRACT

The invention relates methods of reducing the risk or preventing the occurrence of an adverse event (AE) or a serious adverse event (SAE) associated with a medical treatment comprising inhalation of nitric oxide.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted With Initial Filing *OR* ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	135197.00084
First Named Inventor	James S. Baldassarre
COMPLETE IF KNOWN	
Application Number	12/494,598
Filing Date	Herewith
Art Unit	1614
Examiner Name	

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 06/30/2009 as United States Application Number or PCT International Application Number 12/494,598 and was amended on (MM/DD/YYYY) ____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

PTO/SB/01 (07-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☐ A petition has been filed for this unsigned inventor

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City: Doylestown	State: PA	Zip: 18901	Country: US

☑ Additional inventors or a legal representative are being named on the 1 supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

[Page 2 of 2]

Please type a plus sign (+) inside this box → [+]

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
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Inventor's Signature			**Date**
Residence: City	**State**	**Country**	**Citizenship**
Mailing Address			
Mailing Address			
City	**State**	**ZIP**	**Country**

Application Data Sheet

Application Information

Application number:	
Filing Date:	
Application Type:	Continuation
Subject Matter:	Utility
Suggested classification & subclass:	600/481 (surgery/cardiovascular)
Suggested Group Art Unit:	
CD-ROM or CD-R:	None
Number of CD disks:	
Number of copies of CDs:	
Sequence submission::	
Computer Readable Form (CRF):	
Number of copies of CRF:	
Title:	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION
Attorney Docket Number:	I001-0002USC4
Request for Early Publication:	NO
Request for Non-Publication:	NO
Suggested Drawing Figure:	NA
Total Drawing Sheets:	NA
Small Entity:	YES
Latin name:	
Variety denomination name:	
Petition included:	Yes
Petition Type:	Accelerated Examination

Licensed US Govt. Agency:
Contract or Grant Numbers:
Secrecy Order in Parent Appl:

Applicant Information

Applicant Authority Type::	Inventor
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Postal or Zip Code of mailing address::	07930

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Correspondence Customer Number:: 49584

Representative Information

Representative Customer Number:: 49584

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This application	continuation of	12/494,598	06/30/2009

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::

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PTO/SB/06 (12-04)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Date 06/22/10

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PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number
12/821,041

127878875

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	82		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	270		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	110		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =		x$26		OR	x$52	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x$110	110		x$220	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))			195			390	
			TOTAL	572		TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED – PART II

AMENDMENT A	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
					TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
					TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
12/821,041	06/22/2010	3771	572	I001-0002USC4	20	4

CONFIRMATION NO. 3219

FILING RECEIPT

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SPOKANE, WA 99201

Date Mailed: 06/30/2010

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

James S. Baldassarre, Doylestown, PA;
Ralf Rosskamp, Chester, NJ;

Assignment For Published Patent Application

Ikaria Holdings, Inc., Clinton, NJ

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 12/494,598 06/30/2009

Foreign Applications

If Required, Foreign Filing License Granted: 06/30/2010

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/821,041**

Projected Publication Date: 12/30/2010

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****

Title

Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension

Preliminary Class

128

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Application Serial Number	TBD
Confirmation Number	TBD
Filing Date	Herein
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	TBD
Examiner	TBD
Attorney Docket Number	I001-0002USC4

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

From: Christopher P. Rogers (Tel.; Fax 509-323-8979)
Customer Number: 49584
Lee & Hayes, PLLC
601 W. Riverside Avenue, Suite 1400
Spokane, WA 99201

Fees will be paid by credit card through the EFS Web; however the Commissioner is hereby authorized to charge any deficiency of fees and credit any overpayments to Deposit Account Number 12-0769.

06/30/2010 MNGUYEN 00000001 120769 12821041
01 FC:2201 110.00 DA

Respectfully Submitted,

Dated: 21 June 2010

By: [signature]
Christopher P. Rogers
Reg. No. 36,334

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4	3219

49584 7590 07/07/2010
LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE, WA 99201

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1616	

MAIL DATE	DELIVERY MODE
07/07/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

UNITED STATES PATENT AND TRADEMARK OFFICE

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JUL 0 7 2010

LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE WA 99201

In re Application of: BALDASSARRE et al.
Serial No.: 12/821,041

Filed: June 22, 2010
Docket: I001-0002USC4

Title: **METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION**

DECISION ON PETITION TO MAKE SPECIAL FOR NEW APPLICATION UNDER 37 C.F.R. § 1.102 & M.P.E.P. § 708.02

This is a decision on the petition filed on June 22, 2010 to make the above-identified application special for accelerated examination procedure under 37 C.F.R. § 1.102(d).

The petition to make the application special is GRANTED.

The application is eligible for accelerated examination and the petition complies with the conditions for granting the application special status pursuant to the "Change to Practice for Petitions in Patent Applications to Make Special and for Accelerated Examination" published June 26, 2006, in the Federal Register. (71 Fed. Reg. 36323).

The prosecution of the instant application will be conducted expeditiously according to the following guidelines.

1. The application will be docketed to an examiner and taken up for action within two weeks of the date of this decision.

2. Restriction Practice:
   If the examiner determines that the claims are not directed to a single invention, a telephone request to elect one single invention will be made pursuant to MPEP 812.01. As a prerequisite to the grant of this petition, the applicant has agreed to make an oral election, by telephone, without traverse. If the applicant refuses to make an election

without traverse, or the examiner cannot reach the applicant after a reasonable effort, the examiner will treat the first claimed invention (invention defined by claim 1) as having being constructively elected without traverse for examination.

3. Office action:
If it is determined that, after appropriate consultation, there is a potential rejection or any other issue to be addressed, the examiner will telephone the applicant and arrange an interview to discuss and resolve the issue. An Office action, other than a Notice of Allowance and Fee(s) Due (Notice of Allowance), will not be issued unless either: 1) an interview was conducted but did not result in agreed to action that places the application in condition for allowance, or, 2) a determination is made that an interview would be unlikely to result in the application being placed in condition for allowance, and 3) an internal conference has been held to review any rejection of any claim.

4. Time for Reply:
An Office action other than a Notice of Allowance or a final Office action will set a shortened statutory period of one month or thirty days, whichever is longer, for reply with no extension of time available under 37 CFR 1.136(a). Failure to timely file a reply within this non-extendible period for reply will result in the abandonment of the application.

5. Reply by Applicant:
A timely reply to an Office action other than the Notice of Allowance must be submitted electronically via EFS or EFS-web and limited to addressing the rejections, objections and requirement made. Any amendment that attempts to: 1) add claims which would result in more than three pending independent claims or more than twenty pending total claims; 2) present claims not encompassed by the pre-examination search or an updated accelerated examination support document; or 3) present claims that are directed a non-elected invention or an invention other than that previously claimed and examined in the application, will be treated as not fully responsive and will not be entered.

   For any amendment to the claims (including any new claim) that is not encompassed by the accelerated examination support document, applicant must provide an updated accelerated examination support document that encompasses the amended or new claims at the time of filing of the amendment.

   To proceed expeditiously with the examination, it is recommended that a reply with amendments made to any claim or with any new claim being added be accompanied by an updated accelerated examination support document or a statement explaining how the amended or new claim is supported by the original accelerated examination support document.

6. Information Disclosure Statement (IDS):
Any IDS filed during prosecution must be submitted electronically via EFS or EFS-web, accompanied by an updated accelerated examination support document, and be in compliance with 37 CFR 1.97 and 1.98.

7. Post-Allowance Processing:
   To expedite processing of the allowed application into a patent, the applicant must: 1) pay the required fees within one month of the date of the Notice of Allowance, and 2) not file any post allowance papers not required by the Office. In no event may the issue fee be paid and accepted later than three months from the date of the Notice of Allowance.

8. After-Final and Appeal Procedures:
   To expedite prosecution, after receiving the final Office action, applicant must: 1) promptly file a notice of appeal, an appeal brief and appeal fees; and 2) not request a pre-appeal brief conference.

   Any amendment, affidavit or other evidence filed after final Office action must comply with applicable rules and the requirements outlined in numbered paragraphs 5 and 6 above.

   On appeal, the application will proceed according to normal appeal procedures. After appeal, the application will again be treated special.

9. Proceedings Outside the Normal Examination Process:
   If the application becomes involved in a proceeding that is outside the normal examination process (e.g., a secrecy order, national security review, interference proceeding, petitions under 37 CFR 1.181, 182 or 183), the application will be treated special before and after such proceeding.

10. Final Disposition:
    The twelve month goal of this accelerated examination procedure ends with a final disposition. The mailing of a final Office action, a Notice of Allowance, the filing of a Notice of Appeal, or the filing of a Request for Continued Examination (RCE) is the final disposition.

If, during prosecution, a paper is not filed electronically using EFS-web, a reply is filed but is not fully responsive, the application is involved in an appeal, or a proceeding outside normal examination process, the application will still be examined expeditiously, however, the final disposition may occur more than twelve months from the filing of the application.

Any inquiry regarding this decision should be directed to Michael P. Woodward, Quality Assurance Specialist, at (571) 272-8373.

/MP Woodward/
Michael P. Woodward, Quality Assurance Specialist
Technology Center 1600

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4	3219

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LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE, WA 99201

EXAMINER
ARNOLD, ERNST V

ART UNIT	PAPER NUMBER
1616	

MAIL DATE	DELIVERY MODE
07/13/2010	PAPER

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The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

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JUL 13 2010

LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE WA 99201

In re Application of: BALDASSARRE et al.
Serial No.: 12/821,041

Filed: June 22, 2010
Docket: I001-0002USC4

Title: **METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION**

DECISION ON PETITION TO MAKE SPECIAL FOR NEW APPLICATION UNDER 37 C.F.R. § 1.102 & M.P.E.P. § 708.02

This is a decision on the petition filed on June 22, 2010 to make the above-identified application special for accelerated examination procedure under 37 C.F.R. § 1.102(d).

The petition to make the application special is **DENIED**.

REGULATION AND PRACTICE

To be eligible for accelerated examination under 37 C.F.R. § 1.102(d) and pursuant to the "Change to Practice for Petitions in Patent Applications to Make Special and for Accelerated Examination" published in the Federal Register on June 26, 2006 (71 Fed. Reg. 36323), the following conditions must be satisfied:

1. The application must be a non-reissue utility or design application filed under 37 CFR 1.111(a);
2. The application, the petition and the required fees must be filed electronically using the USPTO's electronic filing system (EFS), or EFS-web; if not filed electronically, a statement asserting that EFS and EFS-web were not available during the normal business hours;
3. The application, at the time of filing, must be complete under 37 CFR 1.51 and in condition for examination;

Conditions for Examination: The application must be in condition for examination at the time of filing. This means the application must include the following:

(A) Basic filing fee, search fee, and examination fee, under 37 CFR 1.16 (*see* MPEP section 607(I)),
(B) Application size fee under 37 CFR 1.16(s) (if the specification and drawings exceed 100 sheets of paper) (*see* MPEP section 607(II));
(C) An executed oath or declaration in compliance with 37 CFR 1.63;
(D) A specification (in compliance with 37 CFR 1.52) containing a description (37 CFR 1.71) and claims in compliance with 37 CFR 1.75;
(E) A title and an abstract in compliance with 37 CFR 1.72;
(F) Drawings in compliance with 37 CFR 1.84;
(G) Electronic submissions of sequence listings in compliance with 37 CFR 1.821(c) or (e), large tables, or computer listings in compliance with 37 CFR 1.96, submitted via the USPTO's electronic filing system (EFS) in ASCII text as part of an associated file (if applicable);
(H) Foreign priority claim under 35 U.S.C. 119(a)–(d) identified in the executed oath or declaration or an application data sheet (if applicable);
(I) Domestic benefit claims under 35 U.S.C. 119(e), 120, 121, or 365(c) in compliance with 37 CFR 1.78 (*e.g.*, the specific reference to the prior application must be submitted in the first sentence(s) of the specification or in an application data sheet, and for any benefit claim to a non-English language provisional application, the application must include a statement that: (a) An English language translation, and (b) a statement that the translation is accurate, have been filed in the provisional application) (if applicable);
(J) English language translation under 37 CFR 1.52(d), a statement that the translation is accurate, and the processing fee under 37 CFR 1.17(i) (if the specification is in a non-English language);
(K) No preliminary amendments present on the filing date of the application; and
(L) No petition under 37 CFR 1.47 for a non-signing inventor.

4. The application must contain three or fewer independent claims and twenty or fewer total claims and the claims must be directed to a single invention.

The application as filed is not eligible for the accelerated examination under 37 C.F.R. § 1.102(d) because it was no in condition for examination as evidenced by the presence of four independent claims.

For the above-stated reasons, the petition is denied. The application will therefore be taken up by the examiner for action in its regular turn.

Any inquiry regarding this decision should be directed to Michael P. Woodward, Quality Assurance Specialist, at (571) 272-8373.

/MP Woodward/
Michael P. Woodward, Quality Assurance Specialist
Technology Center 1600

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4	3219

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EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

MAIL DATE	DELIVERY MODE
08/17/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 12/821,041	Applicant(s) BALDASSARRE ET AL.	
	Examiner ERNST V. ARNOLD	Art Unit 1616	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/22/10*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Claims 1-20 are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "serious" in claims 1-3, 6, 10, 13, 15, 17 and 19 is a relative term which renders the claim indefinite. The term "serious" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Furthermore, any adverse effect in a neonate could be considered serious and thus it is redundant. The specification attempts to define the terms in [0025] and [0027] where the “adverse event” is any unfavorable and unintended sign, symptom, or disease temporarily associated with the use of a medicinal/investigational product and is considered clinically significant and “serious adverse event is a significant hazard or side effect which would then make it clinically significant and no different from an “adverse event”. The dependent claims are rejected as being indefinite because they are dependent on indefinite base claims.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 2 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 2 recites: "associations thereof". It is unclear what the metes and bounds of "associations thereof" might encompass as it is unknown what might be associated with the conditions listed. The American Illustrated Medical Dictionary (Dorland, 1914, 7th Ed, page 113) defines "association" as: "The coordination of the functions of similar part." It is unclear what parts are intended to be coordinated by Applicant with "associations thereof". The Examiner suggests deleting the term.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-12 and 17-20 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for reducing the risk in a patient of one or more adverse events associated with a medical treatment comprising inhalation of nitric oxide, does not reasonably provide enablement for *a method of preventing the occurrence in a patient of one or more adverse events associated with a medical treatment comprising inhalation of nitric oxide*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims without an undue amount of experimentation.

Let the Examiner be clear: Applicant is not enabled for *a method of preventing the occurrence in a patient of one or more adverse events associated with a medical treatment comprising inhalation of nitric oxide.*

The factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). Among these factors are: 1) scope or breadth of the claims; 2) nature of the invention; 3) relative level of skill possessed by one of ordinary skill in the art; 4) state of, or the amount of knowledge in, the prior art; 5) level or degree of predictability, or a lack thereof, in the art; 6) amount of guidance or direction provided by the inventor; 7) presence or absence of working examples; and 8) quantity of experimentation required to make and use the claimed invention based upon the content of the supporting disclosure. When the above factors are weighed, it is the Examiner's position that one skilled in the art could not practice the invention without undue experimentation. While all of the factors have been considered, only those required for a *prima facie* case are set forth below.

1) Scope or breadth of the claims

The claims are broader in scope than the enabling disclosure. The specification merely discloses, without more, methods of reducing the risk of adverse events associated with a medical treatment comprising inhalation of nitric oxide. However, Applicant is purporting to prevent adverse events associated with a medical treatment comprising inhalation of nitric oxide.

2) Nature of the invention

The nature of the invention is directed to methods of reducing the risk of adverse events associated with a medical treatment comprising inhalation of nitric oxide.

3) Relative level of skill possessed by one of ordinary skill in the art

MPEP 2141.03 states (in part), "A person of ordinary skill in the art is also a person of ordinary creativity, not an automaton." KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 167 LEd2d 705, 82 USPQ2d 1385, 1397 (2007). "[I]n many cases a person of ordinary skill will be able to fit the teachings of multiple patents together like pieces of a puzzle." Id. Office personnel may also take into account "the inferences and creative steps that a person of ordinary skill in the art would employ." Id. At 1396, 82 USPQ2d at 1396. The "hypothetical person having ordinary skill in the art' to which the claimed subject matter pertains would, of necessity have the capability of understanding the scientific and engineering principles applicable to the pertinent art." Ex parte Hiyamizu, 10 USPQ2d 1393, 1394 (Bd. Pat. App. & Inter. 1988) (The Board disagreed with the examiner's definition of one of ordinary skill in the art (a doctorate level engineer or scientist working at least 40 hours per week in semiconductor research or development), finding that the hypothetical person is not definable by way of credentials, and that the evidence in the application did not support the conclusion that such a person would require a doctorate or equivalent knowledge in science or engineering.).

4) State of, or the amount of knowledge in, the prior art

Beghetti et al. (Journal of Pediatrics, 1997, page 844) teach that: "Inhaled nitric oxide should be administered with caution to babies with LV dysfunction because pulmonary vasoconstriction may act as a protective mechanism of LV overfilling."

Macrae et al. (Intensive Care Med 2004, 30, pp 372-380) teach inhaled nitric oxide therapy in neonates and children and has been performed since 1992 (title and Abstract). Macrae et al. teach using echocardiography to exclude congenital heart disease as a cause of hypoxaemia prior to exposure to iNO and that inhaled NO exposure may even be harmful in some babies with congenital heart disease such as those with severe left ventricular dysfunction (page 373, bottom right to page 374, top left).

Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455) teach inhaled nitric oxide in the neonate with cardiac disease (title). Atz et al. teach that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." (page 452, left column). Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively right to left shunting at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported adverse outcomes in this circumstance." (page 452, left column) (Examiner added emphasis).

Loh et al. (Circulation 1994, 90, 2780-2785) teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract).

Kinsella et al. (The Lancet 1999, 354, pp 1061-1065) teach excluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and page 1062, Methods).

Wessel et al. (Pediatrics, 1997, 100(5), 7 pages) teaches exclusion of patients (newborns) with congenital heart disease or right ventricular dysfunction from treatment with inhaled NO

(Abstract and page 2 of 7, Methods). A patient with left ventricular dysfunction who received treatment died of an intracranial hemorrhage (page 3 of 7, left column to top right column).

5) Level or degree of predictability, or a lack thereof, in the art

Macrae et al. teach that: “Sufficient data are lacking for evaluation of the possible effets of iNO on periventricular haemorrhage or on long-term neurodevelopmental outcome.” (page 377, left column).

6) Amount of guidance or direction provided by the inventor

Applicant was required to provide in the specification additional guidance and direction with respect to how use the claimed subject matter in order for the application to be enabled with respect to the full scope of the claimed invention. Although the instant specification discloses that methods of reducing the risk of adverse events associated with a medical treatment comprising inhalation of nitric oxide, it remains silent on preventing those adverse events.

7) Presence or absence of working examples

The specification fails to provide scientific data and working embodiments with respect to prevention of the adverse effects.

8) Quantity of experimentation required to make and use the claimed invention based upon the content of the supporting disclosure

One of ordinary skill in the art would have to conduct a myriad number of experiments comprising trial and error administration of NO gas to babies without a clue as to how this will affect future development of the child such as on the long-term neurodevelopmental outcome. This is especially difficult when Applicant’s own definition of ‘adverse effects' embraces essentially any effect under the sun. The result of failure is potentially death of the patient.

Essentially, one of ordinary skill in the art has to figure out how to do this themselves. As a result, one of ordinary skill in the art would be required to conduct an undue amount of experimentation to determine if this invention can prevent the myriad number of adverse affects that can be associated with iNO therapy.

Genetech, 108 F.3d at 1366 states that "a patent is not a hunting license. It is not a reward for search, but compensation for its successful conclusion" and "patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable." (*Genentech, Inc. v. Novo Nordisk, A/S*, 108 F.3d 1361, 1365, 42 USPQ2d 1001, 1004 (Fed. Cir. 1997)).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 6, 8, 9, 13 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages).

The NIH discloses that administration of NO has been approved for use in the treatment of term or near term neonates and that that inhaled NO therapy is relative contraindicated for patients with severe left ventricular failure and to beware of increased left ventricular filling associated with rapid changes in pulmonary pressures (pages 2-3 of 13). A contraindication means literally contra- (against) an indication, against something that is indicated as advisable or necessary and a relative contraindication is a condition which makes a particular treatment or procedure inadvisable but does not rule it out. Therefore, in reading the guidelines set forth by the NIH the medical provider of NO inherently is provided with pharmaceutically acceptable NO gas and is informed that excluding patients with pre-existing left ventricular dysfunction would reduce the risk of adverse events because such events are increased with the use of NO thus anticipating instant claims 1, 6 and 9. (The instant specification defines 'children' as being around 4 weeks of age [0023] and that reads on a newborn which is a neonate.) All patients are 'at risk' of the events in instant claim 2, for example anyone receiving the vasodilator NO is at risk of hypotension, and therefore it is inherently anticipated. Since each patient is a case by case situation, then instant claim 8 is anticipated. The practitioner practices the instant method by identifying a patient eligible for iNO treatment and then diagnosing/evaluating/screening for a pre-existing left ventricular dysfunction in the patient and if present exclude the neonate from treatment to avoid/reduce the risk of the adverse events/hazards/complications but administer the treatment if the patient is not contraindicated. Claims 13 and 17 are therefore anticipated.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 13 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455).

Atz et al. disclose methods using inhaled nitric oxide in the neonate with cardiac disease (title and Abstract pages 441-453). Atz et al. disclose that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." (page 452, left column). Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively right to left shunting at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse outcomes*** in this circumstance." (page 452, left column) (Examiner added emphasis). Thus, Atz et al. fairly disclose excluding patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment and consequently any adverse events are reduced. The left ventricular dysfunction is inherently pre-existing and the instant specification defines 'children' as being around 4 weeks old [0023] which is a newborn and hence neonate and therefore instant claim 13 is anticipated. The methods disclosed by Atz et al. are interpreted to mean identifying a patient eligible for NO treatment; diagnosing if the patient has left ventricular dysfunction; excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment. Therefore, instant claim 17 is anticipated.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455) and Kinsella et al. (The Lancet 1999, 354, 1061-1065) and Bolooki (Clinical Application of the Intra-Aortic Balloon Pump 1998, 3rd Ed. Pp 252-253) and Loh et al. (Circulation 1994, 90, 2780-2785) and The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages).

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicant claims

> 1. A method of reducing the risk or preventing the occurrence, in a patient being a child, of one or more adverse events or serious adverse events associated with a medical treatment comprising inhalation of nitric oxide, said method comprising:
>
> a. providing pharmaceutically acceptable nitric oxide gas to a medical provider; and,
>
> b. informing the medical provider that excluding said patients who have pre-existing left ventricular dysfunction from said treatment reduces the risk or prevents the occurrence of the adverse event or serious adverse event associated with said medical treatment.

Determination of the scope and content of the prior art

(MPEP 2141.01)

Atz et al. teach inhaled nitric oxide in the neonate with cardiac disease (title and Abstract and pp 441-453). Atz et al. teach treating children of all ages including 1 month to 1 year olds and older above 15 years of age (Figure 1, page 444). Atz et al. teach that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." (page 452, left column). Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively right to left shunting at the ductus arteriosus, ***<u>NO should be used with extreme caution, if at all</u>***. We and others have reported ***<u>adverse outcomes</u>*** in this circumstance." (page 452, left column) (Examiner added emphasis). Thus, Atz et al. fairly teaches excluding patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets “if at all” to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing. The methods taught by Atz et al. are interpreted to mean identifying a patient eligible for NO treatment; diagnosing if the patient has left ventricular dysfunction; excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment. The left ventricular dysfunction is inherently pre-existing and the instant specification defines ‘children’ as being around 4 weeks old [0023] which is a newborn and hence neonate.

Bolooki teaches using intra-aortic balloon pump as well as nitroglycerin and calcium channel blockers in the treatment of left ventricular dysfunction (pages 252-253).

Kinsella et al. (The Lancet 1999, 354, pp 1061-1065) teach excluding patients from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and page 1062, Methods).

Loh et al. (Circulation 1994, 90, 2780-2785) teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract).

The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages) teaches that inhaled NO therapy is relative contraindicated for patients with severe left ventricular failure and to beware of increased left ventricular filling associated with rapid changes in pulmonary pressures (pages 2-3 of 13). Thus it is a medical mainstream concept that inhaled NO therapy is relative contraindicated for patients with left ventricular dysfunction.

Ascertainment of the difference between the prior art and the claims

(MPEP 2141.02)

1. The difference between the instant application and Atz et al. is that Atz et al. do not expressly teach a method of providing NO gas to a medical provider and informing the medical provider to exclude patients with pre-existing left ventricular dysfunction with a capillary wedge pressure greater than 20 mm Hg but to administer the NO in patients without pre-existing left ventricular dysfunction. This deficiency in Atz et al. is cured by the teachings of Kinsella et al., Loh et al., The NIH and common sense.

2. The difference between the instant application and Atz et al. is that Atz et al. do not expressly teach: reducing the left ventricular afterload with nitroglycerin, calcium channel blocker or intra-aortic balloon pump such that pulmonary edema is reduced/minimized. This deficiency in Atz et al. is cured by the teachings of Bolooki.

Finding of prima facie obviousness

Rational and Motivation (MPEP 2142-2143)

1. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Atz et al. and provide NO gas to a medical provider and inform the medical provider to exclude patients with pre-existing left ventricular dysfunction with a capillary wedge pressure greater than 20 mm Hg but to administer the NO in patients without pre-existing left ventricular dysfunction**,** as suggested by Loh et al., The NIH and Kinsella et al., and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because the practitioner of inhaled NO intrinsically is in possession of pharmaceutically acceptable NO gas and Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with left ventricular dysfunction, which includes children, and the art of Kinsella et al. establishes excluding certain patients from treatment. Furthermore, the NIH teaches that inhaled NO therapy is relative contraindicated for patients with left ventricular dysfunction. ***In other words, the art is already well aware and informed that inhaled NO is contraindicated for patients with left ventricular dysfunction, and consequently it is not inventive to exclude that patient population from treatment when the art already suggests it!*** Thus it is no stretch of the imagination to exclude patients including children with left ventricular dysfunction from inhaled nitric oxide therapy in order to avoid adverse outcomes as taught by Atz et al. and the NIH which intrinsically include all the adverse events recited by Applicant. The ordinary artisan would err on the side of caution for the benefit of the patient. Such patients intrinsically have a pulmonary capillary wedge pressure of greater than 20 mm Hg. In other words, the teachings of Atz et al. include the patients with left ventricular dysfunction intrinsically that have a pulmonary capillary wedge pressure of greater than 20 mm Hg. Inhaled NO increased the wedge pressure as taught by Loh et al. (see entire document)

2. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Atz et al. and reduce the left ventricular afterload with nitroglycerin, calcium channel blocker or intra-aortic balloon pump such that pulmonary edema is reduced/minimized, as suggested by Bolooki, and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because administration of nitroglycerin, calcium channel blocker or intra-aortic balloon pump to treat left ventricular dysfunction is a common technique in the art as taught by Bolooki and intrinsically reduces the left ventricular afterload and reduces pulmonary edema.

In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a).

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re*

Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

1. Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-30 of copending Application No. 12/494598. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction.

The copending application does not expressly teach providing NO gas or informing the medical provider of the adverse events.

However, it is intrinsic to the copending application to provide NO gas for the treatment and inform the medical provider about the adverse events.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

2. Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-19 of copending Application No. 12/820980. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction.

The copending application does not expressly teach providing NO gas or informing the medical provider of the adverse events.

However, it is intrinsic to the copending application to provide NO gas for the treatment and inform the medical provider about the adverse events.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

3. Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-20 of copending Application No. 12/821020. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction.

The copending application does not expressly teach providing NO gas or informing the medical provider of the adverse events.

However, it is intrinsic to the copending application to provide NO gas for the treatment and inform the medical provider about the adverse events.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

4. Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-19 of copending Application No. 12/821866. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more

adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction.

The copending application does not expressly teach providing NO gas or informing the medical provider of the adverse events.

However, it is intrinsic to the copending application to provide NO gas for the treatment and inform the medical provider about the adverse events.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

No claims are allowed. There is no allowable subject matter.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ernst V. Arnold whose telephone number is 571-272-8509. The examiner can normally be reached on M-F (7:15 am-4:45 pm).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Johann Richter can be reached on 571-272-0646. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Ernst V Arnold/
Primary Examiner, Art Unit 1616

Notice of References Cited	Application/Control No. 12/821,041	Applicant(s)/Patent Under Reexamination BALDASSARRE ET AL.	
	Examiner ERNST V. ARNOLD	Art Unit 1616	Page 1 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
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	I	US-			
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FOREIGN PATENT DOCUMENTS

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	N					
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	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	The American Illustrated Medical Dictionary (Dorland, 1914, 7th Ed, page 113)
	V	Beghetti et al. (Journal of Pediatrics, 1997, page 844)
	W	Macrae et al. (Intensive Care Med 2004, 30, pp 372-380)
	X	Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455)

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Notice of References Cited	Application/Control No. 12/821,041	Applicant(s)/Patent Under Reexamination BALDASSARRE ET AL.	
	Examiner ERNST V. ARNOLD	Art Unit 1616	Page 2 of 2

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Kinsella et al. (The Lancet 1999, 354, pp 1061-1065)
	V	The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages).
	W	Bolooki (Clinical Application of the Intra-Aortic Balloon Pump 1998, 3rd Ed. Pp 252-253)
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12821041	BALDASSARRE ET AL.
	Examiner	**Art Unit**
	ERNST V ARNOLD	1616

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
inventor name EAST/PALM	8/11/10	eva
EAST 424/718 text limted all databases	8/11/10	eva
google	8/10/10	eva

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	James S. Baldassarre
	Art Unit	
	Examiner Name	
	Attorney Docket Number	I001-0002USC4

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	5873359		1999-02-23	Zapol, ; et al.	
	2	6063407		2000-05-16	Zapol, ; et al.	
	3	6601580		2003-08-05	Bloch, ; et al.	
	4	7557087		2009-07-07	Rothbard, ; et al.	

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U.S.PATENT APPLICATION PUBLICATIONS

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	1	20040106954	A1	2004-06-03	Whitehurst, Todd K.; et al.	
	2	20090018136	A1	2009-01-15	Oppenheimer; Daniel I.; et al.	
	3	20090029371	A1	2009-01-29	Elliott; C. Gregory	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	James S. Baldassarre
	Art Unit	
	Examiner Name	
	Attorney Docket Number	I001-0002USC4

	4	20090149541	A1	2009-06-11	Stark et al.	
	5	20090176772	A1	2009-07-09	Blackburn et al.	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	EP1682672;(A1)			2006-07-26	COUNCIL SCIENT IND RES [IN]+ (COUNCIL O		☐
	2	WO2005004884;(A2)			2005-01-20	US GOVERNMENT [US]; UN		☐
	3	WO2006127907;(A2)			2006-11-30	MASSACHUSETTS INST TECHNOLOGY [US];		☐
	4	WO2010019540;(A1)			2010-02-18	NOVARTIS AG [CH]; PASC		☐

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	"Inhaled Nitric Oxide and Hypoxic Respiratory Failure in Infants With Congenital Diaphragmatic Hernia", The Neonatal Inhaled Nitric Oxide Study Group (NINOS), PEDIATRICS, Vol. 99, No. 6, 6 June 1997, pp. 838-845.	☐
	2	"Inhaled Nitric Oxide in Full-Term and Nearly Full-Term Infants with Hypoxic Respiratory Failure", The Neonatal Inhaled Nitric Oxide Study Group, N Engl J Med, 1997, Vol. 336, No. 9, pp. 597-605.	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	
	Filing Date	
	First Named Inventor	James S. Baldassarre
	Art Unit	
	Examiner Name	
	Attorney Docket Number	I001-0002USC4

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All participants (applicant, applicant's representative, PTO personnel):

(1) ERNST V. ARNOLD.

(2) Dr. Baldassarre.

(3) Christopher Rogers.

(4) Jonathan Provoost.

Date of Interview: 01 September 2010.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: See Continuation Sheet.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: The interview commenced with Applicant requesting that the Examiner provide a background on his experience with nitric oxide which the Examiner said amounts to about 10 years of graduate school, post doctorate and industry experience prior to joining the USPTO. The inventor Dr. Baldassarre provided a background on the discovery of the invention. Dr. Baldassarre said that iNO was used as a diagnostic tool in children with cardiac problems to determine the pulmonary reactivity. Children with elevated pulmonary capillary wedge pressure need to be excluded from further treatment.Tthe Examiner pointed out that at least instant claim 1 did not require any inhaltion of NO by the patient.The Examiner also acknowledges the reference of Lipshulz (Progress in Pediatric Cardiology 2000, 12, 1-28; of record) which teaches that with respect to ventricular dysfunction in infants/children, data from adults cannot generally be extrapolated for children. Applicant also argued that the cited art in the last Office Action is silent on exclusionary criteria such as a pulmonary capillary wedge pressure (PCWP) of 20 mm Hg as it appears that this value is a key feature of the invention. However, the independent claims do not recite this value and/or language and the independent claims do not even suggest measuring this value via iNO. In fact, the art cited by the Examiner, namely the NIH document (see last Office Action page 8) specifically teaches that iNO is relative contraindicated, which means that it is inadvisable to perform the treatment but does not rule it out, for patients with left ventricular failure and teaches that iNO is approved for use in neonates. The Examiner raised the issue if it was non-obvious to define a sub-set population by the PCWP when the art already teaches that iNO is a relative contraindication for the same condition in the same population. After Applicant files remarks, the Examiner will consult with his supervisor and/or quality assurance specialist.

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4

CONFIRMATION NO. 3219

49584
LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE, WA 99201

PUBLICATION NOTICE

OC000000045212357

Title: Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension

Publication No. US-2010-0331405-A1
Publication Date: 12/30/2010

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/SB/84 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

AUTHORIZATION TO ACT IN A REPRESENTATIVE CAPACITY

In re Application of: James S. Baldassarre et al.

Application No. 12/820,041

Filed: 06/22/2010

Title: METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION

Attorney Docket No.	Art Unit:
I001-0002USC4	1613

The practitioner named below is authorized to conduct interviews and has the authority to bind the principal concerned. (Note: pursuant to 37 CFR 10.57(c), a practitioner cannot authorize other registered practitioners to conduct interviews without consent of the client after full disclosure.) Furthermore, the practitioner is authorized to file correspondence in the above-identified application pursuant to 37 CFR 1.34:

Name	Registration Number
Jonathan N. Provoost	44,292
Henry C. Lebowitz	36,196

This is not a Power of Attorney to the above-named practitioner. Accordingly, the practitioner named above does **not** have authority to sign a request to change the correspondence address, a request for an express abandonment, a disclaimer, a power of attorney, or other document requiring the signature of the applicant, assignee of the entire interest or an attorney of record. If appropriate, a separate Power of Attorney to the above-named practitioner should be executed and filed in the United States Patent and Trademark Office.

SIGNATURE of Practitioner of Record

Signature	/Christopher P. Rogers, Reg. No. 36,334/	Date	January 5, 2011
Name	Christopher P. Rogers	Registration No., if applicable	36,334
Telephone	509-944-4785		

Electronic Acknowledgement Receipt

EFS ID:	9170585
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Daniel Leo Hayes/Jennifer Phipps
Filer Authorized By:	Daniel Leo Hayes
Attorney Docket Number:	I001-0002USC4
Receipt Date:	05-JAN-2011
Filing Date:	22-JUN-2010
Time Stamp:	16:04:41
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	P39272.PDF	43276 e47adc2d90386d1c67ac669235bba1496bfa2585	no	1

Warnings:

Information:

Total Files Size (in bytes):	43276

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4	3219

49584 7590 01/18/2011
LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE, WA 99201

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

NOTIFICATION DATE	DELIVERY MODE
01/18/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

lhpto@leehayes.com

PTOL-90A (Rev. 04/07)

Interview Summary	Application No.	Applicant(s)	
	12/821,041	BALDASSARRE ET AL.	
	Examiner	**Art Unit**	
	ERNST V. ARNOLD	1613	

All participants (applicant, applicant's representative, PTO personnel):

(1) *ERNST V. ARNOLD*.

(2) *Brian Kwon*.

(3) *Chris Rogers + Jeff Smith (telephone), Henry Woods*.

(4) *Dr. Green, Dr. Baldassarre, Jonathan Provost*.

Date of Interview: *10 January 2011*.

Type: a)☐ Telephonic b)☐ Video Conference
c)☒ Personal [copy given to: 1)☐ applicant 2)☒ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: _____.

Identification of prior art discussed: *Loh Circulation 1994; Atz Seminars in Parenatology 1997*.

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *1) Discussed the prior art of record, Loh, Atz and NIH guidelines in great detail. 2) Dr. Greene discussed the differences between the patient populations and the instant invention. 3) Applicant will consider filing claim amendments and arguments for the Examiner's consideration*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
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Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

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The Form provides for recordation of the following information:

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- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	1	AU 2009202685 Office Action dated 06/17/10 (3 pages)	☐
	2	AU 2009202685 Office Action Response dated 07/29/2010, 19 pages	☐
	3	Bates, Inhaled Nitric Oxide: A Selective Pulmonary Vasodilator,	☐
	4	Branson, Inhaled Nitric Oxide in Adults, The Science Journal of the American Association for Respiratory Care 1997 Open Forum Abstracts, December 7, 1997, 2 pages, retrieved at <<http://www.rcjournal.com/abstracts/1997/?id=A00000929>> on 12/22/2010	☐
	5	Braunwald, Heart Failure, chapter 233 of Harrison's Principles of Internal Medicine, 14th Edition, 1998, pp. 1287-1291 & 1360	☐
	6	Clark, et al., Low-Dose Nitric Oxide Therapy for Persistent Pulmonary Hypertension of the Newborn, New England Journal of Medicine, Vol 342, No 7, pp. 469-474	☐
	7	Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing, http://clinicaltrials.gov/archive/NCT00626028/2009_01_12 January 12, 2009	☐
	8	Cox, et al., Factors Associated With Establishing a Causal Diagnosis for Chidren With Cardiology, Pediatrics, Vol 118, No 4, October 4, 2006, pp 1519-1531, published online October 2, 2006	☐
	9	Cuthbertson et al., "UK guidelines for thee use of inhaled nitric oxide therapy in adults ICUs*", Intensive Care Med (1997), 23, Springer-Verlag, 1997, pp#1212-pp#1218	☐
	10	EP 09251949 Office Action dated 10/11/2010, 5 pages	☐
	11	Guideline for Industry; Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, March 1995, 17 pages	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number: 12821041
	Filing Date: 2010-06-22
	First Named Inventor: James S. Baldassarre
	Art Unit: 1616
	Examiner Name: Ernst V. Arnold
	Attorney Docket Number: I001-0002USC4

	12	Headrick, Hemodynamic monitoring of the critically ill neonate, J Perinat Neonatal Nurs 1992; 5(4): 58-67	☐
	13	INO Therapeutics, LLC, "INOflo for Inhalation 800ppm", package leaflet, 2010, 2	☐
	14	JP 2009157623 Office Action dated 02/23/2010, 3 pages	☐
	15	JP 2009157623 Office Action dated 07/30/2010, 6 pages	☐
	16	JP 2009157623 Office Action response filed 06/18/2010, 37 pages (no translation)	☐
	17	JP 2009157623 request for accelerated exam filed 01/15/2010 (60 pages)	☐
	18	JP 2009157623 response filed 11/30/2010, 58 pages	☐
	19	Letter of Acceptance for AU 2010202422, dated 10/7/2010	☐
	20	Letter of acceptance of AU application 2009202685, dated 08/10/2010, 3 pages	☐
	21	Lipschultz, The incidence of pediatric cardiomyopathy in two regions of the United States, New England Journal of Medicine, April 24, 2003. <<http://www.nejm.org/doi/full/10.1056/NEJMoa021715>>	☐
	22	NIH Clinical Center Services, retrieved at <http://www.cc.nih.gov/ccmd/clinical_services.html>> on 08/18/2010	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	23	Notification of Reason for Rejection, mailed 7/30/2010, from Japanese Patent Application No. 2009-157623 (cites foreign references).	☐
	24	Office Action for AU 2010202422 dated 07/09/2010, 3 pages	☐
	25	Office Action from AU 2009202685 dtd 03/15/2010	☐
	26	Office Action from AU 2010206032 dated 08/16/2010 (3 pages)	☐
	27	Office Action Response for AU 2009202685 to 03/15/2010 OA, filed 06/08/2010 (16 pages)	☐
	28	Office Action Response for JP2007157623 filed on 11/12/2009 (no English translation)	☐
	29	Office Action Response to AU 2010202422 OA dated 07/09/2010, response filed 09/01/2010	☐
	30	PCT/US2010/038652 Search Report dated 07/29/2010, 16 pages	☐
	31	Response filed 08/18/2010 to EP Search Report dated 05/10/10 for EP09251949	☐
	32	Search Report from EP 09251949 dated 05/10/10	☐
	33	Towbin, et al., Incidence, Causes, and Outcomes of Dilated Cardiomyopathy in Children, JAMA, October 18, 2006 - Vol 296, No. 15, pp. 1867-1876	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	34	Yoshida, Kiyoshi, " Well-illustrated Diagnostics and Treatment of Heart Failure" Professor of Kawasaki Medical University, cardiovascular internal medicine CIRCULATION Up-to-Date Vol. 2, No. 4, 2007(343), pp. 23-28	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/Christopher P. Rogers, Reg. No. 36,334/	Date Considered	2011-02-17

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ None

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Christopher P. Rogers, Reg. No. 36,334/	Date (YYYY-MM-DD)	2011-02-17
Name/Print	Christopher P. Rogers	Registration Number	36,334

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
Rogers, Christopher...
LEE & HAYES, PLLC
601 West Riverside Avenue Suite 1400
Spokane WA 99201
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 29 July 2010 (29-07-2010)
Applicant's or agent's file reference I001 - 0002PCT	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2010/038652	International filing date *(day/month/year)* 15 June 2010 (15-06-2010)
Applicant Ikaria Holdings, Inc.	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	HODZIC, Iris Tel: +49 (0)89 2399-2084

Form PCT/ISA/220 (July 2009) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annex B).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, International Phase, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet or sheets containing a complete set of claims in replacement of all the claims previously filed must be submitted.

Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively in Arabic numerals (Section 205(a)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]: "Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]: "Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]: "Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or "Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]: "Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1*bis*(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion together, where appropriate, with amendments before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later (Rule 43*bis*.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, National Chapters.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
I001 - 0002PCT		
International application No. PCT/US2010/038652	International filing date (*day/month/year*) 15/06/2010	(Earliest) Priority Date (*day/month/year*) 30/06/2009
Applicant Ikaria Holdings, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into ____________, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. [ ] **Certain claims were found unsearchable** (See Box No. II)
3. [ ] **Unity of invention is lacking** (see Box No III)
4. With regard to the **title,**
   [ ] the text is approved as submitted by the applicant
   [X] the text has been established by this Authority to read as follows:
   METHODS OF IDENTIFYING A PATIENT POPULATION ELIGIBLE FOR TREATMENT WITH NITRIC OXIDE VIA INHALATION
5. With regard to the **abstract,**
   [ ] the text is approved as submitted by the applicant
   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority
6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. ________
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/038652

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The invention relates to methods of reducing the risk or preventing the occurrence of an adverse event (AE) or a serious adverse event (SAE) associated with a medical treatment comprising inhalation of nitric oxide.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/038652

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/21 A61K33/00 A61K45/06 A61P9/08 A61P9/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, PASCAL, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOH EVAN ET AL: "Cardiovascular Effects of Inhaled Nitric Oxide in patients With Left Ventricular Dysfunction" CIRCULATION, vol. 90, no. 6, 1994, pages 2780-2785, XP002577161 ISSN: 0009-7322 the whole document -----	1-30

-/--

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2010	29/07/2010
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Albrecht, Silke

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEMIGRAN MARC J ET AL: "Hemodynamic effects of inhaled nitric oxide in heart failure" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 24, no. 4, 1994, pages 982-988, XP009131903 ISSN: 0735-1097 cited in the application the whole document -----	1-30
X	HAYWARD C S ET AL: "Inhaled nitric oxide in cardiac failure: Vascular versus ventricular effects" JOURNAL OF CARDIOVASCULAR PHARMACOLOGY, vol. 27, no. 1, 1996, pages 80-85, XP009131904 ISSN: 0160-2446 cited in the application the whole document -----	1-30
X	OVODOV ET AL: "Nitric oxide: Clinical applications" SEMINARS IN ANESTHESIA, SAUNDERS, CO, NEW YORK, NY, US LNKD- DOI:10.1053/SA.2000.6785, vol. 19, no. 2, 1 June 2000 (2000-06-01), pages 88-97, XP005426335 ISSN: 0277-0326 page 90, column 1 page 93, column 2 - page 94 -----	1-30
X	HENRICHSEN ET AL: "Inhaled nitric oxide can cause severe systemic hypotension" JOURNAL OF PEDIATRICS, MOSBY-YEAR BOOK, ST. LOUIS, MO, US LNKD- DOI:10.1016/S0022-3476(96)70230-5, vol. 129, no. 1, 1 July 1996 (1996-07-01), page 183, XP022199226 ISSN: 0022-3476 the whole document -----	1-30
X	ADATIA ET AL: "Inhaled nitric oxide and hemodynamic evaluation of patients with pulmonary hypertension before transplantation" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, NEW YORK, NY, US LNKD- DOI:10.1016/0735-1097(95)00048-9, vol. 25, no. 7, 1 June 1995 (1995-06-01), pages 1656-1664, XP005857183 ISSN: 0735-1097 page 1663, column 1 ----- -/--	1-30

4

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CUJEC BIBIANA ET AL: "Inhaled nitric oxide reduction in systolic pulmonary artery pressure is less in patients with decreased left ventricular ejection fraction" CANADIAN JOURNAL OF CARDIOLOGY, vol. 13, no. 9, 1997, pages 816-824, XP002577162 ISSN: 0828-282X the whole document -----	1-30
X	FINDLAY G P: "Paradoxical haemodynamic response to inhaled nitric oxide" INTERNATIONAL JOURNAL OF INTENSIVE CARE 1998 GB, vol. 5, no. 4, 1998, pages 134-139, XP001536771 ISSN: 1350-2794 the whole document -----	1-30
X	BOCCHI E A ET AL: "Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure" AMERICAN JOURNAL OF CARDIOLOGY, CAHNERS PUBLISHING CO., NEWTON, MA, US LNKD- DOI:10.1016/0002-9149(94)90496-0, vol. 74, no. 1, 1 July 1994 (1994-07-01), pages 70-72, XP023278686 ISSN: 0002-9149 [retrieved on 1994-07-01] cited in the application the whole document -----	1-30

4

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) see form PCT/ISA/210 (second sheet)		
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below	
International application No. PCT/US2010/038652	International filing date (*day/month/year*) 15.06.2010	Priority date (*day/month/year*) 30.06.2009
International Patent Classification (IPC) or both national classification and IPC INV. A61K31/21 A61K33/00 A61K45/06 A61P9/08 A61P9/12		
Applicant Ikaria Holdings, Inc.		

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Albrecht, Silke Telephone No. +49 89 2399-7864

Europäisches Patentamt · European Patent Office · Office européen des brevets

Form PCT/ISA/237 (Cover Sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2010/038652

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 16-23

because:

☒ the said international application, or the said claims Nos. 16-23 relate to the following subject matter which does not require an international search *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☐ no international search report has been established for the whole application or for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☒ See Supplemental Box for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non- establishment of opinion with regard to novelty, inventive step and industrial applicability

Independent claims 16, 20 will not be examined in accordance with Rule 67.1(v) PCT, as their subject-matter is limited to mere presentations of information (i.e. informing the medical provider in accordance with feature b of claims 16, 20). Mutatis mutandis dependent claims 17-19, 21-23.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D1-D9) are referred to in this report; the numbering results from the order of citations found in the Search Report (SR) and will be adhered to in the rest of the procedure. The cited passage(s) for each citation will be considered unless otherwise specified.

Claims 1-15, 24-30 relate to subject-matter considered by this Authority to be covered by the provision of Rule 39.1(iv)/67.1(iv) PCT. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Claims 1-15, 24-30 do not comply with the requirements of Article 5 PCT, the reasons being as follows:

These claims comprise i.a. the step of identifying near-term neonate, neonate or child patients eligible for/in need of treatment with NO by inhalation. However, in the present case, the disclosure in the patent application does not give the skilled person any guidance on how to identify these patients (eg method of screening, criteria of inclusion/exclusion etc). Independent of the foregoing claims 1-15, 24-30 also lack clarity in the sense of Article 6 PCT, as the wording of feature (a) of claims 9, 24, 25 and claim 1 is vague and leaves the skilled reader in doubt about the exact scope of these claims. Mutatis mutandis dependent claims 2-8, 10-15, 26-30.

Furthermore, claims 1-15, 24-30 are also considered to be unclear in that they comprise a diagnostic step (eg feature (b) of claims 9, 24, 25), but the said diagnostic procedure is not further defined (e.g. omission of the method steps of data collection, of comparison of data with standard values, of finding of any significant deviation during the comparison). As these features are essential to the definition of the invention, but are nevertheless not mentioned in independent claims 1, 9, 24, 25, these claims do not meet the requirement following from Article 6 PCT that any independent claim must contain all the technical features essential to the definition of the invention.

In addition, claims 28-30 as dependent claims of claims 16, 20 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The reasons are as follows:

Claims 28-30 refer to a medical treatment of a patient (i.e. reducing left ventricular afterload), whereas claims 16, 20 do not relate to any medical treatment of a patient. The method described in claims 16, 20 is merely limited to providing a medical provider with nitric oxide gas and informing him in accordance with feature b of these claims. This contradiction between the subject-matter of claims 16, 20 on one hand and claims 28-30 on the other hand produces a lack of clarity as to the scope of protection afforded by claims 28-30.

In view of the foregoing objections, novelty and inventive step cannot be discussed in detail at present. However, the following should be noted:

The core of the invention appears to reside in the discovery that patients with pre-existing LVD often experience an increased risk of (serious) adverse events when treated with NO by inhalation (cf par.5, 50, 52, 61, 68, 69 of the present application). However, this finding has already been reported in prior art. In particular, D3- D8 explicitly recommend to use inhaled NO with caution in patients with LVD. Furthermore, the authors of D1 state that inhaled NO may have adverse effects in patients with LVD and hence may not be desirable in patients with severe left ventricular failure. D2 reports on the haemodynamic effects of inhaled NO in patients with LVD and concludes that the increase in left ventricular filling pressure seen during NO administration may limit its role to that of a diagnostic agent rather than a therapeutic agent in these patients. As for the specific patient group claimed in the present claims, this cannot contribute to inventive step either, since D5 and D6 refer to neonates and children respectively.

In light of these teachings, it would be obvious for the skilled person to screen patients including neonates and children about to undergo treatment with NO by inhalation and to exclude those with LVD therefrom.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/ WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Daniel Leo Hayes/Jennifer Phipps
Attorney Docket Number:	I001-0002USC4

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	9472003
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Daniel Leo Hayes/Jennifer Phipps
Filer Authorized By:	Daniel Leo Hayes
Attorney Docket Number:	I001-0002USC4
Receipt Date:	17-FEB-2011
Filing Date:	22-JUN-2010
Time Stamp:	18:24:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$180
RAM confirmation Number	4831
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Information Disclosure Statement (IDS) Filed (SB/08)	PK6608.PDF	50515 cf247002e038e868e3343e3af3e849af5f07e8a3	no	6
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Information:					
This is not an USPTO supplied IDS fillable form					
2	NPL Documents	NI3971.PDF	607899 5aa52842927c2ee7ca57b867c54ea1be73db0fe7	no	7
Warnings:					
Information:					
3	NPL Documents	O86044.pdf	153932 96945d60944cf4e06475c57b2ccf5d35497f2fcf	no	4
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4	NPL Documents	O86037.pdf	369742 4a332bdd82d6f9a661ba2a15ce0e7b15d484387f	no	5
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5	NPL Documents	O86041.pdf	727722 40545b06db1277e5c6aa458c60f1a65408c4503a	no	7
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6	NPL Documents	NQ3600.PDF	200468 a68c776d620b936d03dc2d345e1aff6fa8daa817	no	8
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7	NPL Documents	OH8890.PDF	164479 23fc7d9efd65e6829e03ef05a783c3a252d6e90a	no	9
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8	NPL Documents	OE2725.PDF	206048 08852912c18d788810fcf11b8b7828b29cea8686	no	10
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9	NPL Documents	PJ7125.PDF	198204 cc79b3958ad6cf54b1eb1609875cc1a736b64f75	no	3

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18	NPL Documents	PJ7633.PDF	129016 49af91f882b60527f76be352a7c0cccb7882eba8	no	6

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20	NPL Documents	PJ7681.PDF	260513 8f5ac539406d9ff9c966ba83bc654675c3a0ffc4	no	5
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21	NPL Documents	PJ7747.PDF	156607 8ca79138b956c52a91deaf374c9d99bc412dd7b1	no	3
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Warnings:	
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Total Files Size (in bytes):	25754305

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/821,041
Confirmation Number	3219
Filing Date	June 22, 2010
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	1613
Examiner	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY AMENDMENT (37 CFR 1.111)

This communication is responsive to the Non-Final Office Action mailed August 17, 2010, setting a shortened statutory period for reply of 3 months. A 3-month extension of time under 37 CFR 1.136(a) is enclosed herewith. Thus, the Reply is timely and the application remains pending.

Applicant respectfully requests entry of this Reply Amendment, reconsideration of the pending rejections, and allowance of the application. A listing of the claims and amendments thereof is shown starting at page 2.

Remarks to the pending Office Action begin at page 6.

Amendments to the Claims

Please cancel claims 1-20 and add new claims 21-35.

1-20. Canceled.

21. (New) A method of distributing a pharmaceutical product, comprising:

providing a source of nitric oxide gas to a medical provider;

informing the medical provider that a recommended dose of inhaled nitric oxide is 20 ppm nitric oxide;

informing the medical provider that nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood; and

providing an additional warning to the medical provider that in non-adult patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema.

22. (New) The method of claim 21 that further includes the step of providing a warning to the medical provider that patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

23 (New) The method of claim 22 wherein the source of nitric oxide gas is a pressurized cylinder containing nitric oxide and one or more inert gases wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm).

24. (New) A method of reducing the risk of one or more adverse events or serious adverse events in an intended patient population comprising patients under the age of 18 in need of being treated with inhalation of nitric oxide, comprising excluding from such treatment any of such patients under the age of 18 having pre-existing left

ventricular dysfunction whether or not such patients under the age of 18 are dependent on right-to-left shunting of blood.

25. (New) A method of reducing the risk of one or more adverse events or serious adverse events associated with the use of inhaled nitric oxide in patients under the age of 18 for the treatment of hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, comprising:

a. providing a source of pharmaceutically acceptable nitric oxide gas for inhalation to a medical provider;

b. informing the medical provider that inhaled nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood;

c. informing the medical provider that in addition to patients dependent on right-to-left shunt, for patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary wedge pressure leading to pulmonary edema; and

d. informing the medical provider that in addition to patients dependent on right-to-left shunt, patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

26. (New) The method of claim 25 wherein the source of nitric oxide gas is a pressurized cylinder containing nitric oxide and one or more inert gases wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm).

27. (New) The method of claim 25 further including the step of informing the medical provider that a recommended dose of inhaled nitric oxide is 20 ppm.

28. (New) A pharmaceutical product comprising:

a pressurized cylinder containing nitric oxide and one or more inert gases wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm); and

a label comprising:

(i) a statement indicating that a recommended dose of nitric oxide is 20 ppm nitric oxide;

(ii) a statement indicating that nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood; and

(iii) a warning, independent from the contraindication for neonates known to be dependent on right-to-left shunting of blood, said warning indicating that in non-adult patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema.

29. (New) The pharmaceutical product of claim 28 wherein said pharmaceutical product is for the treatment of a term or near term neonates having hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension.

30. (New) The pharmaceutical product of claim 28 wherein the label comprises a further warning, independent from the contraindication for neonates known to be dependent on right-to-left shunting of blood, that non-adult patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

31. (New) A pharmaceutical product comprising:

a source of nitric oxide gas; and

a label comprising:

(i) a statement indicating that nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood;

(ii) a first warning, independent from the contraindication for neonates known to be dependent on right-to-left shunting of blood, said warning indicating that in non-adult patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema; and

(iii) a second warning, independent from the contraindication for neonates known to be dependent on right-to-left shunting of blood, that non-adult patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

32. (New) The pharmaceutical product of claim 31 wherein said pharmaceutical product is for the treatment of term or near term neonates having hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension.

33. (New) The pharmaceutical product of claim 32 wherein the label further comprises a statement indicating that a recommended dose of nitric oxide is 20 ppm nitric oxide.

34. (New) The pharmaceutical product of claim 33 wherein the source of nitric oxide gas is a pressurized cylinder containing nitric oxide and one or more inert gases wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm).

35. (New) The pharmaceutical product of claim 31 wherein the non-adult patients with pre-existing left ventricular dysfunction are characterized by an elevated pulmonary capillary wedge pressure.

36. (New) The pharmaceutical product of claim 32 wherein the pulmonary capillary wedge pressure is greater than 20 mmHg.

REMARKS

Applicant would like to thank Examiner Kwon and Examiner Arnold for providing Applicant an opportunity to discuss the subject matter of the present application during the interviews conducted on September 1, 2010 and January 10, 2011 with respect to the instant application and pending patent application 12/821,041. Applicant further acknowledges receipt of the Interview Summaries dated September 9, 2010 and January 18, 2011, respectively.

Claims 1-20 have been canceled.

Claims 21-36 have been added to more particularly point out and distinctly claim the subject matter applicant regards as the invention and to address matters discussed during the Examiner interviews. The claims have been amended without prejudice. Each of these claims is believed allowable over the prior art of record for at least the reasons described below.

Support for new claims 21-36 is found in the specification of the application, as filed, including the original claims as filed and paragraphs [0007], [0020], and [0023] of the specification. In addition, with respect to the contraindication for neonates dependent on right to left shunting of blood is expressly found in the prescribing label for inhaled nitric oxide incorporated by reference in the specification at paragraph [0020] (copy attached as Appendix B).

Applicant acknowledges entry of the PTO-892 and IDS dated June 22, 2010.[1]

Rejections Under 35 USC § 112

A. Claims 1-20 were rejected under 35 USC § 112, second paragraph on the grounds that the terms "*serious*", "*adverse event*" and "*serious adverse event*" are indefinite. Although claims 1-20 have been cancelled, the terms "*adverse event*" and "*serious adverse event*" remain in new claims 21-36. Applicant respectfully traverses the rejection and assertions thereof, because one of ordinary skill in the medical arts

[1] See Office Action Summary dated August 13, 2010 at attachments 1 and 3.

would clearly recognize and understand what those terms mean, the differences thereof, and the non-overlapping boundaries thereof.

First, the definition of "*adverse event*" and "*serious adverse event*" is clearly set forth in the specification as originally filed (see paragraph **0026** and **0028** respectively).

Further, the FDA, in Federal regulations 21 CFR Part 312, defines adverse events as any untoward medical occurrence that may present itself during treatment or administration with a pharmaceutical product, and which may or may not have a causal relationship with the treatment. In the guideline entitled "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting", the Agency further clarifies and defines serious adverse events stemming from a drug study as any untoward medical occurrence that at any dose results in death; is life-threatening; requires inpatient hospitalization or prolongation of existing hospitalization; creates persistent or significant disability/incapacity, or a congenital anomaly/birth defects. In addition, it is well known that for one of ordinary skill in the medical arts, the terms "*adverse event*" and "*serious adverse event*" are routinely used with respect to clinical use of pharmaceutical products.

Accordingly, for one of ordinary skill in the medical arts, and to the U.S. FDA, the terms adverse events and serious adverse events have a well understood and defined meanings. Reconsideration and withdrawal of the rejection is respectfully requested.

B. The Examiner rejected claim 2 under 35 USC § 112, second paragraph because claim 2 recites "*associations thereof*" and it was alleged that the term was unclear with respect to the metes and bounds of the claimed language. Claim 2 has been cancelled and the new claims do not incorporate the language "associations thereof". Accordingly, the prior rejection is now moot and reconsideration and withdrawal of the rejection is respectfully requested.

C. Lastly, the Examiner rejected claims 1-12 and 17-20 under 35 USC § 112, first paragraph because the specification while being enabling for reducing the risk in a patient of one or more adverse events associated with a medical treatment comprising inhalation of nitric oxide, allegedly does not reasonably provide enablement for "a method of preventing the occurrence in a patient of one or more adverse events associated with a medical treatment comprising inhalation of nitric oxide".

Claims 1-12 and 17-20 have been cancelled. New claims 21-36 no longer incorporate the language "*a method of preventing the occurrence*", but rather claim a method of "*reducing the risk*" of an occurrence. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejections Under 35 USC § 102(b)

Claims 1-20 were rejected as being anticipated by Atz, et al., Seminars in Perinatology, 1997 (hereinafter "Atz") and NIH Clinical Center, Department Policy and Procedure Manual for the Critical Care therapy and Respiratory Care Section; Nitric Oxide Therapy, 2000 (hereinafter the "NIH Manual").

In particular, the Examiner states that the NIH Manual contains a relative contraindication to the use of inhaled nitric oxide in severe left ventricular failure and that such contraindication applies to the treatment of term or near term neonates. During the interview of January 10, 2011, Applicants provided an email from Dr. Dennis T. Brown, Section Chief, at the NIH Hospital in Bethesda, Maryland, to Jeffrey R. Smith, Esq, an attorney and expert witness at the firm representing Applicant, confirming that the NIH manual reference is limited to the use of inhaled nitric oxide therapy in adult patients being treated in the intensive care unit (a reproduction of the email is attached in Appendix A) and that the NIH Hospital does not contain a neonatal intensive care use for the treatment of adults. Further, applicants highlight the fact that section 5.0 of the NIH Manual, Contraindications (both absolute and relative contraindications), is virtually an exact duplicate of the same Contraindications (both absolute and relative contraindications) described in B.H. Cuthbertson, P. Dellinger, O.L., Dyar, T.E. Evens, et al., *UK guidelines for the use of inhaled nitric oxide therapy in adult ICUs*. Intensive Care Med (1997) 23:1212-1218 (see page 1216, Table 1), which is cited in Section 19.0 References of the NIH Manual. Thus, the NIH Manual describes the risks associated with the use of iNO in adult patients, not patients under the age of 18 (e.g., non-adults).

Atz, page 452, states in pertinent part:

> *"Caution should be exercised when administering NO to patients with sever left ventricular dysfunction and pulmonary hypertension. In adults with ischemic cardiomyopathy, sudden pulmonary vasodilation may occasionally unload the right ventricle sufficiently to increase pulmonary blood flow and harmfully augment preload in a compromised left ventricle. The attendant increase in left atrial pressure may produce pulmonary edema. … A different, but related phenomenon may be operative in the newborn with severe left ventricular dysfunction and pulmonary hypertension. In these patients, the systemic circulation may depend in part on the ability of the right ventricle to sustain cardiac output through a right-to-left shunt across the patent ductus arteriosus. Selective pulmonary vasodilation may redirect the right ventricular output to the lungs and away from the systemic circulation. Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively right to left shunting at the ductus arteriosus, NO should be used with extreme caution."*

Thus, Atz describes two distinct phenomenon – (i) the potential effects of inhaled nitric oxide in adults, and (ii) the potential effects of inhaled nitric oxide in newborns dependent on right-to-left shunt across the patent ductus arteriosus.

A. Adults

Atz and the NIH Manual describe the potential effects of inhaled nitric oxide on adults with left ventricular dysfunction due primarily to ischemic or hypertensive cardiomyopathy. As explained during the interviews, adult patents are clearly distinct from non-adult patients due to the fact that the etiology and pathophysiology of the left ventricular dysfunction present in non-adult patients is markedly different from adult patients. Accordingly, adults are not clinically analogous to neonates, particularly with respect to left ventricular dysfunction.

In particular we note that children usually have congenital (structural) defects or cardiomyopathies (muscle diseases), not ischemic or hypertensive heart disease. The distinction is important because left ventricular dysfunction comes in two broad types: diastolic dysfunction (stiff, non-compliant heart that cannot fill properly) or systolic dysfunction (soft flabby heart that cannot push blood out). Adults typically have the former, and this has been previously noted in the medical literature. Non-adults more typically have the latter, and in this case, one would not expect an elevated risk of pulmonary edema or cardiac complications. For this reason, there were no existing concerns about left ventricular dysfunction in non adults until the INOT 22 study was analyzed.

B. Neonates with Right-to-Left Shunt

In addition, Atz is further directed to the potential effects of inhaled nitric oxide in a second, distinct patient population – neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus. As discussed during the interview of January 10, 2011, pre-existing left ventricular dysfunction, as enumerated in the claims, does not include neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus. In particular, patent ductus arteriosus is a congenital disorder in the heart wherein the ductus arteriosus of a neonate (a vascular connection between the pulmonary artery and the aortic arch that allows most of the blood from the right ventricle to bypass the fetus lungs) fails to close naturally after birth. This condition is very specific and rather uncommon; it has essentially no clinical or physiologic overlap with any other condition discussed in this patent application. At the time of the instant invention, it was widely recognized by those of skill in the art that neonates dependent on right-to-left shunting of blood should not be treated with inhaled nitric oxide. In fact, this contraindication has been presented in the prescribing information (sometime referred to as the drug "label") for INOMAX® (nitric oxide) for inhalation, since the approval of the drug by the FDA in December 1999 (see Section 4 of the INOMAX Prescribing Information – Attached hereto as Appendix B). Consequently, patients with this specific condition, were, of course, excluded from the INOT22 study that resulted in the discovery that is the subject of the presently claimed invention. Moreover, in 2009, based on the findings from the INOT22 study, the FDA approved the addition of the following new warnings to the INOMAX® prescribing information, independent of the existing contraindication for neonates dependent on right-to-left shunting of blood:

> "*Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema*" and "*5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*"

One would have to suppose that the FDA would not add new warnings and precautions to the label of a drug that essentially restate a known contraindication already existing on the approved drug label. Indeed, the new FDA-approved warnings for the use of nitric oxide are clinically distinct from the existing, original INOMAX contraindication disclosed by Atz, with respect to neonates with right-to-left shunt.

In contrast to Atz, the NIH Manual and other prior art references cited by the Examiner, the claimed invention relates to an important discovery in a **third patient population**--non-adult patients (i.e., patients under the age of 18) with pre-existing left ventricular dysfunction who are eligible to receive inhaled nitric oxide treatment (i.e., those not dependent on a right-to-left shunting of blood). As discussed during the interview of January 10, 2011, adult patents are clearly distinct from non-adult patients due to the fact that the etiology and pathophysiology of the left ventricular dysfunction present in non-adult patients markedly different from adult patients. Further, the pre-existing left ventricular dysfunction in non-adult patients, as claimed in the present invention, is clinically distinct from the pathophysiology within neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus.

To anticipate a claim, a single prior art reference must be enabled and teach each and every element of the claimed invention. In this case, the NIH manual and Atz (with respect to the adult disclosure within Atz) fail to anticipate the claimed invention in that the prior art disclose the risk associated with the use of inhaled nitric oxide in adults. In addition, the further disclosure in Atz to neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus is not an element of the pending claims.

Rejection and withdrawal of the anticipation rejections in view of Atz and the NIH manual are respectfully requested.

Rejections Under 35 USC § 103(a)

The Examiner rejected claims 1-20 under 35 USC § 103(a) as being obvious over five different references. In addition to Atz and the NIH Manual, the Examiner further sites Kinsella et al. (The Lancet 1999, 354 1061-1065), Bolooki (Clinical Application of the Intra-Aortic Balloon Pump 1998, 3rd Ed. pp 252-253) and Loh et al. (Circulation 1994, 90, 2780-2785).

As explained previously, Atz and the NIH manual describe the use and potential risks of inhaled nitric oxide therapy in adults, a patient population clinically distinct from the patients enumerated in new claims 21-25. Further, Atz describes the well known contraindication with regard to the use of inhaled nitric oxide in neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus.

Kinsella discloses a double blind study that evaluated 80 premature infants with severe hypoxic respiratory failure. The exclusion criteria was "fatal congenital anomalies or congenital heart disease (except atrial and ventricular septal defects" and the study noted the rate and severity of intracranial hemorrhage, pulmonary hemorrhage, duration of ventilation, and, chronic lung disease. Kinsella described the potential adverse effects of inhaled nitric oxide on platelet adhesion and the attendant risks of intracranial hemorrhage Kinsella concluded that low dose iNO improved oxygenation and decreased the need for mechanical ventilation as well as lowered the frequency of chronic lung disease, but did not improve survival in severely hypoxic neonates. Kinsella is silent with respect to the use of inhaled nitric oxide in non-adult patents having pre-existing left ventricular dysfunction.

Bolooki describes uses of an intra-aortic balloon pump in adult patients, as well as nitroglycerin and calcium channel blockers in the treatment of left ventricular dysfunction (pages 252-253). Bolooki is silent respect to the use of inhaled nitric oxide in non-adult patients.

Loh is a study of 19 adult patients with an average age of 52 +/- 3 years suffering from ischemic cardiomyapathy (heart failure due to coronary artery disease and resultant partial cardiac muscle death) and idiopathic dilated cardiomyopathy, which resulted in left ventricular failure. The patients also had reactive pulmonary artery HTN secondary to LV failure. The patients were identified as having heart failure due to LV dysfunction as classified by adult NYHA classifications (class III and class IV). The study found that in patients with heart failure due to LV dysfunction, inhalation of NO causes a decrease in the PVR associated with an increase in LV filling pressure. Unlike the claimed invention, Loh does not disclose the use of inhaled nitric oxide in non-adult patients, a patient population clinically distinct from those treated in Loh and fails to suggest or anticipated an elevated risk for the use of inhaled nitric oxide in non-adult patients subject to distinct cardiac myopathies than those possessed by the adult patient in the Loh study.

As described herein, contrary to the prior art references cited by the Examiner, the claimed invention relates to an important discovery in pediatric patients with pre-existing left ventricular dysfunction who are eligible to receive inhaled nitric oxide treatment (i.e., those not dependent on a right-to-left shunting of blood). As explained during the interviews, those of ordinary skill in the art, prior to the instant invention, would not have found it obvious to withhold inhaled NO treatment from this class of patients based on the prior art cited by the Examiner in the Office Action mailed August 13, 2010, because the etiology and pathophysiology of the left ventricular dysfunction present in adult patient populations and neonates with right-to-left shunt is markedly different from the non-adult patients of the claimed invention. In fact, as described in greater detail below, the members of the INOT22 Screening Committee who designed the study and the approximately 18 Institutional Review Boards and 2 National Health Authorities who reviewed and approved the study prior to its initiation, would have been aware of the cited prior art, but yet failed to predict that any untoward effects would be caused by the administration of inhaled NO in the claimed patient population.

The INOT22 Study

The INOT22 study, entitled “Comparison of supplemental oxygen and nitric oxide for inhalation plus oxygen in the evaluation of the reactivity of the pulmonary vasculature during acute pulmonary vasodilatory testing" was conducted both to access the safety and effectiveness of INOMAX® as a diagnostic agent in patients undergoing assessment of pulmonary hypertension (primary endpoint), and to confirm the hypothesis that iNO is selective for the pulmonary vasculature (secondary endpoint).

During, and upon final analysis of the INOT22 study results, applicants discovered that rapidly decreasing the pulmonary vascular resistance, via the administration of iNO to a patient in need of such treatment, may be detrimental to patients with concomitant, pre-existing left ventricular dysfunction (LVD).

The INOT22 study was an open, prospective, randomized, multi-center, controlled diagnostic trial, with an expected total enrollment of a minimum of 150 patients, in approximately 18 study sites in the US and Europe over approximately 2 years. The expected patient population for enrolment into the INOT22 trial were subjects between the ages of four(4) weeks and eighteen (18) years undergoing diagnostic right heart catheterization scheduled to include pulmonary vasodilation testing to assess pulmonary vasoreactivity. The anticipated study population were subjects with idiopathic pulmonary arterial hypertension, congenital heart disease with pulmonary hypertension and cardiomyopathies.

The INOT22 study was established and designed by the study sponsor, INO Therapeutics LLC (INOT) and a Steering Committee comprising internationally recognized experts in the field of pediatric heart and lung disease.

The original INOT22 protocol contained the following inclusion and exclusion criteria:

Inclusion Criteria

The patient must meet the following criteria:

1. *Have any one of the three disease categories:*

    a. *Idiopathic Pulmonary Arterial Hypertension*

        i. *PAPm >25mmHg at rest, PCWP ≤ 15mmHG, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization*

    b. *CHD with pulmonary hypertension repaired and unrepaired,* *i. PAPm >25mmHg at rest, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization*

c. Cardiomyopathy

i. PAPm >25mmHg at rest, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization

2. Schedule to undergo right heart cateterization to assess pulmonary vasoreactivity by acute pulmonary vasodilation testing.

3. Males or females, ages 4 weeks to 18 years, inclusive

4. Signed IRB/IEC approved informed consent (and assent if applicable).

Exclusion Criteria

The patient will be excluded from enrolment if any of the following are true:

1. Focal pulmonary infiltrates on chest radiograph.

2. diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.

3. Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigation medications, nitroglycerein, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.

4. Pregnant (urine HCG +).

The INOT22 investigational plan and study protocol were further reviewed and approved by the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the participating study institutions, including review by the principal investigator within each study institution.

At no time did any member of the Steering Committee, INOT, nor any member of an IRB, IEC, or individual principal investigator, appreciate, recognize or otherwise suggest that the exclusion criteria be amended to exclude study subjects with pre-existing left ventricular dysfunction, due to an anticipated or predicted risk of adverse events or serious adverse events arising from the use of iNO in patients with pre-existing LVD.

After initiation and enrolment of the first 24 subject in INOT22, there were 5 serious adverse events – a rate much higher than expected based on prior clinical experience. Each of these 5 SAEs were cardiovascular events, and included pulmonary edema, cardiac arrest and hypotension (low blood pressure).

Thereafter, in February 2005, INO and the Steering Committee convened to review the unexpected SAEs described above, and upon review and discussion, expressed concern that the unexpected SAEs may be due to the administration of iNO in subjects having pre-existing LVD. Accordingly, based upon a review of the SAE cases, the exclusion criteria of the INOT22 protocol was amended to thereafter exclude subjects with pre-existing LVD. For the purpose of the study, the exclusion criteria was amended to exclude subjects from enrolment if the subjects demonstrated an elevated pulmonary capillary wedge pressure (PCWP), defined within the study as subject having a PCWP greater than 20 mmHG. All study sites were notified immediately. The exclusion criteria was amended as follows:

Exclusion Criteria

The patient will be excluded from enrolment if any of the following are true:

Focal pulmonary infiltrates on chest radiograph.

diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.

Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigation medications, nitroglycerein, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.

4. Pregnant (urine HCG +).

5. Baseline PCWP > 20 mmHg.

Upon conclusion of the INOT22 study and completion of the final study report, INO noted that subsequent to excluding patients with pre-existing LVD, the rate of serious adverse events (including serious adverse events associated with heart failure) was significantly reduced. There were 5 SAEs amongst the first 24 subjects of this type prior to the additional exclusion criteria, but only 2 SAEs amongst the last 80 subjects in the study after the additional exclusion. Furthermore, there were 2 SAEs amongst the 4 subjects with evidence of pre-existing LVD, but only 5 SAEs amongst the 120 subjects without evidence of LVD.

Therefore, based on this unexpected finding, on February 25, 2009, INO Therapeutics LLC (owner of NDA 20845) submitted a label supplement to the US Food and Drug Administration (FDA) seeking to amend the prescribing information for INOMAX® to include a warning statement for physicians such that the use of iNO in patients with pre-existing LVD could cause serious events, such as pulmonary edema. On August 28, 2009, the FDA approved the INOMAX® label supplement to include the following new information:

> *WARNINGS AND PRECAUTIONS*
>
> *Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).*
>
> *5 WARNINGS AND PRECAUTIONS*
>
> *5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*

Accordingly, as evidenced and supported by the findings of the INOT22 study which gave rise to the present invention, in view of the prior art, one skilled in the art at the time of the invention would not have predicted or anticipated that non-adult patients with pre-existing LVD would be at risk of experiencing adverse events or serious adverse events arising from the treatment with inhaled nitric oxide. Clearly, if such a risk would have been obvious, one of the many skilled medical professionals expert in the field of pediatric cardiology that reviewed the original INOT22 protocol would have noted a predicted increased risk in the claimed patient population. They did not. Rather, it was only after a series of completely unexpected adverse events that the INOT22 protocol was amended to exclude the non-adult patient population enumerated in the claimed invention. As further support of the non-obviousness of the invention, in view of the prior art, Applicants point out that the senior author of Atz, Dr. David Wessel, was a member of the Steering Committee that designed the original INOT22 protocol. As an author of the Atz reference and the disclosures therein, Dr. Wessel did not predict or anticipate that non-adult patients with pre-existing LVD would be at increased risk of experiencing adverse events or serious adverse events arising from the treatment with inhaled nitric oxide.

Turning now to the specific language of the pending claims, new independent claims 21, 24, 25, 28 and 31 are each limited to non-adult patients (i.e., patients under the age of 18), as are their dependents, and accordingly are not anticipated or rendered obvious by the prior art cited by the Examiner in the Office Action mailed August 17, 2010.

In addition, new claims independent claims 21, 24, 25, 28 and 31 expressly enumerate the distinction between the patient population addressed by the Atz reference, (neonates known to be dependent on right-to-left shunting of blood through a patent ductus arteriosus), as to which the potential dangers of inhaled nitric oxide were well known in the prior art and were clearly outlined in a contraindication on the FDA-approved label for inhaled nitric oxide.[2] Conversely, independent claims 21, 24, 25, 28 and 31 pertain to the novel and non-obvious finding that resulted from the INOT22 study, i.e., the new warning added to the Warnings and Precautions section of the prescribing label for inhaled nitric oxide (see page 1, right column) and the additional Section 5.4 of the prescribing label for inhaled nitric oxide (see page 2, left column) containing an additional warning from the INOT22 study. In particular, independent claims 21, 24, 25, 28 and 31 expressly state that the new warnings are independent from the known contraindication, described by Atz, pertaining to neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus.

[2] See Appendix B, label at §4: "Contraindications").

Person of Ordinary Skill in the Art

As is well known, the determination of obviousness, while a question of law, is based on underlying factual inquiries that include the level of ordinary skill in the pertinent art. Factors that may be considered are (i) the educational level of the inventor, (ii) the type of problems encountered in the art, (iii) prior art solutions to those problems; (iv) the rapidity with which innovations are made; (v) the sophistication of the technology; and (vi) the education level of active workers in the field. Applicant notes that the members of the Steering Committee were retained because they were well recognized **experts** in the field, not because they were considered to have "ordinary skill in the art". Thus, although the Steering Committee did not predict or anticipate the risk of adverse events or serious adverse events associated with the use of iNO in study subjects with pre-existing LVD, this level of anticipation is not representative of those of ordinary skill in the art, but rather of those of presumptively **extraordinary** skill. Thus, it is all that much more surprising, and supportive of the non-obviousness of the claims, that the Steering Committee, INOT, the various Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) and the individual Principal Investigators, upon review of the original INOT22 protocol, and in view of prior art cited by the Examiner, did not predict or anticipate the risk of adverse events or serious adverse events associated with the use of iNO in non-adult patients with preexisting LVD.

In light of the above, Applicant respectfully submits that the application as amended is in condition for allowance and respectfully requests the same. Examiner Arnold is invited to contact Chief Patent Counsel for the patent owner, Jonathan Provoost (Reg. No. 44, 292) at 908-238-6392 to discuss any of the amendments or remarks set forth above.

A three-month time extension under 37 CFR 136(a) is submitted herewith. Please apply any additional necessary charges or credits to deposit account **12-0769**, referencing Attorney Docket No. I001-0002USC3.

Respectfully submitted,

/Jonathan N. Provoost, Reg. No. 44,292/ Dated: February 14, 2011

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APPENDIX A

From: Brown, Dennis (NIH/CC/CCMD) [E] [mailto:DBrown@cc.nih.gov]
Sent: Wednesday, December 29, 2010 11:27 AM
To: Jeffrey Smith
Cc: Allen, Sarah (NIH/CC/CCMD) [E]
Subject: RE: NIH Policy/ Procedure

Jeff,
Sorry for the delay in responding to you. We have a process in place for outside inquiries and I wanted to make sure I was in compliance. The answers to your questions are as follows:

So, just to make sure that I am not way off base in my analysis of this document, can you provide me with some direction?
Specifically:

(1) Is this document used as a policy and procedure for RT's in the MICU, giving them guidelines in the delivery of iNO to adult patients?

Yes. This is a policy/procedure in place to provide direction to respiratory therapists in the implementation of NO in the MICU to adult patients in the ICU.

(2) Does the Clinical Center (for which the attached policy pertains) have a neonatal ICU?

No. The Clinical Center at the National Institutes of Health does not have a neonatal ICU nor do we care for neonates.

Once again I apologize for the delay. Hopefully the information will provide you with the requested clarification.

Dennis T. Brown

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From: Jeffrey Smith [mailto:JeffreyS@LeeHayes.com]
Sent: Tuesday, December 21, 2010 12:49 PM
To: Brown, Dennis (NIH/CC/CCMD) [E]
Cc: Allen, Sarah (NIH/CC/CCMD) [E]
Subject: NIH Policy/ Procedure
Importance: High

Hi Dennis:

Last week I contacted Sarah Allen regarding a question about an NIH policy dealing with iNO (see my email to her below). She contacted me this morning and felt like you would be the appropriate contact regarding the request below. I have reattached the document in question.

Thanks for your help. Please let me know if you have any questions. I can be reached at my office, (509-944-4786) or via email.

Have a great day.

Jeff
Jeffrey R. Smith, Esq.
Corporate Practice Group
Life Science Practice Group
(509) 944.4786
jeffreys@leehayes.com

APPENDIX B

INOmax® (nitric oxide) for inhalation

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INOmax safely and effectively. See full prescribing information for INOmax.

INOmax (nitric oxide) for inhalation
Initial U.S. Approval: 1999

RECENT MAJOR CHANGES

Warnings and Precautions, Heart Failure (5.4) 8/2009

INDICATIONS AND USAGE

INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (1.1).

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration (1.1).

Utilize additional therapies to maximize oxygen delivery (1.1).

DOSAGE AND ADMINISTRATION

Dosage: The recommended dose of INOmax is 20 ppm, maintained for up to 14 days or until the underlying oxygen desaturation has resolved (2.1).

Administration:

- INOmax must be delivered via a system which does not cause generation of excessive inhaled nitrogen dioxide (2.2).
- Do not discontinue INOmax abruptly (2.2).

DOSAGE FORMS AND STRENGTHS

INOmax (nitric oxide) is a gas available in 100 ppm and 800 ppm concentrations.

CONTRAINDICATIONS

Neonates known to be dependent on right-to-left shunting of blood (4).

WARNINGS AND PRECAUTIONS

Rebound: Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure (5.1).

Methemoglobinemia: Methemoglobin increases with the dose of nitric oxide; following discontinuation or reduction of nitric oxide, methemoglobin levels return to baseline over a period of hours (5.2).

Elevated NO_2 Levels: NO_2 levels should be monitored (5.3).

Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).

ADVERSE REACTIONS

Methemoglobinemia and elevated NO_2 levels are dose dependent adverse events. Worsening oxygenation and increasing pulmonary artery pressure occur if INOmax is discontinued abruptly. Other adverse reactions that occurred in more than 5% of patients receiving INOmax in the CINRGI study were: thrombocytopenia, hypokalemia, bilirubinemia, atelectasis, and hypotension (6).

To report SUSPECTED ADVERSE REACTIONS, contact INO Therapeutics at 1-877-566-9466 and http://www.inomax.com/ or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Nitric oxide donor agents: Nitric oxide donor compounds, such as prilocaine, sodium nitroprusside, and nitroglycerin, when administered as oral, parenteral, or topical formulations, may have an additive effect with INOmax on the risk of developing methemoglobinemia (7).

Revised: August 2009

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoxic Respiratory Failure

INOmax® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies *[see Clinical Studies (14)]*.

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage

Term and near-term neonates with hypoxic respiratory failure

The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

2.2 Administration

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

Do not discontinue INOmax abruptly, as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

3 DOSAGE FORMS AND STRENGTHS

Nitric oxide is a gas available in 100 ppm and 800 ppm concentrations.

4 CONTRAINDICATIONS

INOmax is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.

5 WARNINGS AND PRECAUTIONS

5.1 Rebound

Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

5.2 Methemoglobinemia

Methemoglobinemia increases with the dose of nitric oxide. In clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide, the methemoglobin levels returned to baseline over a period of hours.

5.3 Elevated NO_2 Levels

In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

5.4 Heart Failure

Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from the clinical studies does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

6.1 Clinical Trials Experience

Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse reactions that occurred in at least 5% of patients receiving INOmax in the CINRGI study with event rates >5% and greater than placebo event rates. None of the differences in these adverse reactions were statistically significant when inhaled nitric oxide patients were compared to patients receiving placebo.

Table 1:
Adverse Reactions in the CINRGI Study

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 *(10%)*	13 *(13%)*
Withdrawal	9 *(10%)*	12 *(12%)*
Atelectasis	8 *(9%)*	9 *(9%)*
Hematuria	5 *(6%)*	8 *(8%)*
Hyperglycemia	6 *(7%)*	8 *(8%)*
Sepsis	2 *(2%)*	7 *(7%)*
Infection	3 *(3%)*	6 *(6%)*
Stridor	3 *(3%)*	5 *(5%)*
Cellulitis	0 *(0%)*	5 *(5%)*

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of INOmax. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to estimate their frequency reliably or to establish a causal relationship to drug exposure. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

7 DRUG INTERACTIONS

No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

8.2 Labor and Delivery

The effect of INOmax on labor and delivery in humans is unknown.

8.3 Nursing Mothers

Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

8.4 Pediatric Use

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

8.5 Geriatric Use

Nitric oxide is not indicated for use in the adult population.

10 OVERDOSAGE

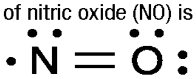
Overdosage with INOmax will be manifest by elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

11 DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:



12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide selectively dilates the pulmonary vasculature, and because of efficient scavenging by hemoglobin, has minimal effect on the systemic vasculature.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

12.2 Pharmacodynamics

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

12.3 Pharmacokinetics

The pharmacokinetics of nitric oxide has been studied in adults.

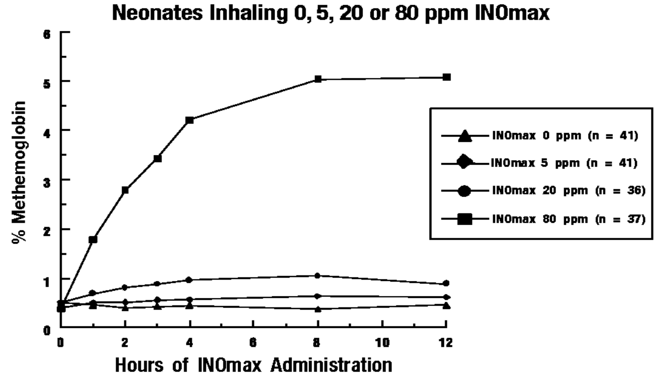
12.4 Pharmacokinetics: Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

12.5 Pharmacokinetics: Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1:
Methemoglobin Concentration – Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groups, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 35% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients, but one patient did not exceed 7% until 40 hours.

12.6 Pharmacokinetics: Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

14 CLINICAL STUDIES

14.1 Treatment of Hypoxic Respiratory Failure (HRF)

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI= mean airway pressure in cm H_2O × fraction of inspired oxygen concentration [FiO_2]× 100 divided by systemic arterial concentration in mm Hg [PaO_2]) and increases PaO_2 *[see Clinical Pharmacology (12.1)]*.

NINOS Study

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 2.

Table 2:
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups *[see Adverse Reactions (6.1)]*. Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

CINRGI Study

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 3.

Table 3:
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO*,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 58%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups *[see Adverse Reactions (6.1)]*.

14.2 Ineffective in Adult Respiratory Distress Syndrome (ARDS)

ARDS Study

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FiO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

16 HOW SUPPLIED/STORAGE AND HANDLING

INOmax (nitric oxide) is available in the following sizes:

Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)
Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure

The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

 SPC-0303 V:4.0

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Daniel Leo Hayes/Pat Palmer
Attorney Docket Number:	I001-0002USC4

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Independent claims in excess of 3	2201	1	110	110
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	2253	1	555	555
Miscellaneous:				
		Total in USD ($)		**665**

Electronic Acknowledgement Receipt

EFS ID:	9472799
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Daniel Leo Hayes/Pat Palmer
Filer Authorized By:	Daniel Leo Hayes
Attorney Docket Number:	I001-0002USC4
Receipt Date:	17-FEB-2011
Filing Date:	22-JUN-2010
Time Stamp:	19:55:30
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$665
RAM confirmation Number	5605
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		PK8567.PDF	424059 609314bb31e9687aa791616e84e1c2b7b10f4e09	yes	28

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	28

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	32028 c2be86ec15dc7c19216d1d0460cf3642c7c60ead	no	2

Warnings:

Information:

Total Files Size (in bytes):	456087

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	
12/821,041	06/22/2010	☐ To be Mailed

APPLICATION AS FILED – PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY ☒ RATE ($)	FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
			TOTAL			TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED – PART II

AMENDMENT

02/17/2011	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 16	Minus	** 20	= 0	X $26 =	0	OR	X $ =	
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $110 =	0	OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE	**0**	OR	TOTAL ADD'L FEE	

AMENDMENT

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/GAIL WOOTEN/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	12/821,041
Filing Date	June 22, 2010
First Named Inventor	Baldassarre, James S.
Art Unit	1613
Examiner Name	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1]Applicant's unique citation designation number (optional). [2]See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6]Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 2

Complete if Known	
Application Number	12/821,041
Filing Date	June 22, 2010
First Named Inventor	Baldassarre, James S.
Art Unit	1613
Examiner Name	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		STEINHORN, "Persistent Pulmonary Hypertension in the Newborn and Infant" 1 (2):287-299 (1987). [downloaded from www.emedicine.com on June 10, 2008].	
		ROBERTS, et al., Nitric Oxide and the Lung, Marcel Dekker, Inc., New York, NY, p. 333-63 (1997).	
		Meyler's Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions, Nitric Oxide, Fifteenth Edition, Elsevier B.V. (2006).	
		ICHINOSE, et al., Circulation, 109: 3106-3111 (2004).	
		INO Therapeutics, NCT00041548 at ClinicalTrials.gov (2005).	
		INO Therapeutics, NCT00551642 at ClinicalTrials.gov (2007).	
		Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NCT00005773 at ClinicalTrials.gov (2005).	
		University of Alabama, NCT00732537 at ClinicalTrials.gov (2008).	
		TRONCY, et al., Can J Anaesth, 44 (9): 973-988 (1997).	
		BLOCH, et al., Cardiovasc. Res. 2007, 75(2): 339-348 (July 15, 2007).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached.

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/821,041
Confirmation Number	3219
Filing Date	June 22, 2010
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	1613
Examiner	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

U.S. Patent and Trademark Office
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Randolph Building
401 Dulany Street
Alexandria, VA 22314

CERTIFICATE OF SERVICE

It is certified that a copy of the attached Third-Party Submission in Published Application Under 37 CFR § 1.99 is being forwarded by First Class Mail on February 23, 2011 to:

Jonathan N. Provost
Associate General Counsel
Ikaria
6 Route 173
Clinton, NJ 08809

Christopher P. Rogers
Lee & Hayes, PLLC
601 W. Riverside Avenue
Suite 1400
Spokane, WA 99201

the Attorneys for the Applicants of U.S. Patent Application No. 12/821,041 referenced above.

Respectfully submitted,

Date: February 25, 2011

William D. Hare

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	William D. Hare
Attorney Docket Number:	I001-0002USC4

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	9538876
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	William D. Hare
Filer Authorized By:	
Attorney Docket Number:	I001-0002USC4
Receipt Date:	25-FEB-2011
Filing Date:	22-JUN-2010
Time Stamp:	17:39:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment Type	Credit Card
Payment was successfully received in RAM	$180
RAM confirmation Number	4862
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Transmittal Letter	041_Third_Party_Submission.pdf	4796 6448be3d6f89fa24920581bb43a39111ca006511	no	1
Warnings:					
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2	Information Disclosure Statement (IDS) Filed (SB/08)	041IDS.pdf	71635 61a3659ca23c3b32a35ca8221c842c66006a50a1	no	2
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11	NPL Documents	UofAStudy1.pdf	101821 2f93f979df5e736edb4541bf9eba1790df2c9aec	no	4
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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Serial Number	12/821,041
Confirmation Number	3219
Filing Date	June 22, 2010
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria Holdings Inc.
Group Art Unit	1613
Examiner	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

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THIRD-PARTY SUBMISSION IN PUBLISHED APPLICATION UNDER 37 CFR § 1.99

Submitted in the above-referenced applications in compliance with 37 CFR § 1.99 are:

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(2) A list of the patents or publications submitted for consideration by the Office, including the date of publication of each patent or publication;

(3) A copy of each listed patent or publication in written form or at least the pertinent portions; and

(4) An English language translation of all the necessary and pertinent parts of any non-English language patent or publication in written form relied upon.

This submission is being served upon the applicant in accordance with § 1.248. A copy of the certificate of service is attached

Respectfully submitted,

Date: February 25, 2011

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

U.S.PATENTS

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	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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Examiner Signature	/Christopher P. Rogers, Reg. No. 36,334/	Date Considered	2011-03-14

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

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☐ See attached certification statement.

☒ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ None

SIGNATURE

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Signature	/Christopher P. Rogers, 36334/	Date (YYYY-MM-DD)	2011-03-14
Name/Print	Christopher P. Rogers	Registration Number	36,334

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Daniel Leo Hayes/Anna Goforth
Attorney Docket Number:	I001-0002USC4

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Utility under 35 USC 111(a) Filing Fees

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Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

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Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Daniel Leo Hayes/Anna Goforth
Filer Authorized By:	Daniel Leo Hayes
Attorney Docket Number:	I001-0002USC4
Receipt Date:	14-MAR-2011
Filing Date:	22-JUN-2010
Time Stamp:	17:58:27
Application Type:	Utility under 35 USC 111(a)

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32	NPL Documents	OI6261.PDF	227013 97120fab9318ed58086ae78bd67d049ada3c9f25	no	11
Warnings:					
Information:					
33	NPL Documents	OI6342.PDF	126443 e184f32669042cfabf820ffebbdfa9cecb99bda0	no	7
Warnings:					
Information:					
34	NPL Documents	OI6517.PDF	139951 81c864c2202ca87243a5a5facd4371552da9f6c7	no	9
Warnings:					
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35	NPL Documents	OI6602.PDF	289637 144304143d42035e47259969d1e42c56324fac0c	no	9

Warnings:					
Information:					
36	NPL Documents	OI7415.PDF	310458 bf072a2265fdbac35890ba46c8298994707 40fba	no	21
Warnings:					
Information:					
37	NPL Documents	OI7522.PDF	78601 b4811f502f03c08bef0710f8e11ae2940014 2ba1	no	3
Warnings:					
Information:					
38	NPL Documents	PS4787.PDF	384261 d2f2bbad8d658cf75d7bc7f782d256876a2 91fc0	no	3
Warnings:					
Information:					
39	NPL Documents	KD6589.PDF	188129 5dfbddab4bd904d4137c2e20bbef4c82bac a028a	no	9
Warnings:					
Information:					
40	NPL Documents	OI8417.PDF	342110 8d1fe14d22147f6308bf4afad2df7a6ca37df 8cd	no	11
Warnings:					
Information:					
41	NPL Documents	PT3002.PDF	224553 6f52ba62c9b7e35678929915c38a4b580af6 7df1	no	2
Warnings:					
Information:					
42	NPL Documents	PT3285.PDF	110728 4231a6373f636407e4bafb7ff540415b1755 eaaa	no	3
Warnings:					
Information:					
43	NPL Documents	PS5790.PDF	124652 e41ec52f45930913820fa218327b35b89f21 e4ce	no	17
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Information:					
44	NPL Documents	NU9264.PDF	318654 bfa5d6be9a90545167eba871ae77595663e 8c7f0	no	5

Warnings:					
Information:					
45	NPL Documents	PR2242.PDF	1065274 11aa669808723b09f22c86eac37e9a58d90913d1	no	12
Warnings:					
Information:					
46	NPL Documents	PR2292.PDF	1289915 d58aca59ec8d5638e8dcab5324b008c7260ea117	no	11
Warnings:					
Information:					
47	NPL Documents	PS4452.PDF	1534950 ed9b3c47f6f8dc5a608a37bf8ebc7bf9e14b3f38	no	11
Warnings:					
Information:					
48	NPL Documents	PR2444.PDF	3558726 4525dc8e0132921a7922ebeecc4c77730db47cff	no	32
Warnings:					
Information:					
49	NPL Documents	PR2490.PDF	628636 f3ff2900daa94db1a8e2d77b5ba9228b822c4bc5	no	7
Warnings:					
Information:					
50	Fee Worksheet (PTO-875)	fee-info.pdf	30461 ef8019274e84ccb665de56361795ad61807d6e8f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	20829707		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 12/821,041
Filing Date 6/22/2010
Confirmation No. 3219
First Named Inventor ....James S. Baldassarre
Assignee ....
Group Art Unit 1616
Examiner ....Ernst V. Arnold
Attorney's Docket No. I001-0002USC4
Title: Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associ

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

From: Christopher P. Rogers (Tel.; Fax 509-323-8979)
Customer Number: 29150
Lee & Hayes, PLLC
601 W. Riverside Avenue, Suite 1400
Spokane, WA 99201

Fees will be paid by credit card through the EFS Web; however the Commissioner is hereby authorized to charge any deficiency of fees and credit any overpayments to Deposit Account Number 12-0769.

Respectfully Submitted,

Dated: March 10, 2011

By: /Christopher P. Rogers, RegNo 36,334/
Christopher P. Roger, Reg. No. 36,334

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/821,041
Confirmation Number	3219
Filing Date	June 22, 2010
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	1613
Examiner	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL REPLY AMENDMENT (37 CFR 1.111)

This supplemental communication is responsive to the Non-Final Office Action mailed August 17, 2010, setting a shortened statutory period for reply of 3 months and is a supplement to the Reply Amendment filed by applicant on February 17, 2011.

Applicant respectfully requests entry of this Supplemental Reply Amendment, reconsideration of the pending rejections, and allowance of the application.

Amendments to the Specification begin on page 2 of this paper.

Supplemental Amendments to the Claims are reflected in the listing of claims beginning on page 3 of this paper.

Amendments to the Specification

Please amend paragraph **[0020]** as follows:

[0020] INOmax® (nitric oxide) for inhalation was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999. Nitric oxide, the active substance in INOmax®, is a selective pulmonary vasodilator that increases the partial pressure of arterial oxygen (PaO2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from the lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios. INOmax® significantly improves oxygenation, reduces the need for extracorporeal oxygenation and is indicated to be used in conjunction with ventilatory support and other appropriate agents. The current FDA-approved prescribing information for INOmax® is incorporated herein by reference in its entirety. Section 4 of the prescribing information, Contraindications, states that INOmax® is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.

Amendments to the Claims

1-20. (Canceled).

21. (Currently Amended) A method of distributing a pharmaceutical product, comprising:

providing a source of nitric oxide gas to a medical provider;

informing the medical provider that a recommended dose of inhaled nitric oxide is 20 ppm nitric oxide;

providing a first warning to ~~informing~~ the medical provider that nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood; and

providing a second ~~an additional~~ warning to the medical provider, separate and distinct from the first warning, that in ~~non-adult~~ pediatric patients with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunting of blood, and whose pre-existing left ventricular dysfunction is characterized by a condition selected from the group consisting of elevated pulmonary capillary wedge pressure, diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease and congenital heart disease, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema.

22. (Previously Presented) The method of claim 21 that further includes the step of providing a warning to the medical provider that patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

23. (Currently Amended) The method of claim 22 wherein the source of nitric oxide gas is a pressurized cylinder containing nitric oxide and one or more inert gases

~~wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm)~~.

24. (Currently Amended) A method of reducing the risk of one or more adverse events or serious adverse events in ~~an intended patient population comprising~~ pediatric patients ~~under the age of 18~~ in need of being treated with inhalation of nitric oxide, but who are not dependent on right-to-left shunting of blood comprising excluding from such treatment any of such patients ~~under the age of 18~~ having pre-existing left ventricular dysfunction characterized by a condition selected from the group consisting of elevated pulmonary capillary wedge pressure, diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease and congenital heart disease ~~whether or not such patients under the age of 18 are dependent on right-to-left shunting of blood~~.

25. (Currently Amended) A method of reducing the risk of one or more adverse events or serious adverse events associated with the use of inhaled nitric oxide in neonate patients receiving ~~under the age of 18 for the~~ treatment ~~of~~ for hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, comprising:

a. providing a source of pharmaceutically acceptable nitric oxide gas for inhalation to a medical provider;

b. informing the medical provider that inhaled nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood; and,

c. informing the medical provider that ~~in addition to~~ separately and independent from patients dependent on right-to-left shunt, for patients with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunting of blood, inhaled nitric oxide may increase pulmonary wedge pressure leading to pulmonary edema~~; and~~

~~d. informing the medical provider that in addition to patients dependent on right-to-left shunt, patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events~~.

26. (Currently Amended) The method of claim 25 wherein the source of nitric oxide gas is a pressurized cylinder containing nitric oxide and one or more inert gases ~~wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm)~~.

27. (Previously Presented) The method of claim 25 further including the step of informing the medical provider that a recommended dose of inhaled nitric oxide is 20 ppm.

28. (Currently Amended) A pharmaceutical product comprising:

a pressurized cylinder containing nitric oxide and one or more inert gases wherein the concentration of nitric oxide in the pressurized cylinder is about 800 parts per million (ppm); and

a label comprising:

(i) a statement indicating that a recommended dose of nitric oxide is 20 ppm nitric oxide;

(ii) a statement indicating that nitric oxide is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood; and

(iii) a warning, independent and separate from the contraindication for neonates known to be dependent on right-to-left shunting of blood, said warning indicating that in ~~non-adult~~ pediatric patients with pre-existing left ventricular dysfunction characterized by a condition selected from the group consisting of elevated pulmonary capillary wedge pressure, diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease and congenital heart disease, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema.

29. (Currently Amended) The pharmaceutical product of claim 28 wherein said pharmaceutical product is for the treatment of a term or near-term neonates having hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension.

30-36. (Canceled).

37. (New) The method of claim 25 further informing the medical provider that separately and independent from patients dependent on right-to-left shunt, patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events.

REMARKS

In the specification, paragraph [0020] was amended to included language from the INOMAX® prescribing information, which was expressly incorporated by reference within paragraph [0020] of the original specification. The language added to paragraph [0020] is material previously incorporated by reference and the amendment to paragraph [0020] contains no new matter (see 37 CFR 1.57(f) and MPEP 608.01(p)).

Claims 1-20 and 30-36 have been canceled.

Claims 21-29 and 37 are pending.

Claims 21, 23-26, 28 and 29 have been amended to more particularly point out and distinctly claim the subject matter applicant regards as the invention.

Claim 37 has been added also to more particularly point out and distinctly claim the subject matter applicant regards as the invention.

The amendments and new claim further address matters discussed during the Examiner Interview conducted on January 10, 2011 (See Interview Summary dated January 18, 2011), and subsequent discussions relating to co-pending application 12/820,866.

Applicant submits that the amendments herein are in compliance with revised 37 CFR 1.121.

Support for the new claim and the present amendments thereto is found in the specification of the application, as filed, including the original claims as filed and paragraphs [0007], [0011], [0014], [0020], [0023] and [0052] of the specification. In addition, with respect to the contraindication for neonates dependent on right to left shunting of blood, this language is expressly found in the prescribing information for inhaled nitric oxide, incorporated by reference in the specification at paragraph [0020], and as per the amendment to the specification above, this language is now expressly included in paragraph [0020].

As amended, claims 21-29 and 37 more clearly distinguish between the step of identifying patients depending on right-to-left shunting of blood at the patent ductus arteriosus, and with respect to the present invention, further identifying patients with pre-existing left ventricular dysfunction, independent of whether such patients have right-to-left shunting of blood at the patent ductus arteriosus. In particular, in claims 21, 24 and 28, pre-existing left ventricular dysfunction is expressly defined to only include patients characterized by a condition selected from the group consisting of elevated pulmonary capillary wedge pressure, diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease and congenital heart disease. This definition more clearly distinguishes the pediatric left ventricular dysfunction of the claimed invention from the disclosure of to right-to-left shunt in the prior art (e.g., Atz et al.).

To reiterate, the prior art (Atz et al., page 452) discloses the well known contraindication (also found on the prescribing information of INOMAX®, submitted with prior communications) that neonates dependent on right-to-left shunting of blood through a patent ductus arteriosus should not be administered inhaled nitric oxide. Indeed, at the time of the invention, it was widely recognized by those of skill in the art that this class of patients should not be given inhaled nitric oxide therapy. In contrast to the prior art and the contraindication, the claimed invention relates to an important discovery of an elevated risk for the use of inhaled nitric oxide within a newly identified and separate patient population - pediatric patients (i.e., children) with pre-existing left ventricular dysfunction, independent and separate from neonates dependent on a right-to-left shunting of blood.

In light of the above, Applicant respectfully submits that the application as amended is in condition for allowance and respectfully requests the same. Examiner Arnold is invited to contact Chief Patent Counsel for the patent owner, Jonathan Provoost (Reg. No. 44, 292) at 908-238-6392 to discuss any of the amendments or remarks set forth above.

Please apply any additional necessary charges or credits to deposit account **12-0769**, referencing Attorney Docket No. I001-0002USC4.

Respectfully submitted,

/Jonathan N. Provoost, Reg. No. 44,292/ Dated: April 13, 2011

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Clinton, NJ 08809
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Cell: (908) 391-3440
Fax (legal dept.): (908) 238-6773
jonathan.provoost@ikaria.com

Electronic Acknowledgement Receipt

EFS ID:	9875001
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Edward John Fain/Shani Hoke
Filer Authorized By:	Edward John Fain
Attorney Docket Number:	I001-0002USC4
Receipt Date:	13-APR-2011
Filing Date:	22-JUN-2010
Time Stamp:	18:21:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response.pdf	79537 3c38b097776683f39f5b156c2cf4be586f713bfd	yes	9

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Supplemental Response or Supplemental Amendment	1	2
	Claims	3	6
	Applicant Arguments/Remarks Made in an Amendment	7	9

Warnings:

Information:

Total Files Size (in bytes):	79537

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
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PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number	Filing Date	
	12/821,041	06/22/2010	☐ To be Mailed

APPLICATION AS FILED – PART I

SMALL ENTITY ☒ OR OTHER THAN SMALL ENTITY

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

SMALL ENTITY OR OTHER THAN SMALL ENTITY

AMENDMENT 04/13/2011	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 10	Minus	** 20	= 0	X $26 =	0	OR	X $ =	
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $110 =	0	OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE	0	OR	TOTAL ADD'L FEE	

AMENDMENT	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/SHARON HARRIS/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	I001-0002USC4	3219

49584 7590 06/27/2011
LEE & HAYES, PLLC
601 W. RIVERSIDE AVENUE
SUITE 1400
SPOKANE, WA 99201

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

NOTIFICATION DATE	DELIVERY MODE
06/27/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

lhpto@leehayes.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No.	Applicant(s)
	12/821,041	BALDASSARRE ET AL.
	Examiner	Art Unit
	ERNST ARNOLD	1613

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 April 2011*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21-29 and 37* is/are pending in the application.

4a) Of the above claim(s) *21-23,28 and 29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *24-27 and 37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/17/11, 2/25/11 and 3/14/11*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12821041	BALDASSARRE ET AL.
	Examiner	**Art Unit**
	ERNST V ARNOLD	1616

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
inventor name EAST/PALM	8/11/10	eva
EAST 424/718 text limted all databases	8/11/10	eva
google	8/10/10	eva
various disucssions with QAS Bennett Celsa and Jean Vollano concering proper incorporation by reference as well as patentabilityn	6/18/11	eva

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

DETAILED ACTION

Claims 1-20 were canceled and claims 21-36 were introduced in the amendment filed 2/17/11. In the amendment filed 3/14/11, claims 30-36 have been canceled and claim 37 is new. Accordingly, claims 21-29 and 37 are pending and under examination. Applicant's amendments have necessitated a new ground of rejection. Accordingly, this action is FINAL.

Election/Restrictions

Newly submitted claims 21-23 and 28 and 29 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: Claims 21-23 directed to methods of distributing a pharmaceutical product and 28 and 29 are directed to a pharmaceutical product comprising a pressurized cylinder of nitric oxide which would have been restricted had these claims been originally presented because the inventions drawn to the pharmaceutical product and methods of reducing the risk of one or more adverse events or serious adverse events in a pediatric patient are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case the method can use nitric oxide produced from NO donor compounds rather than NO in a pressurized cylinder. Inventions drawn to methods of reducing the risk of one or more adverse events or serious adverse events in a pediatric patient and methods of distributing a pharmaceutical product are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions have different steps with different expected outcomes.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 21-23, 28 and 29 are withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03. Claims 24-27 and 37 are under examination.

Information Disclosure Statement

The information disclosure statements (IDS) submitted on 2/17/11, 2/25/11 and 3/14/11 were filed after the mailing date of the Office Action on 8/17/10. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. References without a date have not been considered.

Specification

The disclosure is objected to because of the following informalities: The attempt to incorporate subject matter into the specification by reference to INOmax® is defective because the subject matter being incorporated into the claims must also be present in the specification. The same exact language is not present in the specification as used in the claims. See MPEP 608.01(p).

The incorporation by reference will not be effective until correction is made to comply with 37 CFR 1.57(b), (c), or (d). If the incorporated material is relied upon to meet any outstanding objection, rejection, or other requirement imposed by the Office, the correction must be made within any time period set by the Office for responding to the objection, rejection, or other requirement for the incorporation to be effective. Compliance will not be held in abeyance with respect to responding to the objection, rejection, or other requirement for the incorporation to be effective. In no case may the correction be made later than the close of prosecution as defined in 37 CFR 1.114(b), or abandonment of the application, whichever occurs earlier.

Any correction inserting material by amendment that was previously incorporated by reference must be accompanied by a statement that the material being inserted is the material incorporated by reference and the amendment contains no new matter. 37 CFR 1.57(f).

Appropriate correction is required.

Withdrawn rejections:

Applicant's amendments and arguments filed 2/17/11 and 4/13/11 are acknowledged and have been fully considered. Any rejection and/or objection not specifically addressed below is herein withdrawn. Claims 1, 2, 6, 8, 9, 13 and 17 were rejected under 35 U.S.C. 102(b) as being anticipated by The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages). Claims 13 and 17 were rejected under 35 U.S.C. 102(b) as being anticipated by Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455). Claims 1-20 were rejected under 35 U.S.C.

103(a) as being unpatentable over Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455) and Kinsella et al. (The Lancet 1999, 354, 1061-1065) and Bolooki (Clinical Application of the Intra-Aortic Balloon Pump 1998, 3rd Ed. Pp 252-253) and Loh et al. (Circulation 1994, 90, 2780-2785) and The NIH (Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, May 2000, 13 pages). Applicant has canceled these claims which renders the rejections moot. Accordingly, the rejections are withdrawn.

The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set of rejections and/or objections presently being applied to the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 26 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 26 introduces new matter as the claim recites the limitation: "a pressurized cylinder containing nitric oxide and one or more inert gases" There is no support in the specification for this limitation. The limitation of: " a pressurized cylinder containing nitric oxide and one or more inert gases" was not described in the specification as filed, and person skilled in the art would not recognize in the applicant's disclosure a description of the invention as presently claimed. The specification discloses "a gaseous blend of NO and nitrogen...cylinders as a compressed gas under high pressure" [0021] but does not describe the instantly claimed limitation. There is no guidance in the specification to select "a pressurized cylinder containing nitric oxide and one or more inert gases" which is broader in scope and represents a new concept. From MPEP 2163.06: "Applicant should therefore specifically point out the support for any amendments made to the disclosure." Applicant has directed the Examiner to paragraphs 7, 11, 14, 20, 23 and 52 as well as the original claims for support in the specification for the amendments. However, the Examiner did not find such support. Therefore, it is the Examiner's position that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of filing of the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 37 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 26 introduces new matter as the claim recites the limitation: "even for short durations". There is no guidance in the specification to select "even for short durations" which represents a new concept. From MPEP 2163.06: "Applicant should therefore specifically point out the support for any amendments made to the disclosure." Applicant has not directed the Examiner to the support in the specification for the amendments. Therefore, it is the Examiner's position that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of filing of the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 37 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 37 contains the relative term "short durations". It is unclear what the metes and bounds of this limitation might be. It could mean 1 second or it could mean 10 minutes. Correction is required. The claim will be examined as it reads on any duration.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 24-27 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455) and Kinsella et al. (The Lancet 1999, 354, 1061-1065) and Loh et al. (Circulation 1994, 90, 2780-2785).

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicants claims, for example:

> 24. (Currently Amended) A method of reducing the risk of one or more adverse events or serious adverse events in ~~an intended patient population comprising~~ pediatric patients ~~under the age of 18~~ in need of being treated with inhalation of nitric oxide, but who are not dependent on right-to-left shunting of blood comprising excluding from such treatment any of such patients ~~under the age of 18~~ having pre-existing left ventricular dysfunction characterized by a condition selected from the group consisting of elevated pulmonary capillary wedge pressure, diastolic dysfunction, hypertensive cardiomyopathy, systolic dysfunction, ischemic cardiomyopathy, viral cardiomyopathy, idiopathic cardiomyopathy, autoimmune disease related cardiomyopathy, drug-related cardiomyopathy, toxin-related cardiomyopathy, structural heart disease, valvular heart disease and congenital heart disease ~~whether or not such patients under the age of 18 are dependent on right-to-left shunting of blood~~.

Determination of the scope and content of the prior art

(MPEP 2141.01)

Atz et al. teach methods using inhaled nitric oxide in the **neonate**, which is a pediatric patient, with cardiac disease, hence an **identified patient** in need of nitric oxide treatment, (title and Abstract) which intrinsically provides pharmaceutically acceptable NO gas for inhalation to a medical provider to provide to the patient. Atz et al. warn that sudden pulmonary vasodilation may produce **pulmonary edema** (page 452, left column). Atz et al. teach that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." (page 452, left column). Since the patients have pulmonary hypertension as claimed in instant claim 25 then they also intrinsically have hypoxic respiratory failure in the absence of evidence to the contrary. It is irrelevant how the hypoxic respiratory failure is associated with clinical or echocardiographic evidence of pulmonary hypertension because the hypoxia is intimately tied to the pulmonary hypertension regardless of how it is evidenced. Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively ***right to left shunting*** at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse outcomes*** in this circumstance." (page 452, left column) (Examiner added emphasis). Therefore, it is known in the art that patients who had pre-existing LVD treated with NO for any duration may experience adverse outcomes. Artz et al. thus identify conditions in the patients which is screening of the patient. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.

To summarize, the methods disclosed by Atz et al. are interpreted to mean:

- identifying a patient eligible for NO treatment;
- diagnosing/identifying if the patient has left ventricular dysfunction;
- excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment.

Atz et al. teach neonates with pulmonary hypertension (Abstract and page 442, left column to right column) thus the hypertension is diagnosed in the patient population.

Kinsella et al. teach excluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and page 1062, Methods). Since left ventricular dysfunction is a congenital heart disease, as acknowledged by Applicant, (see specification [0028]), and it would be pre-existing, then the methods of Kinsella et al. intrinsically exclude this patient population from the method. The patients also had pulmonary hypertension which would be associated with the cardiac function (Abstract). Thus, one or more adverse events are reduced in the neonates excluded from the method. The neonate must breathe oxygen to survive. Furthermore, if the patients are already excluded then any further limitations on the treatment are truly irrelevant. The intended patient population is intrinsically at risk of one or more adverse events. Patients are intrinsically identified for nitric oxide inhalation treatment, diagnosed for congenital heart disease which intrinsically includes left ventricular dysfunction, and if the patient meets the criteria than treatment with NO is performed thereby reducing the risk of adverse events associated with the treatment. The neonate must breathe oxygen to survive.

Loh et al. teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract). Loh et al. clearly teaches that patients with pulmonary artery wedge pressure, which is synonymous with the instantly claimed pulmonary capillary wedge pressure, ***of greater than or equal to 18 mm Hg*** had a greater effect of inhaled NO due to the greater degree of reactive pulmonary hypertension present in such patients (page 2784, left column). Loh et al. state: "*Since the degree of reactive pulmonary hypertension is generally related to the severity of hemodynamic compromise in patients with LV failure, it might be* _anticipated that patients with more severe heart failure will have a more marked hemodynamic response to inhaled NO_." Loh et al. examined this prediction further and verified it (page 2784, left column).

Ascertainment of the difference between the prior art and the claims

(MPEP 2141.02)

1. The difference between the instant application and Atz et al. is that Atz et al. do not expressly teach identifying patients with a LVD characterized by the conditions of instant claim 24 and informing the medical provider that separately and independent from patients dependent on right to left shunt that patients with pre-existing LVD who are not dependent on right to left shunting of blood iNO may increase PWCP leading to pulmonary edema or using a pressurized cylinder of NO and recommending a dose of 20 ppm NO. This deficiency in Atz et al. is cured by the teachings of Kinsella et al. and Loh et al. and the common sense of the ordinary artisan.

Finding of prima facie obviousness

Rational and Motivation (MPEP 2142-2143)

1. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Atz et al. and identify patients with a LVD characterized by the conditions of instant claim 24 and informing the medical provider that separately and independent from patients dependent on right to left shunt that patients with pre-existing LVD who are not dependent on right to left shunting

of blood iNO may increase PWCP leading to pulmonary edema or using a pressurized cylinder of NO and recommending a dose of 20 ppm NO**,** as suggested by Loh et al., and Kinsella et al., and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because:1) if the pediatric patient is not healthy and has left ventricular dysfunction (LVD), which would intrinsically be characterized by any of the instantly claimed conditions of instant claim 24, then Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with LVD which would also render obvious all conditions/risk factors associated with LVD; 2) the art of Kinsella et al. establishes excluding patients from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease which are structural heart diseases as claimed in instant claim 24; 3) Loh et al. establish the guidepost for PCWP limits (discussed in more detail below); and 4) it is nothing more than routine optimization to determine the amount of NO to recommend for delivery to the patient, such as 20 ppm, as this would have to be done on a patient to patient basis. Thus it is no stretch of the imagination to exclude patients with LVD, with right to left shunting of the blood or with or without the myriad number of other conditions independent and separate from the first risk factor from inhaled nitric oxide therapy in order to avoid adverse outcomes as taught by Atz et al. which intrinsically include all the adverse events recited by Applicant including pulmonary edema as discussed above. It is the Examiner's position that the ordinary artisan would err on the side of caution for the benefit of the patient, especially when this can be a life or death type of decision of the most extreme consequence to the patient and exclude any patient with any form of LVD from iNO therapy to avoid the risk of adverse and serious adverse events. This is common sense especially when Loh et al. point to elevated PCWP as in instant claims 21 or 24 *which Applicant defines as a second warning* which is separate and distinct from the first warning or Kinsella et al. who teaches excluding patients from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease which are structural heart diseases and *secondary warning as defined by Applicant* which is separate and distinct from the first warning as claimed in instant claims 21 and 24.

Furthermore, it is already known through the teachings of Loh et al. that a pulmonary capillary wedge pressure (PCWP) of greater than 18 mg Hg serves as a guidepost for alerting the artisan to adverse events from inhaled NO. Thus, it is not inventive to exclude patients with a PCWP of greater than 20 mm Hg when the art already suggests the risk of trouble of treating patients with a PCWP of 18 mm Hg because inhaled NO increases the wedge pressure as taught by Loh et al. (see entire document).

In summary, it remains the position of the Examiner, which is in alignment with the written opinion of the international search authority, that it is simply not inventive to 'inform' a medical provider that a neonate with LVD is at risk of adverse/serious adverse events from iNO therapy when the art already has established that fact and the ordinary artisan is alerted to this fact. *If the patient has LVD then they are at risk of adverse and/or serious adverse events from iNO therapy and it is not inventive to further identify other secondary conditions associated with LVD and provide further warnings for secondary conditions that are separate and independent from the first condition but nevertheless associated with LVD to the medical provider. Screening for conditions that predispose* *the patient to adverse/serious adverse effects from medical treatment of iNO is obvious given the teachings above.* Respectfully, the instantly claimed method steps are in the realm of common sense and not in the realm of invention because it is already known in the art that patients with pre-existing LVD are at risk of adverse effects from iNO. It is obvious to the ordinary artisan that if the neonate has LVD with or without any number of conditions then, in order to avoid the risk of adverse or serious adverse events associated with iNO, to then exclude the neonate from iNO therapy. In other words, given *the art as a whole*, determination of further conditions associated with LVD that would exclude the neonate from iNO therapy is obvious given the teachings in the art as discussed above which direct the artisan to screen neonates about to undergo treatment with NO by inhalation and to exclude those with LVD from such treatment.

In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a).

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Response to Arguments:

The Examiner has considered Applicants arguments as they pertain to the previous rejections of record. This is a new rejection of record and arguments directed to the previous rejections of record are now moot since those rejections have been rendered moot by the cancellation of all previously pending claims and introduction of all new claims.

With respect to the art of Atz et al., Applicant asserts that it "in contrast to the prior art and the contraindication, the claimed invention relates to the an important discovery of an elevated risk for the use of inhaled NO within a newly identified and separate patient population - pediatric patients (i.e., children) with pre-existing left ventricular dysfunction, independent and separate from neonates dependent on a right-to-left shunting of the blood." Respectfully, after careful consideration of Applicant's arguments and the art as a whole, it is the Examiner's position that it is not inventive to provide yet another warning when the art already suggests that patients with pre-existing LVD be screened prior to iNO therapy because of the risk of adverse events such as edema. In other words, there is ample teaching in the art as a whole that if the patient has pre-existing LVD, which embraces all forms of LVD including second conditions claimed by Applicant, then to exclude that patient population from iNO therapy to avoid the risk of adverse conditions such as edema. This is known in the art.

Applicant asserts that it requires extraordinary skill to recognize the risk of adverse or serious adverse events associated with the use of iNO in study subjects with pre-existing LVD. Respectfully, the Examiner cannot agree with Applicant’s assertions because there is clear and convincing evidence of increased risk of adverse or serious adverse events associated with the use of iNO in study subjects with pre-existing LVD including separate and distinct conditions from right-to-left shunting of blood as discussed in the rejection above.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

1. Claims 24-27 and 37 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 21-28 of copending Application No. 12/820980. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

2. Claims 24-27 and 37 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 21-30 of copending Application No. 12/821020. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

3. Claims 24-27 and 37 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 20-27 of copending Application No. 12/820866. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy by informing the medical provider of the risks.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ERNST ARNOLD whose telephone number is (571)272-8509. The examiner can normally be reached on M-F 7:15-4:45.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Kwon can be reached on 571-272-0581. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

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BIB DATA SHEET

CONFIRMATION NO. 3219

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
12/821,041	06/22/2010 RULE	424	1613	I001-0002USC4

APPLICANTS
James S. Baldassarre, Doylestown, PA;
Ralf Rosskamp, Chester, NJ;

** **CONTINUING DATA** *************************
This application is a CON of 12/494,598 06/30/2009 ABN

** **FOREIGN APPLICATIONS** *************************

** **IF REQUIRED, FOREIGN FILING LICENSE GRANTED** ** ** SMALL ENTITY **
06/30/2010

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /ERNST V ARNOLD/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		PA	0	20	4

ADDRESS

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SPOKANE, WA 99201
UNITED STATES

TITLE

Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension

FILING FEE RECEIVED		
682	FEES: Authority has been given in Paper No.__________ to charge/credit DEPOSIT ACCOUNT No.__________ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other ________ ☐ Credit

BIB (Rev. 05/07).

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	12821041
Filing Date	2010-06-22
First Named Inventor	James S. Baldassarre
Art Unit	1616
Examiner Name	Ernst V. Arnold
Attorney Docket Number	I001-0002USC4

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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FOREIGN PATENT DOCUMENTS

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	12821041 12821041 - GAU: 1613
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	Examiner Name: Ernst V. Arnold
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	23	Notification of Reason for Rejection, mailed 7/30/2010, from Japanese Patent Application No. 2009-157623 (cites foreign references).	☐
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Application Number	12/821,041
Filing Date	June 22, 2010
First Named Inventor	Baldassarre, James S.
Art Unit	1613
Examiner Name	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC4

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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NON PATENT LITERATURE DOCUMENTS

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	Examiner Name	Ernst V. Arnold
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Attorney Docket No.: 26047-0003003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010		
Title	: Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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Under 37 CFR §3.73(b), Ikaria Holdings, Inc., a corporation, certifies that it is the assignee of the entire right, title, and interest in the patent application identified above, by virtue of an assignment from the inventors of the patent application identified above, which assignment was recorded in the Patent and Trademark Office at Reel 026606, Frame 0158 on July 18, 2011.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

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The undersigned, acting on behalf of the assignee, hereby revokes all powers of attorney previously granted in the application and appoints the attorneys and agents associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, said appointment to be to the exclusion of the inventors and their attorney(s) and/or agent(s) in accordance with the provisions of 37 CFR §3.71, et seq. of the Patent Office Rules of Practice.

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Respectfully submitted,

Date: July 21, 2011

JONATHAN N. PROVOOST
Associate General Counsel
Ikaria, Inc.

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EFS ID:	10570215
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	49584
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	I001-0002USC4
Receipt Date:	21-JUL-2011
Filing Date:	22-JUN-2010
Time Stamp:	15:29:06
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	revpower26047_003003.pdf	54407 8b35119d22854909e337a8eefb9464773e10f417	no	1

Warnings:

Information:

Total Files Size (in bytes):	54407

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/821,041	06/22/2010	James S. Baldassarre	26047-0003003

CONFIRMATION NO. 3219

POA ACCEPTANCE LETTER

94169
Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

OC000000048985934

Date Mailed: 07/29/2011

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/21/2011.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/ddinh/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Attorney Docket No.: 26047-0003005 / 3000-US-0008CON4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219

Title : METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

NOTICE OF APPEAL

Applicant hereby appeals to the Board of Patent Appeals and Interferences from the action dated June 27, 2011, finally rejecting claims 24-27 and 37.

The fee for an extension of time under 37 CFR §1.136 to extend the time to respond to the final rejection for 3 month(s) up to and including December 27, 2011, is enclosed.

The appeal fee of $310 is being paid concurrently herewith. Please apply any other necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: December 27, 2011

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22763751.doc

CERTIFICATE OF TRANSMISSION
I hereby certify under 37 CFR §1.8(a) that this correspondence is being filed via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4), on the date indicated below.
December 27, 2011
Date of Transmission
/Nancy Bechet/
Signature
Nancy Bechet
Typed or Printed Name of Person Signing Certificate

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Nancy Bechet
Attorney Docket Number:	26047-0003005

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Notice of appeal	2401	1	310	310
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	2253	1	635	635
Miscellaneous:				
		Total in USD ($)		**945**

Electronic Acknowledgement Receipt

EFS ID:	11711532
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	27-DEC-2011
Filing Date:	22-JUN-2010
Time Stamp:	16:02:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$945
RAM confirmation Number	2233
Deposit Account	061050
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Notice of Appeal Filed	noticeofappeal0003005.pdf	63698 538a21fc65be18c144daf8e262164630202a5383	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32109 c8e0c41940f64d77bf466ec5fd8c92ed48a510ab	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	95807	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	James S. Baldassarre	Nonprovisional Application Number (if known):	12/821,041
Title of Invention:	Methods Of Treating Term And Near-Term Neonates Having Hypoxic Respiratory Failure Associated With Clinical Or Echocardiographic Evidence Of Pulmonary Hypertension		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, examination fee, and any required excess claims and application size fees are filed with the request or have been already been paid.
2. The application contains or is amended to contain no more than four independent claims and no more than thirty total claims, and no multiple dependent claims.
3. The applicable box is checked below:

I. ☐ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed oath or declaration under 37 CFR 1.63 is filed with the application.

II. ☒ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form,
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Janis K. Fraser/	Date January 6, 2012
Name (Print/Typed) Janis K. Fraser, Ph.D., J.D.	Practitioner Registration Number 34,819

***Note**: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.*

☒ *Total of 1 forms are submitted.

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PTO/SB/30 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	12/821,041
Filing Date	June 22, 2010
First Named Inventor	James S. Baldassarre
Art Unit	1613
Examiner Name	Ernst V. Arnold
Attorney Docket Number	26047-0003005

This is a Request for Continued Examination (RCE) under 37 CFR 1 .114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on ______

ii. ☐ Other ______

b. ☒ Enclosed

i. ☒ Amendment/Reply with Exhibits A-G

ii. ☐ Affidavit(s)/ Declaration(s)

iii. ☐ Information Disclosure Statement (IDS)

iv. ☒ Other Statement under 37 CFR 1.57(f) and Exhibit 1; Request for Prioritized Examination

2. Miscellaneous

Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a

a. ☐ period of ______ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

b. ☐ Other ______

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
The Director is hereby authorized to charge the following fees any underpayment of fees or credit any overpayments to

a. ☒ Deposit Account No. 06-1050 .

i. ☒ RCE fee required under 37 CFR 1.17(e)

ii ☐ Extension of time fee (37 CFR 1.136 and 1.17)

iii ☒ Other Fees for Request for Prioritized Examination, including processing and publication fees; any deficiencies

b. ☐ Check in the amount of $ ______ enclosed

c. ☐ Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Janis K. Fraser/	Date	January 6, 2012
Name (Print/Type)	Janis K. Fraser, Ph.D., J.D.	Registration No.	34,819

22764021.doc

Attorney Docket No.: 26047-0003005 / 3000-US-0008CON4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	James S. Baldassarre et al.	Art Unit	:	1613
Serial No.	:	12/821,041	Examiner	:	Ernst V. Arnold
Filed	:	June 22, 2010	Conf. No.	:	3219

Title : METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN REPLY TO ACTION OF JUNE 27, 2011

A Notice of Appeal was filed on December 27, 2011. A Request for Continued Examination and a Statement under 37 CFR 1.57(f) are being filed with this Reply.

Please amend the above-identified application as follows:

Amendments to the Specification:

Replace the title on page 1 with the following new title:

Methods of reducing the risk of occurrence of pulmonary edema in term or near-term neonates in need of treatment with inhaled nitric oxide

Replace paragraph **[0020]** with the following amended paragraph:

[0020] INOmax® (nitric oxide) for inhalation was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999. Nitric oxide, the active substance in INOmax®, is a selective pulmonary vasodilator that increases the partial pressure of arterial oxygen (PaO2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from the lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios. INOmax® significantly improves oxygenation, reduces the need for extracorporeal oxygenation and is indicated to be used in conjunction with ventilatory support and other appropriate agents. The current FDA-approved prescribing information for INOmax® is incorporated herein by reference in its entirety. The CONTRAINDICATIONS section of the prescribing information for INOmax® states that INOmax® should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood.

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-37. (Canceled)

38. (New) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, the method comprising:

(a) identifying a term or near-term neonate patient in need of inhaled nitric oxide treatment, wherein the patient is not known to be dependent on right-to-left shunting of blood;

(b) determining that the patient identified in (a) has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

39. (New) The method of claim 38, wherein the patient has pulmonary hypertension.

40. (New) The method of claim 38, wherein the patient has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg.

41. (New) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, said method comprising:

(a) identifying a term or near-term neonate patient in need of inhaled nitric oxide treatment, wherein the patient is not known to be dependent on right-to-left shunting of blood;

(b) determining by diagnostic screening that the patient identified in (a) has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from treatment with inhaled nitric oxide based on the determination that the patient has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

42. (New) The method of claim 41, wherein the diagnostic screening comprises echocardiography.

43. (New) The method of claim 41, wherein the patient has pulmonary hypertension.

44. (New) The method of claim 41, wherein the patient has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg.

45. (New) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, the method comprising:

(a) identifying a plurality of term or near-term neonate patients who are in need of inhaled nitric oxide treatment, wherein the patients are not known to be dependent on right-to-left shunting of blood;

(b) determining that a first patient of the plurality has pre-existing left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) determining that a second patient of the plurality does not have pre-existing left ventricular dysfunction;

(d) administering the inhaled nitric oxide treatment to the second patient; and

(e) excluding the first patient from treatment with inhaled nitric oxide, based on the determination that the first patient has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

46. (New) The method of claim 45, wherein the first and second patients have pulmonary hypertension.

47. (New) The method of claim 45, wherein the second patient has congenital heart disease.

48. (New) The method of claim 45, wherein the first patient has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg.

49. (New) The method of claim 45, wherein determining that the first patient of the plurality has pre-existing left ventricular dysfunction and the second patient of the plurality does not have pre-existing left ventricular dysfunction comprises diagnostic screening.

50. (New) A method of reducing the risk of occurrence of pulmonary edema associated with medical treatment comprising inhalation of nitric oxide gas, the method comprising:

(a) providing to a medical provider a source of pharmaceutically acceptable nitric oxide gas suitable for inhalation by a term or near-term neonate patient in need of treatment with inhaled nitric oxide; and

(b) informing the medical provider that if said patient has pre-existing left ventricular dysfunction, treatment of said patient with inhaled nitric oxide may cause pulmonary edema.

51. (New) The method of claim 50, wherein step (b) further comprises informing the medical provider that if said patient has pre-existing left ventricular dysfunction, treatment with inhaled nitric oxide may increase pulmonary wedge pressure.

52. (New) The method of claim 50, wherein the source of nitric oxide gas is a cylinder containing a compressed gaseous blend of nitric oxide and nitrogen.

REMARKS

The above claim amendment cancels all of the pending claims and adds new claims 38-52. The new claims are supported throughout the application and claims as originally filed. For example, the concept of a "plurality" of patients in new claim 45 is supported by the discussion of "patients" (plural) at paragraph [0004] and "a patient population" at paragraph [0007]. Paragraph [0007] also supports the recitation in claim 45 of a first patient who is determined to have left ventricular dysfunction and so is excluded from treatment with inhaled nitric oxide. The claim 45 recitation of a second patient who is determined not to have left ventricular dysfunction and is administered inhaled nitric oxide is supported, e.g., at paragraph [0008]. (Of course, the terms "first" and "second" in claim 45 are merely standard linguistic devices useful to distinguish between two patients, and do not imply any particular temporal order.) The remaining limitations of claim 45, and of the other new claims, are supported throughout the specification and original claims, e.g. at paragraphs [0004]- [0008], [0012], [0018], [0020] (as amended herein), [0021], [0028], [0033], [0051], and [0052]. No new matter has been added.

Applicants have replaced the title of the application with a new title that more accurately reflects the presently claimed invention. In addition, paragraph [0020] has been amended to insert language that was previously incorporated by reference. This amendment and the accompanying Statement under 37 CFR 1.57(f) are discussed below.

I. Election/Restriction

The Final Office Action dated June 27, 2011 (the "Final Action") asserts at page 2 that claims 21-23, 28, and 29 are directed to an invention that is independent or distinct from the invention originally claimed, as these claims are directed to methods of distributing a pharmaceutical product (claims 21-23) or to a pharmaceutical product (claims 28 and 29), rather than to a method of reducing the risk of one or more adverse events or serious adverse events in a pediatric patient. The Final Action thus withdrew claims 21-23, 28, and 29 from prosecution.

The present amendment cancels all of the pending claims, including claims 21-23, 28, and 29, rendering the issue moot. All of the newly presented claims are directed to methods of reducing risk of a particular adverse event (pulmonary edema) in a category of pediatric patients (term or near-term neonates), so are within the group the Examiner says was originally constructively elected in this application.

II. Objection to Specification

The Office action at pages 3-4 objects to the specification, alleging that the attempt to incorporate subject matter into the specification by reference is defective. In the Supplemental Reply Amendment filed on April 13, 2011, Applicants had requested that paragraph [0020] of the specification be amended to include certain language quoted from the US Food & Drug Administration (FDA)-approved prescribing information for INOmax®. That Applicants are entitled to do so is clear, because the specification as originally filed included the statement, "**The current FDA-approved prescribing information for INOmax® is incorporated herein by reference in its entirety**." See paragraph [0020] in the original specification. Applicants understand the current objection to the specification to be based on the requirement under 37 CFR 1.57(f) that the amendment inserting material in the specification be accompanied by a statement that the material being inserted is the material incorporated by reference and the amendment contains no new matter.

As the amendment to the specification filed April 13, 2011, was deemed defective, Applicants assume it was not entered. Thus, a new amendment to paragraph [0020] (with slightly different wording to more closely track the language of the INOmax® prescribing information that was current at the June 30, 2009, priority date) is provided above. The amendment adds the following language to paragraph [0020]: "The CONTRAINDICATIONS section of the prescribing information for INOmax® states that INOmax® should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood." The requisite Statement under 37 CFR 1.57(f) is being filed concurrently herewith, along with a copy of the FDA-approved prescribing information for INOmax® that was current as of the June 30, 2009, priority date. The language incorporated by reference is quoted from the second page of the prescribing information, left column, second paragraph (CONTRAINDICATIONS). Each of new independent claims 38, 41, and 45 includes a limitation based on the material incorporated by reference: **"wherein the patient(s) is/are not known to be dependent on right-to-left shunting of blood.**"

If something more is needed to effect this amendment, the Examiner is respectfully asked to clarify.

III. Rejections under 35 USC § 112, first paragraph

Claim 26 was rejected under 35 USC § 112, first paragraph, as allegedly failing to comply with the written description requirement because it included the claim language "a pressurized cylinder containing nitric oxide and one or more inert gases." See Final Action at pages 5-6. Without acquiescing in the rejection, Applicants have canceled this claim, rendering the rejection moot. Applicants understand the Examiner's concern to be based in particular on the phrase "and one or more inert gases," a phrase that does not appear in any of the new claims now pending. New claim 52 says, "the source of nitric oxide gas is a cylinder containing a compressed gaseous blend of nitric oxide and nitrogen," language that has unequivocal written description support in the specification at paragraph [0021].

Claim 37[1] was rejected under 35 USC § 112, first paragraph, as allegedly failing to comply with the written description requirement because it included the claim language "even for short durations." See Final Action at page 6-7. Without acquiescing in the rejection, Applicants have canceled claim 37, rendering the rejection moot. The objected-to language does not appear in any of the new claims now pending.

IV. Rejections under 35 USC § 112, second paragraph

Claim 37 was rejected under 35 USC § 112, second paragraph, as allegedly being indefinite because it includes the term "short durations." Without acquiescing in the rejection, Applicants have canceled claim 37, rendering the rejection moot. The objected-to language does not appear in any of the new claims now pending.

[1] Claim 37 is the claim referenced at page 6, last full sentence. Applicants assume the reference to "claim 26" on page 7, line 2, of the Final Action was inadvertent, and that "claim 37" was intended.

V. Rejections under 35 USC § 103(a)

Claims 24-27 and 37 were rejected as allegedly obvious over Atz & Wessel (Seminars in Perinatology 1997, 21(5), p441-455), Kinsella et al. (The Lancet 1999, 354, 1061-1065), and Loh et al. (Circulation 1994, 90, 2780-2785). All of those claims have been canceled, rendering the rejection moot as to them. To the extent the rejection may be applied to the new claims now pending in the case, Applicants respectfully traverse.

New independent claim 38 reads:

> 38. (New) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, said method comprising:
>
> (a) identifying a term or near-term neonate patient in need of inhaled nitric oxide treatment, wherein the patient is not known to be dependent on right-to-left shunting of blood;
>
> (b) determining that the patient identified in (a) has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and
>
> (c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

Claim 38 and all of the other claims now pending are limited to methods involving a term or near-term neonate patient, defined in paragraph [0033] as being ">34 weeks gestation." In claim 38, the patient is further defined by the following three criteria: (1) "in need of inhaled nitric oxide treatment;" (2) "not known to be dependent on right-to-left shunting of blood;" and (3) having "pre-existing left ventricular dysfunction and so [being] at particular risk of pulmonary edema upon treatment with inhaled nitric oxide." For purposes of the discussion below, term or near-term neonate patients who meet all of those criteria are referred to as the "Claimed Patient Population."

The rejection for obviousness as stated in the Final Action begins with a discussion of Atz & Wessel. According to the Final Action at pages 9-10,

> Atz et al. warn that sudden pulmonary vasodilation may produce **pulmonary edema** (page 452, left column). Atz et al. teach that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction[2] and pulmonary hypertension." (page 452, left column)…. Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively ***right to left shunting*** at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse outcomes*** in this circumstance." (page 452, left column) (Examiner added emphasis). Therefore, it is known in the art that patients who had pre-existing LVD treated with NO for any duration may experience adverse outcomes…. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.
>
> To summarize, the methods disclosed by Atz et al. are interpreted to mean:
>
> - identifying a patient eligible for NO treatment;
> - diagnosing/identifying if the patient has left ventricular dysfunction;
> - excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment.

(Emphasis in the original.)

Applicants submit that the Examiner's interpretation of the Atz & Wessel reference is far broader than what it really says. This is clear from a careful parsing of the crucial paragraph on page 452 of the reference, and is supported by factual evidence submitted herewith.

The cited Atz & Wessel paragraph begins with the general introductory statement "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." The rest of the paragraph elaborates on this brief introductory statement, explaining that the patients to whom this cautionary warning applies are not <u>all</u> patients with LVD, but rather only those that fall into either of two limited categories:

[2] Left ventricular dysfunction is frequently abbreviated as "LVD" in this Reply.

(a) **adults with ischemic cardiomyopathy** (hereafter the "Adult Population"), who may experience pulmonary edema when NO relieves their pulmonary hypertension; and

(b) **newborns who exhibit a combination of all of the following three conditions:** ***severe LVD, pulmonary hypertension, predominantly left to right shunting at the foramen ovale,*** **and** ***exclusively right to left shunting at the ductus arteriosus*** (hereafter the "Shunt-Reliant Population"), who may experience a catastrophic decrease in systemic circulation when NO relieves their pulmonary hypertension.

In order to help elucidate what Atz & Wessel was communicating to those of skill in the art in the page 452 paragraph, and to show that the authors were focused solely on LVD patients who fall into the two very limited patient populations described above in (a) and (b) (i.e., not all LVD patients), Applicants provide below a detailed discussion of the two patient populations to which Atz & Wessel refers and the very different population referenced in the present claims.

The Adult Population. With respect to this population, Atz & Wessel state the following:

> In adults with ischemic cardiomyopathy, sudden pulmonary vasodilation may occasionally unload the right ventricle sufficiently to increase pulmonary blood flow and harmfully augment preload in a compromised left ventricle. The attendant increase in left atrial pressure may produce pulmonary edema.[3]

As the Examiner will recognize, this part of the disclosure is explicitly limited to adult patients and does not address the potential impact of inhaled nitric oxide on neonates. As explained in the enclosed Declaration of Douglas A. Greene, M.D. under 37 C.F.R. § 1.132, dated April 29, 2011 (originally filed in a sister application, US. Serial No. 12/820,866, and attached hereto as Exhibit A; "the First Greene Declaration"), the hearts of the Adult Population are clinically distinct from the hearts of the Claimed Patient Population due to the fact that the etiology and pathophysiology of the left ventricular dysfunction present in the Claimed Patient Population is markedly different from what is present in the Adult Population. Left ventricular dysfunction

[3] Atz & Wessel at 452.

comes in two broad types: systolic and diastolic. As detailed in paragraphs 15-16 of the First Greene Declaration, left-sided ventricular dysfunction in the Claimed Patient Population is generally associated with a soft, overly elastic heart that cannot push blood out, resulting in impaired emptying, i.e., *systolic dysfunction*. Conversely, in the Adult Population, left-sided ventricular dysfunction is generally ischemic or hypertensive in origin, and is associated with a stiff, non-compliant left ventricle that cannot fill properly, i.e., *diastolic dysfunction*. In addition, the Adult Population is inherently different than the Claimed Patient Population in that the Adult Population has, as stated by Atz & Wessel, ischemic cardiomyopathy (the result of a heart attack due to a blocked blood vessel), and does not suffer from congenital heart disease.[4] Given these dramatic anatomical differences between adult LVD and neonatal LVD, those skilled in the art do not consider LVD in adults with ischemic cardiomyopathy as analogous to, or predictive of risks associated with, LVD in the Claimed Patient Population.[5]

Because of this important clinical and etiological distinction, one would have had no reason to expect an elevated risk of pulmonary edema or cardiac complications (or any other adverse event) when using inhaled nitric oxide in the Claimed Patient Population. For this reason, the Examiner's inference that Atz & Wessel's disclosure concerning the Adult Population would be read as a general warning concerning all patients with LVD is simply incorrect, and unsupported by the facts.

The Shunt-Reliant Population. The second patient population addressed by Atz & Wessel on page 452 is neonates suffering from LVD that are reliant on right-to-left shunting of blood at the patent ductus arteriosus, i.e., the Shunt-Reliant Population. See the discussion of these neonates at ¶ 9 of the Declaration of Douglas A. Greene, M.D. under 37 C.F.R. § 1.132, dated July 7, 2011, that is attached hereto as Exhibit B (the "Second Greene Declaration"). The Shunt-Reliant Population is entirely distinct from the Claimed Patient Population, and the physiological reasons why inhaled nitric oxide is not recommended for use the Shunt Reliant Population are wholly different from the physiological reasons underlying the claimed invention.

With respect to the Shunt-Reliant Population, Atz & Wessel disclose:

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[4] Congenital heart disease is a problem with the heart's structure and function due to abnormal heart development before birth. "Congenital" means present at birth. National Heart Lung and Blood Institute, http://www.nhlbi.nih.gov/health/health-topics/topics/chd/.

[5] First Greene Declaration, ¶¶ 15-16.

> A different but related phenomenon may be operative in the newborn with severe left ventricular dysfunction and pulmonary hypertension. In these patients, the systemic circulation may depend in part on the ability of the right ventricle to sustain cardiac output through a right-to-left shunt across the patent ductus arteriosus. Selective pulmonary vasodilation may redirect the right ventricular output to the lungs and away from the systemic circulation. **Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively right to left shunting at the ductus arteriosus, NO should be used with extreme caution, if at all.** We and others have reported adverse outcomes in this circumstance.[6]

Importantly, Atz & Wessel's "caution" regarding use of inhaled nitric oxide in neonates is explicitly limited to neonates who present with a combination of three conditions, i.e.:

severe left ventricular dysfunction

AND

predominantly left to right shunting at the foramen ovale

AND

exclusively right-to-left shunting at the ductus arteriosus.[7]

These are three different conditions that can occur separately or together in a neonate. (Azt & Wessel describes only the situation when all occur together.) Left ventricular dysfunction by definition involves the left ventricle in particular. In contrast, the two "shunting" conditions mentioned by Atz & Wessel involve structures of the heart separate from the left ventricle, and cannot in any way be characterized as forms of left ventricular dysfunction. The foramen ovale is a hole between the left atrium and right atrium of the heart that, when it remains abnormally open after birth, permits blood to flow ("shunt") between the two atria.[8] The ductus arteriosus is a passageway between the pulmonary artery (on the right side of the heart) and the systemic circulation (on the left side of the heart). When the ductus arteriosus remains abnormally open after birth, it permits blood to flow ("shunt") from the right side of the heart directly into the systemic circulation.[9] The particular foramen ovale condition mentioned in Atz & Wessel

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[6] Atz & Wessel at 452 (emphasis added).
[7] *Id.*
[8] First Greene Declaration, ¶¶ 11-12.
[9] *Id.*, ¶¶ 10-13.

involves a flow of blood through the foramen ovale predominantly from the left atrium to the right atrium (i.e., "predominantly left to right"). The ductus arteriosus condition mentioned in the reference involves shunting that is exclusively from (a) the pulmonary artery exiting the right heart to (b) the systemic circulation on the left side of the heart (i.e., "exclusively right to left"). The two shunting conditions (predominantly left to right shunting at the foramen ovale and exclusively right-to-left shunting at the ductus arteriosus) can occur separately, but can also co-exist, and when they do, they may work in tandem to create a flow of blood from the left atrium to the right atrium (via the foramen ovale) and from there into the systemic circulation (via first the right ventricle, then the pulmonary artery, and then through the ductus arteriosus to the systemic circulation). When a newborn has both shunting conditions as well as LVD so severe that his/her left ventricle cannot do its normal work of pumping sufficient blood into the systemic circulation to sustain life (i.e., the combination of the three conditions described in the above-quoted sentence from Atz & Wessel), the combination of the three conditions provides an alternate route that bypasses the severely dysfunctional left ventricle and provides life-sustaining blood flow to the systemic circulation. Thus, the newborn with these three conditions is relying for survival on the flow of blood pumped by the right ventricle into the pulmonary artery and shunted right to left through the ductus arteriosus into the systemic circulation.[10] In other words, the newborn is dependent on right-to-left shunting of blood. Atz & Wessel teach that such a newborn should not be given inhaled NO because this may abrogate the right-to-left shunt on which the newborn depends for survival:

> In these patients, the systemic circulation may depend in part on the ability of the right ventricle to sustain cardiac output through a right-to-left shunt across the patent ductus arteriosus. Selective pulmonary vasodilation may redirect the right ventricular output to the lungs and away from the systemic circulation. Therefore [in these patients], NO should be used with extreme caution, if at all. We and others have reported adverse outcomes in this circumstance.[11]

(See also the explanation in the First Greene Declaration at ¶ 14.) A neonate's dependency on right-to-left shunting of blood for survival has long been known to be a contraindication for inhaled NO use and has been cited as such on the prescribing information for INOmax® nitric

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[10] *Id.*, ¶¶ 13-14.
[11] Atz & Wessel, page 452.

oxide for inhalation ever since this product was first approved for marketing in 1999.[12] (See page 2, left column, "CONTRAINDICATIONS" in the 2007 version of the prescribing information attached to the Statement under 37 CFR § 1.57(f) filed concurrently herewith.) Such neonates constitute the "Shunt-Reliant Population." Thus, Atz & Wessel were simply pointing out what was already widely known in the art about the danger of giving inhaled NO to neonates who have a combination of the three conditions (severe left ventricular dysfunction and predominantly left to right shunting at the foramen ovale and exclusively right-to-left shunting at the ductus arteriosus) and therefore are dependent on right-to-left shunting of blood for survival. Note that the Atz & Wessel reference includes no discussion whatsoever concerning neonates with LVD who do *not* have right-to-left shunting at the ductus arteriosus, so are *not* dependent on right-to-left shunting of blood (i.e., the Claimed Patient Population). Atz & Wessel discloses only a different set of neonates who are at risk from treatment with inhaled NO: those who have severe LVD as well as the two shunting conditions, with the combination of all three conditions leaving them dependent on the right-to-left shunt to maintain their systemic circulation. Their reliance on the right-to-left shunt is absolutely key to the risk that inhaled NO poses for them. There is no suggestion that any other group of neonates might be at risk of systemic circulatory collapse when given inhaled NO, and certainly no reason to suspect that these or any other neonates might ever be at risk of some other serious adverse event, such as pulmonary edema, upon inhalation of NO. **Indeed, if Atz & Wessel had intended to state that those at risk included not only the two populations previously identified as at risk (i.e., the Adult Population and the Shunt-Reliant Population), but also a population that was never previously known to be at risk (i.e., the Claimed Patient Population), it stands to reason that they would have made the case very explicitly, as that would be new information of intense interest to their readers.**

Furthermore, one could not have predicted from the disclosure of Atz & Wessel that neonates who have LVD but are not dependent on a right-to-left shunting of blood (i.e., the Claimed Patient Population) are at risk.

[12] First Greene Declaration, ¶ 14.

The risk in the Shunt-Reliant Population discussed by Atz & Wessel is directly related to this population's dependence on the right-to-left shunt, so is not predictive of any risk in the Claimed Patient Population, which *a priori* has no such dependence on a right-to-left shunt. Furthermore, the risk in the Shunt-Reliant Population (collapse of the systemic circulation) has nothing to do with pulmonary edema, as specified in the present claims, and is certainly not predictive of same.

For entirely different reasons, the risk in the Adult Population discussed by Atz & Wessel is also not predictive of any risk in the Claimed Patient Population. The risk in the Adult Population occurs when inhaled NO suddenly relieves pulmonary vasoconstriction, thereby increasing blood flow through the lungs and then to the left side of the heart in a volume too large for the dysfunctional and stiff left ventricle to contain. As Atz & Wessel put it, this may "harmfully augment preload" in the stiff left ventricle, resulting in increased left atrial pressure, engorgement of the pulmonary blood vessels, and pulmonary edema.[13] Because a patient within the Claimed Patient Population does not suffer from a stiff left ventricle, but rather from the opposite (an overly-elastic left ventricle), those of ordinary skill in the art had no reason to expect that increasing pulmonary blood flow due to inhalation of NO would produce a similar problem of "harmfully augmenting preload" (and the resulting pulmonary edema) in the Claimed Patient Population.

Appellant's reading of the Atz & Wessel reference is supported by the enclosed Declaration of David L. Wessel, M.D. under 37 CFR § 1.132 (the "Wessel Declaration"; originally filed in a sister application, US. Serial No. 12/820,866, and attached hereto as Exhibit C). In particular, Dr. Wessel states that he and his co-author (Atz) "did not disclose or predict…that neonatal patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at greater risk of adverse events."[14] Dr. Wessel also declares that "it was unanticipated and surprising that children with left ventricular dysfunction who are not dependent on right-to-left shunting [i.e., the Claimed Patient Population] would be at increased risk of adverse events when administered iNO."[15]

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[13] Atz & Wessel, page 452; see also First Greene Declaration, ¶¶ 15-16.
[14] Wessel Declaration, ¶¶ 7-8 (emphasis in original).
[15] *Id.* ¶ 9.

In particular, Dr. Wessel states:

> Neither the Atz et al. article that I co-authored, nor the medical literature or medical experience of which I was aware at the time, predict this risk. Instead, Atz et al. describes two distinct, independent precautions with respect to the use of iNO. First, with respect to adults, Atz et al., stated that iNO may be more effective in newborns than in older patients, and noted that it should be used with caution in adults with ischemic cardiomyopathy in whom a risk of pulmonary edema is a consideration (see page 452, left column). Second, with respect to neonates, we stated the well-known contraindication (currently found in the INOMAX® prescribing information) that iNO should not be used in newborns dependent upon right-to-left shunting of blood across a patent ductus arteriosus to avoid circulatory collapse. What we did **not** disclose or predict was that neonatal patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at greater risk of adverse events.
>
> It is ironic that my own publication would be cited to suggest that it would have been obvious to predict the adverse events and outcomes of the INOT22 Study when I, the senior author of Atz et al., failed to anticipate or predict these unexpected outcomes at the time I participated in drafting the original INOT22 Study protocol. If so, I would have been acting either negligently or intentionally to harm babies, and I most certainly was not.[16]

Thus, Dr. Wessel himself explained that the caution in his Atz & Wessel paper did not apply to all patients with LVD, but rather was limited to adults with LVD and neonates with LVD who are dependent on right-to-left shunting of blood across a patent ductus arteriosus, the same neonate population explicitly contraindicated in the INOmax® prescribing information current at the time. This evidence flatly contradicts the Examiner's unduly broad interpretation of what Atz & Wessel discloses, undermining the factual basis for the entire *prima facie* obviousness rejection. Contrary to the Final Action's above-quoted summary of the Examiner's interpretation of Atz & Wessel, this reference does not teach, even by implication, that any and all patients diagnosed as having LVD should be excluded from treatment with inhaled NO. In fact, it is clear that the warning in Atz & Wessel regarding excluding certain newborns is based solely upon their dependence on a right-to-left shunt in conjunction with LVD (since that warning focuses on the danger of abrogating the right-to-left shunt by redirecting blood to the lungs and away from the systemic circulation in these newborns), and not because of the LVD

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[16] *Id.* ¶ 7-8 (emphasis in original).

itself. Claims 38-49 make it clear that the subject patient is not either of the two types of patients (adult or shunt-reliant neonate) to which Atz & Wessel refers, by specifying that the patient is a neonate who "is not known to be dependent on right-to-left shunting of blood." Claims 50-52 likewise specify that the subject patient is a neonate (so not the adult described by Atz & Wessel); these claims further specify a step of informing the medical provider that, if the neonate has pre-existing LVD, treatment with inhaled NO may cause pulmonary edema (rather than harmfully redirecting blood flow away from the systemic circulation, as taught by Atz & Wessel for neonates dependent on a right-to-left shunt across the patent ductus arteriosus).

As noted by the Court of Appeals of the Federal Circuit: "It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art."[17] Yet the Examiner has done exactly what the Court warns is impermissible: i.e., the Examiner has "picked and chosen" only the LVD aspect of Atz & Wessel's disclosure, excluding the rest of that reference's disclosure from his summary of this reference on page 10 of the Final Action and thereby entirely distorting what the reference says in a way that supports the rejection, but is plainly inaccurate. If, as the Examiner implies, the authors of Atz & Wessel had been aware that all patients with LVD (including neonates who are not dependent on a right-to-left shunting of blood through a patent ductus arteriosus) were at increased risk for adverse events when treated with inhaled nitric oxide, or even had suspected that would be the case, one would expect they would have mentioned it in their publication, rather than focusing solely on the Adult Population and the Shunt-Reliant Population. The Examiner offers no rationale as to why Atz & Wessel failed to note this supposedly "obvious" risk to the Claimed Patient Population.

The Final Action cites Kinsella et al. as allegedly teaching:

> [E]xcluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease....Since left ventricular

[17] *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 796 F.2d 443, 448 (Fed. Cir. 1986) (internal citations omitted).

> dysfunction is a congenital heart disease…and it would be pre-existing, then the methods of Kinsella et al. intrinsically exclude this patient population from the method.[18]

It is not clear what Kinsella et al. adds to the present obviousness rejection. The Examiner acknowledges that Kinsella et al.'s study concerned premature neonates. Premature neonates are not encompassed by the claims currently pending, all of which specify "term or near-term neonate." With respect to present claims 39, 43, and 46, Appellants further note that the subjects studied by Kinsella et al. had severe respiratory failure due to immature lungs and surfactant deficiency, and did not suffer from pulmonary hypertension.[19] Since they did not have pulmonary hypertension, treatment of Kinsella et al.'s subjects with inhaled NO would not be expected to increase blood flow to their lungs and from there to the left side of the heart. There is therefore no basis whatsoever to suppose that inhaled NO would put Kinsella et al.'s subjects at risk of increased pressure on the left side of the heart, leading to pulmonary edema. The claimed methods, all of which specify term or near-term neonates and pulmonary edema, simply do not apply to Kinsella et al.'s subjects.

Furthermore, Kinsella et al.'s motivation for excluding certain premature neonates from that study is entirely irrelevant to the presently claimed methods. The reason Kinsella et al. designed their study protocol to exclude patients who have fatal congenital anomalies or congenital heart disease was likely related to a desire to minimize confounding variables (such as deaths from underlying conditions unrelated to the condition being studied) that would complicate the study results, and not from a desire to protect LVD patients from undue risk from the inhaled NO treatment. The Second Greene Declaration explains this point as follows:

> For example, clinical trial inclusion and exclusion criteria are often chosen to define or restrict the study population in order to maximize homogeneity, thereby minimizing the presence of potentially confounding factors. This exclusion greatly facilitates the interpretation of the study results, and increases the soundness of the conclusions reached in the study. Accordingly, patients with background disease sufficiently severe to overwhelm or confound an expected treatment effect are systematically identified and excluded quite independently from considerations of anticipated safety or efficacy of the test article in this particular patient group. For example, patients with malignancy are often excluded from non-oncologic clinical trials, not because the test agents are unsafe, pose any specific risk in this population, or will not work, but rather because the clinical

[18] Final Action, page 11.
[19] Second Greene Declaration, ¶ 17.

> results will be confounded by the wholly unrelated effects of the underlying malignancy, thereby reducing the power of the clinical trial to answer a specific hypothesis regarding the test treatment. As a specific example, exclusion of patients with malignancy or advanced heart failure from cholesterol lowering trials does not imply that statins are unsafe or ineffective in these patients, but rather that their inclusion would confound the potential effects of statins on overall mortality or cardiovascular events. In the specific case of Kinsella et al., it is clear that one of ordinary skill in the art would understand that the patients having fatal congenital anomal[]ies or congenital heart disease were excluded not because of a suspected safety risk of treating these patients with inhaled NO (e.g., a risk of pulmonary edema), but rather solely because the inclusion of such patients would have made it much more difficult – if not impossible - for Kinsella et al. to interpret the target outcomes of the study (i.e., would have "confounded" the results).[20]

That Dr. Greene's above-described view of Kinsella et al. is the view that would be shared by those of ordinary skill in the art upon reading that reference is clear from the post-filing publication Fraisse & Wessel, "Acute pulmonary hypertension in infants and children: cGMP-related drugs," *Pediatric Critical Care Med.* 2010, Vol. 11, No. 2 (Suppl.), pages S37-S40; attached as Exhibit D. The abstract of this article states:

> Inhaled nitric oxide is extremely efficacious in increasing cGMP and selectively reducing mean pulmonary arterial pressure in pediatric cardiac patients. It is considered standard treatment in most centers.

This view of the value of inhaled nitric oxide for treating pediatric patients with congenital heart disease is confirmed in the body of the article, where the authors again state:

> [I]nhaled NO is extremely efficacious in selectively reducing mean pulmonary arterial pressure (PAP) in cardiac patients and is considered standard treatment in most centers.[21]

These statements extolling the benefits of inhaled nitric oxide as being "extremely efficacious" and a "standard treatment" in pediatric cardiac patients, most of whom have congenital heart disease, were made by the authors in 2010, *eleven years* after Kinsella et al. was published. If those of skill in the art had read Kinsella et al.'s exclusion criteria to mean that infants with congenital heart disease in general should be excluded from treatment with inhaled nitric oxide for safety reasons, this treatment would certainly not have achieved its present status

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[20] *Id.*, ¶¶ 18-20.
[21] Fraisse & Wessel at S37.

of a "standard treatment" for pediatric cardiac patients. The quoted statements from Fraisse & Wessel are cogent, objective evidence that, if the Final Action was interpreting Kinsella et al. to constitute a general teaching that congenital heart disease patients in need of treatment with inhaled NO should be excluded from the treatment for safety reasons, such an interpretation is unwarranted. If this is not the Examiner's point in citing Kinsella et al., then it is unclear what the point is. Properly interpreted, Kinsella et al. does not support the present rejection at all.

The Final Action at pages 11-12 describes Loh et al. as follows:

> Loh et al. teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure ... Loh et al. clearly teaches that patients with pulmonary artery wedge pressure...***of greater than or equal to 18 mm Hg*** had a greater effect of inhaled NO due to the greater degree of reactive pulmonary hypertension present in such patients.... Loh et al. state: "*Since the degree of reactive pulmonary hypertension is generally related to the severity of hemodynamic compromise in patients with LV failure, it might be anticipated that patients with more severe heart failure will have a more marked hemodynamic response to inhaled NO*." Loh et al. examined this prediction further and verified it. (Emphasis in original)

From the first sentence quoted above from the Final Action regarding Loh et al., it appears that the Examiner may be reading Loh et al. as broadly teaching that that inhaled nitric oxide may have adverse effects in all patients with LVD. If so, Applicants point out that this is simply not an appropriate reading of the reference. Loh et al. is solely about adult patients who have a form of LVD associated with congestive heart failure characterized as New York Heart Association functional class III or IV heart failure due to coronary artery disease or dilated cardiomyopathy (see Loh et al., "Study Population" on page 2780, right column, and the Second Greene Declaration at ¶ 21). Loh et al. says nothing about neonates, who typically have a very different type of LVD arising from very different etiology. The marked distinctions between the LVD of the adults studied by Loh et al. and the LVD of the Claimed Patient Population have been explained above and are further elaborated in the First Greene Declaration at ¶¶ 15-16 and in the Second Greene Declaration at ¶ 22. Briefly, the adults studied by Loh et al. had a form of LVD in which their left ventricles are stiff and unable to fill properly with blood (diastolic dysfunction), while the form of LVD found in neonates is typically a congenital form that leaves their left ventricles overly elastic and unable to contract properly to expel blood (systolic dysfunction). As explained above, these vast differences would lead of ordinary skill in the art to realize that the problems caused by inhaled NO observed by Loh et al. in adults with LVD would not likely translate to similar problems in neonates with LVD. Further, the underlying causes of pulmonary vasoconstriction (i.e., the condition treated with inhaled NO) in the Adult Population studied by Loh et al. and the Claimed Patient Population are entirely different (see the discussion of this point in the Second Greene Declaration at ¶ 22), giving further reason to assume that the hemodynamic response to inhaled NO in one of these populations is not predictive of the hemodynamic response to inhaled NO in the other. As explained by Dr. Greene, one cannot reasonably predict from Loh et al.'s observations in adults that there would have been any particular risk in the Claimed Patient Population. Thus, those skilled in the art would not consider Loh et al.'s teachings to broadly cover all patients with all kinds of LVD, and certainly would not consider those teachings to cover the Claimed Patient Population, with its entirely different pathology.

Applicants submit that the combination of Atz & Wessel, Kinsella et al., and Loh et al. does not render the present claims obvious. Once these references are read properly (as any person of ordinary skill in the art would have done), nothing in any of the references taken alone or in combination suggests a reason to carry out the method of any of the present claims. In addition, nothing in the cited references provides a reasonable expectation that the claimed methods will be successful in reducing the risk of pulmonary edema, as required by the present claims, for at least the reason that the prior art had no idea that inhaled NO might cause pulmonary edema in the Claimed Patient Population.

As further evidence of the nonobviousness of the presently claimed methods, Applicants submit herewith the evidence described below.

Beginning in 2004, INO Therapeutics LLC ("INOT") sponsored a clinical trial formally entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilatory Testing" and known as the INOT22 Study. See ¶ 4 of the enclosed Declaration of James S. Baldassarre, M.D. under 37 C.F.R. § 1.132, dated September 29, 2010 ("First Baldassarre Declaration"; originally filed in a sister application, US. Serial No. 12/820,866, and attached hereto as Exhibit E). The purpose of the study was to assess the safety and effectiveness of inhaled nitric oxide as a diagnostic agent in pediatric patients undergoing assessment of pulmonary hypertension (primary objective), and to confirm the hypothesis that inhaled NO is selective for the pulmonary vasculature (secondary objective).[22]

As described in the First Baldassarre Declaration, "the INOT22 Study was an open, prospective, randomized, multi-center, controlled diagnostic trial, with an expected total enrollment of a minimum of 150 patients, in approximately 18 study sites in the US and Europe over approximately 2 years."[23] "The expected patient population for enrollment into the INOT22 Study were subjects between the ages of 4 weeks and 18 years with idiopathic pulmonary arterial hypertension, congenital heart disease with pulmonary hypertension and cardiomyopathies, and who were undergoing diagnostic right heart catheterization scheduled to include pulmonary vasodilation testing to assess pulmonary vasoreactivity."[24]

The INOT22 Study was designed by the study sponsor (INO Therapeutics, or INOT) and a Steering Committee made up of internationally recognized experts in the field of pediatric heart and lung disease.[25]

The Steering Committee consisted of:

a. **David L. Wessel, MD**, presently Division Chief, Pediatric Critical Care Medicine at Children's National Medical Center, Washington, DC;
b. **Robyn J. Barst, MD**, presently Professor Emeritus of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York; and
c. **Duncan J. Macrae, MD**, presently Director, Pediatric Intensive Care, Royal Brompton Hospital, London, U.K.[26]

The original exclusion criteria for the INOT22 Study did **not** exclude patients with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunting of blood.[27] In particular, the original INOT22 Study protocol contained the following inclusion and exclusion criteria:

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22 First Greene Declaration, ¶ 18.
23 First Baldassarre Declaration, ¶ 5.
24 *Id.* ¶ 6.
25 *Id.* ¶ 7.
26 *Id.* ¶ 8.
27 *Id.* ¶ 11.

<u>Inclusion Criteria</u>

The patient must meet the following criteria:

1. Have any one of the three disease categories:

a. Idiopathic Pulmonary Arterial Hypertension

i. PAPm >25mmHg at rest, PCWP ≤ 15mmHg, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization

b. CHD [Congenital Heart Disease] with pulmonary hypertension repaired and unrepaired,

i. PAPm >25mmHg at rest, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization

c. Cardiomyopathy

i. PAPm >25mmHg at rest, and PVRI > 3 u.m^2 or diagnosed clinically with no previous catheterization

2. Scheduled to undergo right heart catheterization to assess pulmonary vasoreactivity by acute pulmonary vasodilation testing.

3. Males or females, ages 4 weeks to 18 years, inclusive.

4. Signed IRB/IEC approved informed consent (and assent if applicable).

<u>Exclusion Criteria</u>

The patient will be excluded from enrollment if any of the following are true:

1. Focal pulmonary infiltrates on chest radiograph.

2. Diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.

3. Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.

4. Pregnant (urine HCG +).[28]

The original INOT22 Study investigational plan and study protocol were reviewed and approved by the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the 18 participating study institutions, including review by the principal investigator within each study institution.[29] The original study protocol was also reviewed by experts at FDA and each National Health Authority (European equivalent to FDA) within the four European countries

[28] *Id.* ¶ 9.
[29] *Id.* ¶ 10.

participating in the INOT22 Study: United Kingdom, France, Netherlands, and Spain.[30] In addition, INOT regularly requested input and scientific guidance on clinical trials, such as the INOT22 Study, from its own Scientific Advisory Board (SAB). See the Declaration of James S. Baldassarre, M.D. under 37 C.F.R. § 1.132, dated July 7, 2011 ("Second Baldassarre Declaration"; originally filed in a sister application, US. Serial No. 12/820,866, and attached hereto as Exhibit F) at ¶ 8.

At no time did the study sponsor, any of the experts on the Steering Committee, any of the principal investigators, any of the IRBs, any of the IECs, any of the SAB members, any of the FDA experts, or any of the European Health Authority experts (altogether estimated to total at least 115 medical professionals) suggest that the exclusion criteria for the INOT22 Study protocol be amended to exclude patients who have LVD but were not dependent on a right-to-left shunt.[31] **In other words, of the estimated 115+ medical professionals tasked with the duty to consider potential safety issues for INOT22 Study patients, none—*not a single one*--suggested there was a chance that inhaled nitric oxide might increase the likelihood of pulmonary edema or other adverse events in the Claimed Patient Population.**[32]

Upon administration of inhaled nitric oxide to the first 24 subjects enrolled in the INOT22 Study, five serious adverse events were recorded – a rate much higher than expected based on prior clinical experience with inhaled nitric oxide. Each of these five serious adverse events (SAEs) was a cardiovascular event, such as pulmonary edema, cardiac arrest or hypotension (low blood pressure).[33]

In February 2005, INOT and the Steering Committee convened to review the unexpected SAEs described above, and upon review and discussion, submitted a protocol amendment to FDA to thereafter exclude subjects from enrollment if they demonstrated an elevated pulmonary capillary wedge pressure (PCWP), defined within the study as subjects having a PCWP greater than 20 mmHg, a symptom of LVD. All study sites were notified immediately.[34]

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[30] Second Greene Dec. ¶ 26.
[31] *Id.*
[32] *Id.* ¶ 11.
[33] First Baldassarre Dec. ¶ 12.
[34] *Id.* ¶ 13.

After conclusion of the study, analysis of the data revealed that modification of the exclusion criteria significantly reduced the rate of serious adverse events (including serious adverse events associated with heart failure). This analysis demonstrated that there were 5 SAEs among the first 24 subjects (i.e., those enrolled prior to amendment of the exclusion criteria), but only 2 SAEs among the next 80 subjects in the study (i.e., enrolled after amendment of the exclusion criteria). Further analysis of the data showed that a total of four subjects had pre-existing LVD, and of these four, 50% experienced SAEs. Of the 120 subjects not found to have evidence of LVD, only 4% experienced SAEs. This result was unexpected and came as a great surprise to those working on the study.[35]

In light of this important and unexpected result, on February 25, 2009, INOT submitted a label supplement to the FDA seeking to amend the prescribing information for INOmax® to include a new warning statement for physicians stating that use of inhaled nitric oxide in patients with LVD could cause serious adverse events, such as pulmonary edema.[36] On August 28, 2009, FDA approved an INOmax® label supplement (attached hereto as Exhibit G) that included the following two new warnings:

> *WARNINGS AND PRECAUTIONS*
>
> *Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).*
>
> *5 WARNINGS AND PRECAUTIONS*
>
> *5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*

Thereafter, similar warnings regarding this risk were added to the INOmax® label in Japan, Europe, Canada, and Australia.[37]

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[35] *Id.* ¶¶ 14-15; *See also* Wessel Declaration, ¶ 9.
[36] First Baldassarre Declaration, ¶ 15.
[37] Second Greene Declaration, ¶ 15.

Dr. Wessel did not find the claimed methods to be obvious

Dr. David Wessel (the author of the Wessel Declaration discussed above) chaired the INOT22 Steering Committee that in 2005 designed the original protocol for the INOT22 Study.[38] This same Dr. Wessel is the senior author of the Atz & Wessel reference cited in the rejection.[39]

As senior author of Atz & Wessel, Dr. Wessel was obviously well aware of that reference and its teachings. It is therefore telling that Dr. Wessel did not initially exclude LVD patients (other than those who are dependent on a right-to-left shunting of blood) from the INOT22 Study. As Dr. Wessel explains in his declaration, he did not exclude such LVD patients from the INOT22 Study because it was unanticipated at the time the protocol was first designed that "a child with left ventricular dysfunction who is not dependent on right-to-left shunting of blood would be at additional risk when treated with inhaled nitric oxide (iNO)."[40]

Over 100 other medical professionals did not find the claimed methods to be obvious

Dr. Wessel was not alone in this conclusion. It was seconded by literally more than one hundred other medical professionals belonging to the IRBs and IECs at each of the 18 medical institutions in the United States and Europe that participated in the study. Each of these IRBs and IECs reviewed the original INOT22 Study protocol design prior to study initiation and enrollment. This included review by the principal investigator within each study institution.[41]

As described in the Second Baldassarre Declaration, FDA regulations require an IRB to comprise a group of professionals appropriately constituted and formally designated to review and monitor biomedical research involving human subjects.[42] In accordance with FDA regulations, an IRB has the authority to approve, require modifications in (to secure approval), or disapprove research. This group review serves an important role and responsibility in the protection of the rights and welfare of human research subjects and in ensuring that appropriate steps are taken to protect human subjects participating in clinical research. An IRB must have at least five members, and each member must have enough expertise to make an informed decision

38 Wessel Declaration, ¶ 5.
39 *Id.* ¶ 8.
40 *Id.* ¶ 6.
41 Second Baldassarre Declaration, ¶¶ 8-11.
42 *Id.* ¶ 9.

on whether the research is ethical, the informed consent is sufficient, and the appropriate safeguards to protect patient safety have been put in place prior to starting a clinical trial.[43]

In Europe, the analog of an IRB is an IEC, an independent body consisting of healthcare professionals and non-medical members whose responsibility is to protect the rights, safety, and wellbeing of human subjects involved in a clinical trial and to provide public assurance of that protection by expressing an opinion on a proposed clinical trial protocol, the suitability of the investigators, and the adequacy of facilities involved in a trial. Like an IRB, an IEC will review a clinical trial protocol with the intent of protecting patient safety prior to clinical enrollment.[44]

In sum, IRBs and IECs are composed of qualified medical professionals tasked with reviewing all clinical trial protocols proposed at their respective institutions and empowered to make or suggest changes to a given protocol that are deemed necessary to best ensure patient safety during the clinical trial. *Naturally, any obvious safety concerns arising from a proposed clinical trial protocol will be identified by an IRB/IEC and the protocol will be amended to avoid obvious and unnecessary clinical risks.*[45] *If a given safety issue is not flagged by the reviewing IRB/IEC, it by definition is not obvious to the members of the IRB/IEC.*

<u>Officials of FDA and four European Health Authorities did not find the claimed methods to be obvious</u>

As further evidence that those of skill in the art did not consider the claimed methods to be obvious, Applicants note that FDA did not require the INOmax drug label to include a warning or exclusion for the Claimed Patient Population until <u>after</u> Applicants discovered the risk to this population. Furthermore, FDA and four European Health Authorities who reviewed the original INO22 Study protocol did not flag any risk to the Claimed Patient Population.

Under the Food, Drug and Cosmetic Act (FDCA), FDA is charged with ensuring not only that drugs are safe and effective,[46] but also that their labels contain adequate directions for use,

43 *Id.*
44 *Id.* ¶ 10.
45 *Id.* ¶¶ 9-10.
46 The FDA was first given responsibility for ensuring the efficacy of prescription drugs under the 1962 Kefauver-Harris amendments to the Food, Drug, and Cosmetic Act, Pub. L. No. 87-781, 76 Stat. 780 (1962) (FDCA; codified as amended at 21 U.S.C. § 301 et. seq. (1998)).

including appropriate disclosure of known safety issues.[47] A drug Sponsor, typically a pharmaceutical company, will work with FDA to design clinical trials for testing the safety and efficacy of any new, unapproved drug (typically consisting of Phase 1, 2a, 2b and 3 clinical trials). Upon completion of the clinical trial process, the Sponsor will submit a New Drug Application (NDA) to FDA to obtain marketing approval for a drug within the U.S. The NDA will contain extensive and detailed data regarding the safety and efficacy of the drug that the Sponsor obtained during its research and development. These data include the results of clinical trials, pharmacology and toxicology data, chemistry and manufacturing data, and proposed packaging and labeling information. Throughout the process, FDA and the Sponsor communicate through in-person meetings, telephone conferences, letters, e-mails, and faxes.[48]

Toward the end of the review process, FDA and the Sponsor negotiate the drug's final package label.[49] Each element of the label requires FDA approval, including the indications, dosing, directions for use, and safety information. Once all the reviews are complete, the division director and/or the office director evaluate the reviews and make FDA's decision.[50]

Inhaled NO was approved as a drug by FDA in December 1999, after extensive clinical study and FDA review. Upon approval, and up to the time the present invention was made, the INOmax® label[51] contained language communicating, in pertinent part, the following general warnings and contraindication (emphasis added):

> *INOmax® should not be discontinued abruptly, as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2).*
>
> *Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax....*
>
> *Methemoglobinemia increases with the dose of nitric oxide. ... Following discontinuation or reduction of nitric oxide the methemoglobin levels returned to baseline over a period of hours....*

47 See FDCA, § 502(f) (codified as amended at 21 U.S.C. § 352(f) (1998)).
48 21 C.F.R. § 312.
49 21 C.F.R. § 201.
50 21 C.F.R. § 314.105.
51 *See, e.g.,* Exhibit 1 attached to the Statement under 37 CFR § 1.57(f), page 2, in the "Dosage and Administration," "Precautions" and "Contraindications" sections.

> *INOmax should be administered with monitoring for PaO_2, methemoglobin and NO_2....*
>
> ***INOmax® should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood.***

Thus, although the original INOmax® label included an express contraindication for the Shunt-Reliant Population, it did not include any warning or precaution with respect to the Claimed Patient Population, and in fact was entirely silent about the latter.[52]

Moreover, neither FDA nor other National Health authorities reviewing the original protocol for the INOT22 Study suggested that the Claimed Patient Population should be excluded from this study.[53] Sponsors of clinical investigations are required to provide oversight to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality and integrity of the resulting data submitted to FDA.[54] Accordingly, the original INOT22 Study protocol was submitted to and approved by FDA prior to starting enrollment in the study.[55] It was similarly submitted to and approved by the National Health Authorities of each country containing a clinical trial center participating in the INOT22 Study (United Kingdom, France, Netherlands and Spain).[56] Not a single individual in any of these regulatory organizations suggested that administering inhaled nitric oxide to the Claimed Patient Population might lead to an increased risk of adverse events such as pulmonary edema.[57]

The evidence shows, however, that FDA did require a label change upon being notified by the INOT22 Study sponsor of the newly discovered risk to the Claimed Patient Population.[58] FDA does not take drug warnings lightly, and would not approve changes to a drug label that merely restate existing warnings.[59] Upon conclusion of the INOT22 Study and completion of the final study report, applicants discovered that the Claimed Patient Population was at increased

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[52] *Id.* After approval by FDA, INOmax® was also approved for use in Europe, Canada, Australia, Mexico and Japan by the National Health Authorities of those countries. Like the U.S. label, the original INOmax® drug labels in those countries did not contain any warning or precaution to refrain from administrating inhaled nitric oxide to the Claimed Patient Population.
[53] Second Baldassarre Dec. ¶ 8.
[54] *See generally* Responsibilities of Sponsors and Investigators, 21 C.F.R. § 312, subpart D; *See also* Responsibilities of Sponsors, 21 C.F.R. § 812, subpart C.
[55] Second Baldassarre Dec. ¶ 8.
[56] *Id.*
[57] *Id.*
[58] First Greene Declaration, ¶ 22.
[59] Second Greene Declaration, ¶ 15.

risk for adverse events. Because this was an important and unexpected finding, INOT submitted a label supplement to the FDA on February 25, 2009, seeking to amend the prescribing information for INOmax® to include a warning statement for physicians.[60] On August 28, 2009, FDA approved the INOmax® label supplement to include the following new information:

> *WARNINGS AND PRECAUTIONS*
>
> *Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).*
>
> *5 WARNINGS AND PRECAUTIONS*
>
> *5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*[61]

Thereafter, similar warnings regarding the Claimed Patient Population were added to the INOmax® label in Japan, Europe, Canada and Australia.[62]

The above facts illustrate that, prior to applicants' invention, medical professionals working in the real world did not exclude the Claimed Patient Population from inhaled NO therapy.[63] Over 100 experts worldwide and the regulatory authorities of five countries considered what patient populations to exclude from the INOT22 Study and did not exclude the Claimed Patient Population from that study.[64] Their actions definitively demonstrate that excluding the Claimed Patient Population from inhaled NO therapy was not obvious to those skilled in the art at the time of Appellant's invention.

VI. Rejections for obviousness-type double patenting

Beginning at page 17 of the Final Action, the Examiner asserts that the claims then pending in the case were provisionally rejected on the ground of nonstatutory obviousness-type

[60] First Baldassarre Declaration, ¶ 15.
[61] First Baldassarre Declaration, ¶ 16.
[62] Second Greene Declaration, ¶ 15.
[63] First Baldassarre Declaration, ¶¶ 9-11.
[64] Second Baldassarre Declaration, ¶ 11.

double patenting as being unpatentable over various claims of copending Application Nos. 12/820980, 12/821020, and 12/820866. As none of these copending applications has yet been allowed, the rejections are all provisional. Applicants intend to file a Terminal Disclaimer to overcome this ground of rejection, if the rejection becomes non-provisional and such a Terminal Disclaimer is appropriate at the time the present claims are otherwise deemed allowable.

CONCLUSION

It is believed that all grounds for rejection have been addressed and overcome. Applicants respectfully ask the Examiner to reconsider and withdraw the rejections, and to allow the new claims now pending. If a telephone conference would be helpful in advancing the case, the Examiner is invited to telephone the undersigned at the number provided below.

This reply is being filed with Exhibits A-G; a Statement under 37 CFR § 1.57(f) with its attached Exhibit 1; a Request for Continued Examination (RCE); and a Request for Prioritized Examination. The required fees are being paid concurrently herewith on the Electronic Filing System (EFS) by way of Deposit Account authorization. Please apply these and any other necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: January 6, 2012

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EXHIBIT A

Exhibit A

USSN: 12/820,866

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/820,866
Confirmation Number	2913
Filing Date	22-JUN-2010
Title of Application	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION
First Named Inventor	JAMES S. BALDASSARRE
Assignee	IKARIA, INC.
Group Art Unit	1616
Examiner	ARNOLD, ERNST V.
Attorney Docket Number	I001-0002USC1

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DOUGLAS A. GREENE, M.D.
UNDER 37 C.F.R. § 1.132

I, Douglas A. Greene, do hereby declare the following:

1. I currently hold the position of Executive Vice President and Head, Research and Development at INO Therapeutics LLC ("INO"). A copy of my *curriculum vitae* is attached as **Exhibit 1.**

2. I received an undergraduate degree in biology (*cum laude*) from Princeton University in 1966 and a doctoral degree in medicine (M.D.) from Johns Hopkins School of Medicine in 1970.

3. I spent the next thirty years of my medical career (1970-2000) practicing and teaching medicine at some of America's foremost academic medical centers, including Johns Hopkins, Penn, Pitt, and the University of Michigan. At Michigan, I was a full professor of internal medicine, director of the Michigan Diabetes Research and Training Center, and chief of the Division of Endocrinology and Metabolism.

4. In 2000, I left Michigan to join Merck as Executive Vice President in charge of clinical sciences and product development. In this role, I supervised and directly managed all clinical research at Merck Research Laboratories, among other duties.

5. In 2003, I left Merck for Sanofi-Aventis, where I became a Senior Vice President and Chief Medical Officer. My duties at Sanofi-Aventis included overseeing all aspects of pre-clinical and clinical regulatory development of the company's products and overseeing all medical aspects of the company's US business.

6. In 2010, I joined INO, where – as noted above – I am presently Executive Vice President and Head of Research and Development.

7. INO markets pharmaceutical grade nitric oxide (NO) gas under the brand name INOmax®. INOmax® is administered to patients using INO's proprietary INOvent® and INOmax® DS devices.

8. INOmax® was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999 for the treatment of term and near-term (≥ 34 weeks gestational age) neonates with hypoxic respiratory failure ("HRF") associated with clinical or echocardiographic evidence of pulmonary hypertension, a condition also known as persistent pulmonary hypertension in the newborn ("PPHN"). From 2000 to the present, INO has been selling INOmax® throughout the United States, Canada and certain other overseas markets.

9. In addition to the approved indication, physicians employ INOmax® to treat or prevent pulmonary hypertension and improve blood oxygen levels in a variety of other clinical settings, including in both pediatric and adult patients suffering from acute respiratory distress syndrome ("ARDS"), pediatric and adult patients undergoing cardiac or transplant surgeries, pediatric and adult patients for testing to diagnose reversible pulmonary hypertension, and in pediatric patients with congenital diaphragmatic hernia. In most, if not all, of these applications, INOmax® acts by preventing or treating reversible pulmonary vasoconstriction, and improves pulmonary gas exchange.

10. The mechanism of action of INOmax® - the selective relaxation of pulmonary blood vessels - is particularly relevant to the transition of the newborn from the fetal to the neonatal environment. During *in utero* development, the fetal lungs are not filled with air. Accordingly, the fetus obtains oxygen from the mother across the placenta into the systemic circulation, whereas the circulation through the lungs is largely shut down because the pulmonary vessels are tightly constricted. Instead of the blood being pumped from the right side of the heart through the fetal lungs and then returning to the left side of the heart to be pumped to the rest of the body, as it is normally after birth, blood from the right side of the fetal heart bypasses the fetal lungs through a patent ductus arteriosis, a blood vessel connecting the outflow of the right heart directly to the systemic circulation.

11. In addition to the patent ductus arteriosis, the fetal heart contains a second anatomical distinction from the neonatal heart - the foramen ovale - as a means for fetal blood to circumvent the nonfunctional fetal lungs while the fetus obtains its oxygen from the placenta. The foramen ovale is a "hole" located in the wall that separates the right and left atria of the heart. The foramen ovale is usually covered by a flap of tissue known as the septum primum, which is located on the inner wall of the left atrium. The septum primum and the foramen ovale together act as a one-way valve that permits blood to be shunted from the right atrium, where blood pressure is usually high due to the high vascular resistance present in the non-functional fetal lungs, into the left atrium for distribution to the body via the left ventricle. As discussed below, nonclosure of a patent foramen ovale after birth, as well as other forms of congenital heart disease, are often associated with a large persistently patent ductus arteriosis.

12. After birth, the pressure in the pulmonary circulatory system drops, reducing the right atrial pressure below that of the left atrium. This shift in pressure causes the septum primum to close off the foramen ovale, and this flap of tissue eventually becomes incorporated into the intra-atrial wall. In certain instances, however, the foramen ovale may remain open or "patent" after birth. In one such case, elevation of pressure in the pulmonary circulatory system (i.e.: pulmonary hypertension due to various causes) can prevent the pressure shift that leads to the closure of the foramen ovale. This condition is known as patent foramen ovale, and the use

of inhaled nitric oxide to decrease pulmonary hypertension is known to be a successful treatment for right-to-left shunting through a patent foramen ovale.[1]

13. At birth, the ductus arteriosis closes and pulmonary vessels relax, thereby redirecting the outflow of the right heart to the now oxygenated lungs, with oxygenated blood then returning to the left side of the heart to be pumped to the rest of the body from the left ventricle. However, in some instances, neonates are born with severe congenital heart disease involving the left ventricle, wherein the left side of the heart lacks the ability to pump blood to the rest of the body. In these instances, a ductus arteriosis that remains open or "patent" is actually beneficial, and in fact is life-saving when combined with pulmonary hypertension, because the reverse pressure created by the pulmonary hypertension creates a right-to-left shunt through the patent ductus arteriosis, thereby permitting the right ventricle to pump oxygenated blood directly to the systemic circulation to maintain organ function; simply put, the patent ductus arteriosis permits the right ventricle to subsume the role of nonfunctional left ventricle in circulating blood to the body. In these circumstances, stealing blood circulation away from the ductus arteriosis would be potentially fatal, and significantly, pulmonary vasoconstriction is also absolutely essential for survival in order to divert sufficient blood from the right heart through the patent ductus arteriosis to the systemic circulation, thus bypassing the non-functional left side of the heart to maintain life. The terminology to describe this situation is "neonates dependent upon right-to-left shunting of blood" for survival.

14. Administration of inhaled nitric oxide (iNO) in the context of such right-to-left shunting would be catastrophic, because reducing or eliminating the pulmonary vasoconstriction would permit blood to be diverted to the lungs and away from the patent ductus arteriosis.[2] Accordingly, an absolute contraindication for the use of iNO in babies dependent upon right-to-

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1 *See* Fessler MB et al., *Right-to-left shunting through a patent foramen ovale in right ventricular infarction: improvement of hypoxemic and hemodynamics with inhaled nitric oxide*, J. Clin. Anesth. 15: 371-4, 1993, at 371.

2 *See, e.g.*, Atz AM, Wessel DL. *Inhaled nitric oxide in the neonate with cardiac disease.* Sem. Perinatol. 21:441-455, 1997, at 452.

left shunting of blood has been contained in the INOmax® prescribing information since the original approval of INOmax® by the FDA in December, 1999.[3]

15. Pulmonary engorgement also occurs in adults with serious left-sided heart disease due to coronary artery disease ("ischemic cardiomyopathy"), hypertensive heart disease ("hypertensive cardiomyopathy") or obstructive valvular disease or other conditions that similarly restrict the inflow of blood to the left side of the heart such that engorgement of the pulmonary blood vessels ensues. It is important to note that restriction of left-sided inflow is particularly prominent in the above cardiomyopathies, and is described as diastolic dysfunction.[4] Diastolic dysfunction is extremely common in adult heart disease, especially in the elderly, but is extremely rare in childhood heart disease, which is generally caused by either congenital malformations or viral infections.[5]

16. To summarize, in adults, left-sided ventricular dysfunction is generally ischemic or hypertensive in origin, and is associated with a stiff, non-compliant left ventricle that cannot

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3 *See*, Exhibit 2, section 4, Prescribing Information, INOMAX.

4 *See* "Diastolic Dysfunction" American Heart Association "Learn and Live" website visited April 13, 2011: "The heart contracts and relaxes with each heartbeat. The contraction part of this cycle is called systole (SIS'-to-le). The relaxation portion is called diastole (di-AS'-to-le). In some people with heart failure, the contraction function is normal but there's impaired relaxation of the heart. This affects the heart's lower, pumping chambers (the ventricles) specifically. If the relaxation part of the cycle is abnormal, it's called diastolic (di"as-TOL'-ik) dysfunction. Because the ventricle doesn't relax normally, the pressure in it increases and exceeds what's normal as blood for the next heartbeat. (It's harder for all of the blood to go into the ventricle.) This can cause increased pressure and fluid in the blood vessels of the lungs. (This is called pulmonary congestion.) It can also cause increased pressure and fluid in the blood vessels coming back to the heart. (This is called systemic congestion.) People with certain types of cardiomyopathy (kar"-de-o-my-OP'-ah-the) may also have diastolic dysfunction."

5 Diastolic dysfunction in children has been described in rare genetic diseases such as Marfan's syndrome [that directly affects the elasticity of connective tissue of the heart and elsewhere], Kawasaki's disease [that creates cardiac ischemia similar to that in adult ischemic cardiomyopathy] or sickle cell disease [that produces fibrotic scars in the myocardium].

fill properly ("diastolic dysfunction"). In contrast, in children, left-sided ventricular dysfunction is generally not of ischemic or hypertensive in origin and is not associated with impaired filling, but rather is associated with a soft, overly elastic heart that cannot push blood out, resulting in impaired emptying ("systolic dysfunction"). Thus, adult left ventricular diastolic dysfunction, but not childhood left ventricular systolic dysfunction, would lead to pulmonary vascular engorgement, requiring caution in the use of iNO.

17. Since the approval of iNO in December 1999, INO has from time-to-time sponsored, supported or otherwise facilitated - under its own FDA Investigational New Drug (IND) application or IND applications filed by other investigators - clinical research exploring the efficacy and safety of iNO in clinical contexts outside the approved indication for PPHN. The results of these investigations are submitted to the FDA and are often published in the medical literature. In May 2004, following detailed consultations with an expert steering committee composed of leading world authorities in pediatric heart and lung disease,[6] INO initiated a multinational randomized controlled 150-patient study entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing" ("INOT22"). Prior to its initiation, the INOT22 study was reviewed and approved by the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the 18 participating study institutions, and by two independent National Health Authorities (the U.S. FDA and the European Medicines Agency (EMEA)). At no time did any of the members of these boards, committees or agencies counsel against giving inhaled nitric oxide to the proposed patient population because of the risk of severe adverse events in pediatric patients (i.e., children) with left ventricular dysfunction.

18. INOT22 was designed and purposed to compare the diagnostic utility of short-term (10 minute) inhalation of iNO alone, iNO plus oxygen ("O_2") or O_2 alone to children between the ages of 4 weeks and eighteen years with either idiopathic pulmonary arterial

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6 The steering committee included Dr. David Wessel of the Department of Cardiology, Children's Hospital and the Department of Pediatrics, Harvard Medical School.

hypertension, congenital heart disease with pulmonary arterial hypertension, or childhood forms of cardiomyopathy undergoing diagnostic right heart catheterization and acute pulmonary vasodilatation testing to assess pulmonary vasoreactivity. The rationale for INOT22 were: (1) that in patients with right ventricular failure and lung disorders, the prognosis and course of treatment are determined by acute pulmonary vasodilatation testing (APVT); (2) a reduction in the mean pulmonary artery pressure and pulmonary vascular resistance with acute vasodilator treatment may be used to predict therapeutic efficacy of long-term vasodilator medication; and (3) APVT is also used to evaluate patients being considered for heart or heart/lung transplantation; elevated pulmonary artery pressures and pulmonary vascular resistance place a strain on the right ventricle leading to an increased risk of perioperative morbidity and mortality due to right heart failure post heart transplant. Accordingly, the primary objective of INOT22 was to compare the number of patients who exhibited reversible pulmonary hypertension (vasoreactivity) in response to iNO or iNO plus and oxygen as compared to 100% oxygen alone.

19. Under the direction of the expert steering committee, inclusion and exclusion criteria were established that were intended to ensure the safe use of iNO during the conduct of the study. For example, patients dependent on right-to-left shunting and thereby contraindicated for iNO treatment were not included. Patients also were excluded if they had focal pulmonary infiltrates on chest radiograph, a diagnosis of severe obstructive or restrictive pulmonary disease that significantly contributed to the patient's pulmonary hypertension, had received treatment with iNO within 30 days prior to study initiation or were on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin, or were pregnant.

20. However, since the inclusion criteria included congenital heart disease or cardiomyopathy, many of the patients had, by design, significant childhood heart disease. This was not considered to pose a significant risk by the experts on the steering committee (1) based on the exclusion of right-to-left shunt-dependent patients, (2) based on prior extensive safe experience with iNO in pediatric patients with congenital heart disease or cardiomyopathy by the

investigators and published in the medical literature,[7] and (3) the very different nature of non-ischemic non-hypertensive childhood heart disease from the ischemic or hypertensive adult form marked by diastolic dysfunction.

21. Surprisingly and unexpectedly, severe adverse events including pulmonary edema and death were noted during the early phase of the study, and the study was stopped. Analysis of the cases revealed that the patients suffering severe adverse events had severe left ventricular dysfunction, largely due to viral cardiomyopathy, and exhibited during their right-sided cardiac catheterizations an increased pulmonary capillary wedge pressure ("PCWP") of greater than 20 mm Hg, indicative of elevated pressures in the upper chamber of the left side of the heart (the left atrium).

22. To determine if there was a correlation between the severe adverse events and the left ventricular dysfunction of the patients that had suffered them, a protocol amendment was submitted to FDA to exclude – on an ongoing basis - patients with severe left ventricular dysfunction with a PCWP greater than 20 mm Hg from further enrollment in the study. The study was then completed. On analyzing the data from the study, the inventors concluded that a correlation did, in fact, exist between the severe adverse events that had occurred during the study and the left ventricular dysfunction of the patients that had suffered them. Accordingly, INO subsequently requested that the FDA add an additional warning to the product labeling for INOmax concerning use of the drug within patients with left ventricular dysfunction. The FDA agreed and included an additional warning in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information (in the US and worldwide).[8]

23. Competent practitioners would understand that the warnings included in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information are intended as a separate warning generally applicable to all patients with left ventricular dysfunction and not limited to those patients having left ventricular dysfunction that also rely on

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7 *See* Atz AM et al. *Combined effects of nitric oxide and oxygen during acute pulmonary vasodilator testing.* J. Amer. Coll. Cardio. 33:813-819, 1999, at 814, 818.

8 *See* <u>EXHIBIT 2</u>.

right to left shunting of blood. This second category of patients is the subject of a separate section of the US Package Insert which expressly provides that INOmax is contraindicated for patients with this condition. The fact that administration of INOmax would be harmful to patients dependent on right to left shunting of blood has been well known for many years as demonstrated by several of the references that are of record in the present case including [e.g., Atz AM, Wessel DL. *Inhaled nitric oxide in the neonate with cardiac disease.* Sem. Perinatol. 21:441-455, 1997].

24. Furthermore, no competent practitioner would understand the separate warnings in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information, or the disclosure in the present application of the potential for severe adverse events in patients with left ventricular dysfunction as referring to patients dependent on right to left shunting of blood, since it has long been known that the use of INOmax is contraindicated in such patients. Rather, the competent practitioner would understand the additional warnings added at section 5.4 and within the Warnings and Precautions section of the INOmax prescribing information, and the disclosure in the present application of the potential for severe adverse events in patients with left ventricular dysfunction, as a distinct and separate warning and disclosure that administration of INOmax to patients with left ventricular dysfunction generally (even those not dependent on right to left shunting of blood) may result in serious adverse events.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '359 patent.

26.

Dated: April 29, 2011

Douglas A. Greene, M.D.

EXHIBIT 1

CURRICULUM VITAE

PERSONAL DATA

Name: Douglas Alan Greene, M.D.

EDUCATION

High School	Columbia High School, South Orange, NJ, 1962
Undergraduate	Princeton University, Princeton, NJ, BA Biology(cum laude), 1962-1966
Graduate/Professional	Johns Hopkins School of Medicine, Baltimore, MD, M.D., 1966-1970

POSTDOCTORAL TRAINING

Medical Internship:	Department of Medicine, Johns Hopkins, Baltimore, MD, 1970-1971
Medical Residency:	Department of Medicine, Johns Hopkins, Baltimore, MD, 1971-1972
Fellowship:	Medical Fellowship, Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, 1970-1972
	Post-doctoral Research Fellow, Diabetes, George S. Cox Medical Research Institute; Hospital of the University of Pennsylvania, Philadelphia, PA (Dr. Albert I. Winegrad, preceptor), 1972-1975
	Medical Fellowship, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA, 1972-1975

NON-ACADEMIC EMPLOYMENT

2000-2003 Executive Vice President, Clinical Sciences and Product Development (CSPD), Merck Research Laboratories, Rahway, New Jersey, and Corporate Officer, Merck, Inc. Supervised and directly managed all clinical research, regulatory affairs, clinical and non-clinical quality assurance and pharmaco-vigilance at Merck Research Laboratories.

2003-2006 Vice President, Head Corporate Regulatory Development, Sanofi-Aventis, Bridgewater, NJ. Overseeing all aspects of corporate regulatory development of all pre-clinical and clinical development projects/life-cycle products in Research & Development.

2006-2009 Senior Vice President, Chief Medical Officer, Sanofi-Aventis, Bridgewater, NJ. Overseeing medical, regulatory, pharmocovigilance, risk management, education and medical communications for US region, Member US Executive Committee, Member Committee Operational de Development, International Clinical Development.

2009-present Senior Vice President, Senior Scientific Advisor, Sanofi-Aventis, Bridgewater, New Jersey. Member Corporate Portfolio Valuation Process and Drug Development Committees. The position at the interface between the Research and Development and Pharmaceutical Operations is responsible for providing key scientific and medical guidance for sanofi-aventis' scientific strategy within U.S. and global contexts to enhance the quality and effectiveness of the company's research and product portfolio, including assessment and guidance of internal R&D product pipeline and franchise portfolio and external commercial and academic innovation opportunities.

ACADEMIC APPOINTMENTS

1975-1980	Assistant Professor of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania
1980-1986	Associate Professor of Medicine, Director, General Clinical Research Center and Diabetes Research Laboratories, University of Pittsburgh, School of Medicine
1986-2000	Professor of Internal Medicine, Director, Michigan Diabetes Research and Training Center, University of Michigan School of Medicine
1991-2000	Chief, Division of Endocrinology & Metabolism, University of Michigan School of Medicine
2000-Present	Adjunct Professor, Internal Medicine, Division of Endocrinology & Metabolism, University of Michigan, School of Medicine

SELECTED SCIENTIFIC ACTIVITIES

1988-1994	Chairman, Endocrinologic and Metabolic Drug Advisory Board, Food and Drug Administration, Washington D.C (Chair, 1990-1994)
1994-2000	Chairman, Merck Scientific Board of Advisors

SELECTED SCIENTIFIC PRIZES AND AWARDS

1986	First Annual Raymond A. and Robert L. Kroc Lecturer, Eisenhower Medical Center, Palm Springs, California
1987	Moore Award, The American Association of Neuropathologists, Seattle, Washington
1987	Carol Sinicki Manuscript Award (The Diabetes Educator), American Association of Diabetes Educators, Chicago, Illinois
1988	Kellion Lecture, International Diabetes Federation, Sydney, Australia
1989	Banting and Best Lecture, Toronto General Hospital, Toronto, Canada
1994	Charles H. Best Lecturer, Toronto Diabetes Association, Toronto, Canada
1996	Invited Speaker, Seventy-fifth Anniversary Celebrating the Discovery of Insulin, Toronto, Canada
1996	First Alan Robinson Lecturer, University of Pittsburgh
1998	Outstanding Foreign Investigator Award, Japan Society of Diabetic Complications

Douglas A. Greene, M.D.
updated 05/28/10

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Peer-Reviewed Publications (Selected from over 170 peer-reviewed articles):

1. Greene DA, DeJesus PV, Winegrad AI: Effect of insulin and dietary Myo-Inositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. *J. Clin. Invest.* 55:1326-1336, 1975.

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3. Greene DA, Lattimer SA: Sodium- and energy dependent uptake of myo-inositol by rabbit peripheral nerve. Competitive inhibition by glucose and lack of an insulin effect. *J. Clin. Invest.* 70:1009-1018, 1982.

4. Greene DA, Lattimer SA: Impaired rat sciatic nerve sodium-potassium ATPase in acute streptozocin diabetes and its correlation by dietary myo-inositol supplementation. *J. Clin. Invest.* 72:1058-1063, 1983.

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13. Greene DA, Chakrabarti S, Lattimer SA, Sima AAF: Role of sorbitol accumulation and myo-inositol depletion in paranodal swelling of large myelinated nerve fibers in the insulin-deficient spontaneously diabetic bio-breeding rat: Reversal by insulin replacement, an aldose reductase inhibitor, and myo-inositol. *J. Clin. Invest.* 79:1479-1485, 1987.

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diacylglycerol in cultured human retinal pigment epithelial cells. *J. Clin. Invest.* 93:2718-2724, 1994.

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EXHIBIT 2

INOmax® (nitric oxide) for inhalation

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INOmax safely and effectively. See full prescribing information for INOmax.

INOmax (nitric oxide) for inhalation
Initial U.S. Approval: 1999

RECENT MAJOR CHANGES

Warnings and Precautions, Heart Failure (5.4) 8/2009

INDICATIONS AND USAGE

INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (1.1).

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration (1.1).

Utilize additional therapies to maximize oxygen delivery (1.1).

DOSAGE AND ADMINISTRATION

Dosage: The recommended dose of INOmax is 20 ppm, maintained for up to 14 days or until the underlying oxygen desaturation has resolved (2.1).

Administration:

- INOmax must be delivered via a system which does not cause generation of excessive inhaled nitrogen dioxide (2.2).
- Do not discontinue INOmax abruptly (2.2).

DOSAGE FORMS AND STRENGTHS

INOmax (nitric oxide) is a gas available in 100 ppm and 800 ppm concentrations.

CONTRAINDICATIONS

Neonates known to be dependent on right-to-left shunting of blood (4).

WARNINGS AND PRECAUTIONS

Rebound: Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure (5.1).

Methemoglobinemia: Methemoglobin increases with the dose of nitric oxide; following discontinuation or reduction of nitric oxide, methemoglobin levels return to baseline over a period of hours (5.2).

Elevated NO_2 Levels: NO_2 levels should be monitored (5.3).

Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).

ADVERSE REACTIONS

Methemoglobinemia and elevated NO_2 levels are dose dependent adverse events. Worsening oxygenation and increasing pulmonary artery pressure occur if INOmax is discontinued abruptly. Other adverse reactions that occurred in more than 5% of patients receiving INOmax in the CINRGI study were: thrombocytopenia, hypokalemia, bilirubinemia, atelectasis, and hypotension (6).

To report SUSPECTED ADVERSE REACTIONS, contact INO Therapeutics at 1-877-566-9466 and http://www.inomax.com/ or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Nitric oxide donor agents: Nitric oxide donor compounds, such as prilocaine, sodium nitroprusside, and nitroglycerin, when administered as oral, parenteral, or topical formulations, may have an additive effect with INOmax on the risk of developing methemoglobinemia (7).

Revised: August 2009

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoxic Respiratory Failure

INOmax® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies *(see Clinical Studies (14))*.

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage

Term and near-term neonates with hypoxic respiratory failure

The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

2.2 Administration

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

Do not discontinue INOmax abruptly, as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

3 DOSAGE FORMS AND STRENGTHS

Nitric oxide is a gas available in 100 ppm and 800 ppm concentrations.

4 CONTRAINDICATIONS

[illegible]

5 WARNINGS AND PRECAUTIONS

5.1 Rebound

Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

5.2 Methemoglobinemia

Methemoglobinemia increases with the dose of nitric oxide. In clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide, the methemoglobin levels returned to baseline over a period of hours.

5.3 Elevated NO_2 Levels

In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

5.4 Heart Failure

[illegible]

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from the clinical studies does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

6.1 Clinical Trials Experience

Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse reactions that occurred in at least 5% of patients receiving INOmax in the CINRGI study with event rates >5% and greater than placebo event rates. None of the differences in these adverse reactions were statistically significant when inhaled nitric oxide patients were compared to patients receiving placebo.

Table 1:
Adverse Reactions in the CINRGI Study

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 (10%)	13 (13%)
Withdrawal	9 (10%)	12 (12%)
Atelectasis	8 (9%)	9 (9%)
Hematuria	5 (6%)	8 (8%)
Hyperglycemia	6 (7%)	8 (8%)
Sepsis	2 (2%)	7 (7%)
Infection	3 (3%)	6 (6%)
Stridor	3 (3%)	5 (5%)
Cellulitis	0 (0%)	5 (5%)

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of INOmax. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to estimate their frequency reliably or to establish a causal relationship to drug exposure. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

7 DRUG INTERACTIONS

No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

8.2 Labor and Delivery

The effect of INOmax on labor and delivery in humans is unknown.

8.3 Nursing Mothers

Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

8.4 Pediatric Use

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

8.5 Geriatric Use

Nitric oxide is not indicated for use in the adult population.

10 OVERDOSAGE

Overdosage with INOmax will be manifest by elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

11 DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:

·N̈ = Ö:

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide selectively dilates the pulmonary vasculature, and because of efficient scavenging by hemoglobin, has minimal effect on the systemic vasculature.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

12.2 Pharmacodynamics

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

12.3 Pharmacokinetics

The pharmacokinetics of nitric oxide has been studied in adults.

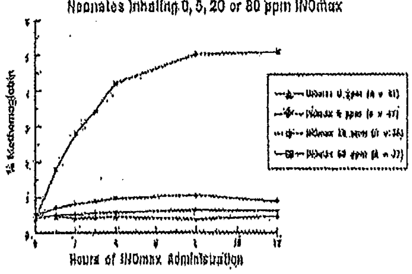
12.4 Pharmacokinetics: Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

12.5 Pharmacokinetics: Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1:
Methemoglobin Concentration – Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groups, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 35% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients, but one patient did not exceed 7% until 40 hours.

12.6 Pharmacokinetics: Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

14 CLINICAL STUDIES

14.1 Treatment of Hypoxic Respiratory Failure (HRF)

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI= mean airway pressure in cm H_2O × fraction of inspired oxygen concentration [FiO_2]× 100 divided by systemic arterial concentration in mm Hg [PaO_2]) and increases PaO_2 *[see Clinical Pharmacology (12.1)]*.

NINOS Study

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 2.

Table 2:
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups *[see Adverse Reactions (6.1)]*. Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

CINRGI Study

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 3.

Table 3:
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO*,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 58%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups *[see Adverse Reactions (6.1)]*.

14.2 Ineffective in Adult Respiratory Distress Syndrome (ARDS)

ARDS Study

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FiO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

16 HOW SUPPLIED/STORAGE AND HANDLING

INOmax (nitric oxide) is available in the following sizes:

Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)
Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure

The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

 SPC-0303 V:4.0

EXHIBIT B

Exhibit B

USSN: 12/820,866

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/820,866
Confirmation Number	2913
Filing Date	22-JUN-2010
Title of Application	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION
First Named Inventor	JAMES S. BALDASSARRE
Assignee	IKARIA, INC.
Group Art Unit	1616
Examiner	ARNOLD, ERNST V.
Attorney Docket Number	I001-0002USC1

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DOUGLAS A. GREENE, M.D.
UNDER 37 C.F.R. § 1.132

I, Douglas A. Greene, do hereby declare the following:

1. I currently hold the position of Executive Vice President and Head of Research and Development at INO Therapeutics LLC ("INO"), which is a wholly-owned subsidiary of Ikaria, Inc. A copy of my *curriculum vitae* is attached as **Exhibit 1**.

2. I received an undergraduate degree in biology (*cum laude*) from Princeton University in 1966 and a doctoral degree in medicine (M.D.) from Johns Hopkins School of Medicine in 1970.

3. I spent the next thirty years of my medical career (1970-2000) practicing and teaching medicine at some of America's foremost academic medical centers, including Johns Hopkins, Penn, Pitt, and the University of Michigan. At Michigan, I was a full professor of internal medicine, director of the Michigan Diabetes Research and Training Center, and chief of the Division of Endocrinology and Metabolism.

4. In 2000, I left Michigan to join Merck as Executive Vice President in charge of clinical sciences and product development. In this role, I supervised and directly managed all clinical research at Merck Research Laboratories, among other duties.

5. In 2003, I left Merck for Sanofi-Aventis, where I became a Senior Vice President and Chief Medical Officer. My duties at Sanofi-Aventis included overseeing all aspects of pre-clinical and clinical regulatory development of the company's products and overseeing all medical aspects of the company's US business.

6. In 2010, I joined INO, where – as noted above – I am presently Executive Vice President and Head of Research and Development.

7. I have been shown a Non-Final Office Action issued by the United States Patent and Trademark Office (USPTO) on June 8, 2011 in a pending patent application having US serial number 12/820,866. This Non-Final Office Action rejected the pending claims of 12/820,866 as "obvious" based on clinical interpretations presented by the USPTO regarding the teaching and disclosure of Atz & Wessel. (Seminars in Perinatology 1997, 21(5), 441-455), Kinsella et al. (Lancet 1999, 354 1061-1065) and Loh et al. (Circulation 1994, 90, 2780-2785). Below is my professional opinion and interpretation of the arguments and clinical interpretations presented by the USPTO within the Non-Final Office Action of June 8, 2011, for 12/820,866 (the "Office Action).

8. On page 7 of the Office Action, the Examiner states:

> *"Atz et al. teach that: 'Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension.' (page 452, left column)."*

A more complete excerpt from Atz & Wessel, p. 452, left column is as follows:

> "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension. In **adults with ischemic cardiomyopathy**, sudden pulmonary vasodilation may occasionally

> unload the right ventricle sufficiently to increase pulmonary blood flow and harmfully augment preload in a compromised left ventricle. The attendant increase in left atrial pressure may produce pulmonary edema. ... A different but related phenomenon may be operative in the newborn ...." (emphasis added)

Thus, although Atz & Wessel warns that "[c]aution should be exercised when administering nitric oxide (NO) to patients with severe left ventricular dysfunction and pulmonary hypertension[,]" this caution is specifically limited to two populations of patients. In the first population, the statement in Atz & Wessel p. 452, left column, is directed to adult patients with ischemic cardiomyopathy who also exhibit severe left ventricular dysfunction and pulmonary hypertension. This patient population is clearly different from the neonatal population that is the object of the teaching of the present claims.

9. Further in the same paragraph, Atz & Wessel specifically refers to a second patient population, which is also distinct from that of the present patent application, to whom inhaled NO should not be administered, namely, neonates depending on right-to-left shunting of blood:

> "A different but related phenomenon may be operative in the newborn with severe left ventricular dysfunction and pulmonary hypertension. In these patients, the **systemic circulation may depend in part on the ability of the right ventricle to sustain cardiac output through a right-to-left shunt across the patent ductus arteriosus.** Selective pulmonary vasodilation may redirect the right ventricular output to the lungs and away from the systemic circulation." (emphasis added)

For this second patient population, Atz & Wessel state that these patients exhibit a "different but related phenomenon" from that observed in adults with ischemic cardiomyopathy. This second population of patients consists of newborn patients with congenital heart disease and left ventricular dysfunction who are **dependent on a right-to-left shunt through a ductus arteriosus** in order to maintain peripheral circulation necessary to survive. In these patients, a patent ductus provides the only alternate pathway for blood being pumped by the right ventricle to **bypass the dysfunctional left ventricle** and thereby substitute for the dysfunctional left ventricle in providing life-sustaining blood flow to the peripheral circulation. Blood emerging from the right ventricle has only two possible pathways, either through the pulmonary circulation and then back to the dysfunctional left ventricle, or to pass through the patent ductus arteriosus in a right-to-left shunt to reach the systemic circulation. Inhaled NO dilates the pulmonary circulation, and therefore would divert blood to the lungs at the expense of the patent ductus arteriosus and systemic circulation, causing systemic vascular collapse and death. Again, this second patient population described by Atz & Wessel is also completely different from the patient population addressed in the present claims, which is term or near term neonates with left ventricular dysfunction who are **NOT dependent upon right-to-left shunting.**

10. The risk of circulatory collapse in the subset of newborns with congenital heart disease and severe left ventricular dysfunction **who are dependent upon a right-to-left shunt** through a patent ductus arteriosus was well known in this field long before the Atz & Wessel publication, as evidenced by the contraindication stated in the US Food and Drug Administration (FDA) prescribing information for INOMAX® (nitric oxide) for inhalation from the time of its initial approval by the FDA in 1999: "**CONTRAINDICATIONS:** Neonates known to be dependent on right-to-left shunting of blood".

11. As a result of the INOT22 study, it was recognized that a second population of neonates existed, distinct from the population described in Atz & Wessel, that had an increased risk of adverse events when inhaled NO was administered, namely: pediatric patients with left ventricular dysfunction **who are not dependent upon right-to-left shunting of blood.** In view of this newly identified risk, the FDA imposed the addition of a distinct and separate precaution to the prescribing information for INOMAX specifically cautioning about an additional risk of pulmonary edema for patients with left ventricular dysfunction (see paragraph 15). It is important to note that patients covered in the pre-existing contraindication (specifically neonates known to be dependent on right-to-left shunting of blood) were completely excluded from INOT22 by virtue of the labeled contraindication. The newly discovered risk of adverse events in neonates and children with left ventricular dysfunction **who are not dependent on right-to-left shunting** was not addressed, suggested or otherwise inferred from the teachings of Atz & Wessel, because when Atz and Wessel recommend that inhaled NO should be used with caution

"if at all", that warning relates to neonates **who are dependent upon right-to-left shunting of blood** – a completely different population of patients than the population that is addressed in the present claims.

12. On page 7 of the Office Action, the Examiner further states:

> *"Since pulmonary hypertension is instantly claimed, then the subject intrinsically has hypoxic respiratory failure."*

This statement is not medically accurate. Pulmonary hypertension occurs in many conditions other than hypoxic respiratory failure, such as congenital heart disease, maternal use of serotonin reuptake inhibitors, idiopathic pulmonary hypertension, etc.

13. On page 7 and 8 of the Office Action, the Examiner states:

> *"Atz et al. continues with: 'Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and **exclusively right to left shunting at the ductus arteriosus**, NO should be used with extreme caution, if at all. We and others have reported adverse outcomes in this circumstance.' (p. 452, left column) (emphasis differing from original)."*

This statement merely reiterates the "caution" delivered by Atz & Wessel for the second population of patients identified in that publication, namely neonates **dependent upon a right-to-left shunt** at the ductus arteriosus. In this statement, Atz & Wessel simply teach that patients with severe left ventricular dysfunction dependent upon an exclusively right-to-left shunt at the ductus arteriosus often have coexistent predominantly left-to-right shunt at the foramen ovale. This additional left-to-right shunt at the foramen ovale, upstream from the dysfunctional left ventricle, permits blood to bypass the dysfunctional left ventricle and enter the right side of the heart, thereby enhancing the ability of the right ventricle to pump sufficient blood through the ductus arteriosus to maintain the systemic circulation. The population of patients dependent upon right-to-left shunting of blood (with or without shunting at the foramen ovale) was already excluded by the pre-existing FDA-mandated contraindication for inhaled NO, and is distinct from the patient population addressed in the present claims.

14. On page 8 of the Office Action, the Examiner states:

> *"Atz et al. thus identify conditions in the patients which is screening of the patient. Thus, Atz et al. fairly teaches excluding patients which include neonates with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing."*

This statement misinterprets the teaching of Atz & Wessel. Specifically, "if at all" refers to the second patient population, wherein no treatment is allowed in the population of newborn "patients dependent upon right-to-left shunting of blood" who are at risk for circulatory collapse. Because these patients were already contraindicated in the drug labeling for inhaled NO prior to INOT22 (see paragraph 10 above), they were excluded from INOT22 and more importantly, are distinct from the patients identified in the new inhaled NO safety warnings mandated by the FDA in view of the risk that was newly identified as a result of the INOT22 study.

15. On February 25, 2009, INO Therapeutics LLC (owner of NDA 20845) submitted a label supplement to the FDA seeking to amend the prescribing information (i.e., the "label") for INOMAX® (nitric oxide) for inhalation, to include a new warning statement based on the unexpected outcome of the INOT22 study On August 28, 2009, the FDA approved the INOMAX® label supplement to include the following new information:

> *<u>WARNINGS AND PRECAUTIONS</u>*
>
> *Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).*
>
> *<u>5 WARNINGS AND PRECAUTIONS</u>*
>
> *5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*

Thereafter, similar warnings were added to the INOMAX label by Health Authorities in Japan, Europe, Canada and Australia. The FDA (and it's counterparts in foreign nations) would not add new warnings and precautions to the label of an approved drug that merely restate a known contraindication already existing on the approved drug label. Indeed, the new FDA-approved warnings for the use of nitric oxide are clinically distinct from the existing, original INOMAX contraindication disclosed by Atz & Wessel, with respect to neonates dependent on right-to-left shunt.

16. On page 8 and 9 of the Office Action, the Examiner states:

> *"Kinsella et al. teach excluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and p. 1062, Methods). Since left ventricular dysfunction is a congenital heart disease, as acknowledged by Applicant, (see specification [0028]), and it would be pre-existing, then the methods of Kinsella et al. intrinsically exclude this patient population from the method. ... The intended patient population is intrinsically at risk of one or more adverse events. Patients are intrinsically identified for nitric oxide inhalation treatment, diagnosed for congenital heart disease which intrinsically includes left ventricular dysfunction, and if the patient meets the criteria then treatment with NO is performed thereby reducing the risk of adverse events associated with the treatment."*

Based on these statements, it is clear that the Examiner fails to understand several critical aspects of the study of Kinsella et al.

17. First and foremost, the patients included in the Kinsella et al. trial were premature neonates who have severe respiratory failure due to immature lungs and surfactant deficiency, rather than term and near-term neonates suffering from pulmonary hypertension. In addition, none of the premature neonates enrolled in Kinsella et al. suffered from pulmonary hypertension. Thus, the patients included in Kinsella et al. were clinically differentiated, by age, etiology and pathophysiology, from the term and near-term neonates addressed in the present claims.

18. Secondly, exclusion of patients from a particular study may occur for a variety of reasons. For example, clinical trial inclusion and exclusion criteria are often chosen to define or restrict the study population in order to maximize homogeneity, thereby minimizing the presence of potentially confounding factors. This exclusion greatly facilitates the interpretation of the

study results, and increases the soundness of the conclusions reached in the study. Accordingly, patients with background disease sufficiently severe to overwhelm or confound an expected treatment effect are systematically identified and excluded quite independently from considerations of anticipated safety or efficacy of the test article in this particular patient group.

19. For example, patients with malignancy are often excluded from non-oncologic clinical trials, not because the test agents are unsafe, pose any specific risk in this population, or will not work, but rather because the clinical results will be confounded by the wholly unrelated effects of the underlying malignancy, thereby reducing the power of the clinical trial to answer a specific hypothesis regarding the test treatment. As a specific example, exclusion of patients with malignancy or advanced heart failure from cholesterol lowering trials does not imply that statins are unsafe or ineffective in these patients, but rather that their inclusion would confound the potential effects of statins on overall mortality or cardiovascular events.

20. In the specific case of Kinsella et al., it is clear that one of ordinary skill in the art would understand that the patients having fatal congenital anomalities or congenital heart disease were excluded not because of a suspected safety risk of treating these patients with inhaled NO (e.g., a risk of pulmonary edema), but rather solely because the inclusion of such patients would have made it much more difficult – if not impossible - for Kinsella et al. to interpret the target outcomes of the study (i.e., would have "confounded" the results).

21. On page 9 of the Office Action, the Examiner states:

> *Loh et al. teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract). Loh et al. clearly teaches that patients with pulmonary artery wedge pressure, which is synonymous with the instantly claimed pulmonary capillary wedge pressure, of greater than or equal to 18mm Hg had a greater effect of inhaled NO due to the greater degree of reactive pulmonary hypertension present in such patients (p. 2784, left column). Loh et al. state: "Since the degree of reactive pulmonary hypertension is generally related to the severity of hemodynamic compromise in patients with LV failure, it might be*

> *anticipated that patients with more severe heart failure will have a more marked hemodynamic response to inhaled NO." Loh et al. examined this prediction further and verified it (p. 2784, left column).*

The Examiner apparently neglects to consider that the acute hemodynamic effect of inhaled NO was studied by Loh et al. only in **adult** patients with New York Heart Association Class III or IV congestive failure due to coronary artery disease or dilated cardiomyopathy, not in term or near-term neonates who were not dependent upon right-to-left shunting. Thus, their observations do not teach, or even suggest, the risk of inhaled NO in neonates or children with pulmonary hypertension and left ventricular dysfunction who are not dependent on right-to-left shunting of blood, the population that is addressed in the present claims.

22. The underlying etiologies and hemodynamic characteristics of both the primary heart disease and the increased pulmonary vascular resistance are drastically different from adults, as compared to non-adults, such that one cannot readily assume or anticipate clinical results within adults to translate into neonates or children. In particular, left ventricular dysfunction in neonates with congenital heart disease is primarily due to developmental structural disease of the heart, inborn errors of metabolism that impair energy generation in the heart muscle, or viral infection. Class III or class IV congestive heart failure in adults (in contrast to congenital heart disease in neonates or children) is due to ischemic or dilated cardiomyopathy, mostly secondary to coronary artery disease and/or chronic systemic hypertension. Pulmonary hypertension associated with neonatal congenital heart disease is secondary to chronic hypoxemia, developmental abnormalities of the pulmonary blood vessels and/or pulmonary vascular damage from abnormally high blood flow and/or pressure through the pulmonary vasculature, resulting in evident disease of the lung vasculature. In contrast, increased pulmonary vascular resistance in adult Class III or IV congestive heart failure is due to reactive pulmonary vasoconstriction secondary to increased sympathetic tone or circulating vasoactive molecules (Loh et al., p. 2780, left column) in otherwise structurally normal blood vessels. Therefore, the hemodynamic responses to pulmonary vasodilation by inhaled NO in children or neonates, without right-to-left shunting of blood, but with significant pulmonary hypertension and left ventricular dysfunction cannot be reasonably predicted from the hemodynamic responses to pulmonary vasodilation by inhaled NO of adults with advanced

atherosclerotic congestive heart failure and reactive neuro-humoral pulmonary vascular constriction (with or without pulmonary hypertension) as described by Loh et al.

23. On page 10 of the Office Action, the Examiner states:

> *"It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Atz et al. and identify patients with a second condition/risk factor and administer iNO to patients that do not have the first or second condition/risk factors of instant claims 20-27 and inform the medical provider that patients with a pulmonary capillary wedge pressure greater than 20 mm Hg that may increase pulmonary edema, as suggested by Loh et al., and Kinsella et al., and produce the instant invention."*

24. Atz & Wessel do not recommend exercising "caution" when treating term or near-term neonates who are not dependent upon right-to-left shunting, but rather refer to two other patient populations, namely (i) neonatal patients whose systemic circulation is dependent upon right-to-left shunting of blood and who therefore might suffer from systemic circulatory collapse if given inhaled NO (a well-known contraindication for inhaled NO) and (ii) adult patients with New York Heart Association Class III-IV heart failure due to ischemic or dilated cardiomyopathy and increased neuro-humorally-mediated pulmonary vascular resistance might be hemodynamically at risk for pulmonary edema if given inhaled NO (the same population discussed by Loh et al.).

25. On page 10 of the Office Action, the Examiner states:

> *"One of ordinary skill in the art would have been motivated to do this because: 1) it is common sense that if the neonate is healthy then INO therapy can be performed safely; 2) if the neonate is not healthy and has left ventricular dysfunction (LVD), then Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with LVD which would also render obvious all conditions/risk factors associated with LVD; and 3) the art of Kinsella et al. establishes excluding certain patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease."*

The conclusion presented by the Examiner is not clinically accurate, nor does it accurately reflect the expectations or motivations of a clinician of ordinary skill in the art at the time of the invention. Their expectation would have been quite the opposite. It is by no means "*1) ... common sense that if the neonate is healthy then iNO therapy can be performed safely; 2) if the neonate is not healthy and has left ventricular dysfunction (LVD), then Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with LVD.*" Firstly, inhaled NO would have no utility in healthy neonates, and is safely used in very severely ill neonates on a routine basis. Secondly, Atz & Wessel teach "using extreme caution or not using NO at all" only in neonates dependent upon right-to-left shunting of blood in order to avoid systemic circulatory collapse, and makes no statement regarding neonates with left ventricular dysfunction **who are not dependent upon right-to-left shunting.** Kinsella et al. do not teach about the safe or unsafe use of inhaled NO in neonates or children, let alone term or near-term neonates not dependent upon right-to-left shunting, but merely noted that they had excluded premature babies with fatal malformations or congenital heart disease from a clinical trial of inhaled NO in premature babies suffering from the respiratory distress of prematurity. Loh et al. teach about the effect of inhaled NO on hemodynamic measurements in adults with advanced heart failure and secondary neuro-humorally-mediated increased pulmonary vascular resistance, and speculate that these adults may be at increased risk for pulmonary edema, but do not teach anything about the use of inhaled NO in term or near-term neonates not dependent upon right-to-left shunting.

26. On page 11 of the Office Action, the Examiner states:

> "*Furthermore, it is already known through the teachings of Loh et al. that a pulmonary capillary wedge pressure (PCWP) of greater than 18 mg Hg serves as a guidepost for alerting the artisan to adverse events from inhaled NO. Thus, it is not inventive to exclude patients with a PCWP of greater than 20 mm Hg when the art already suggests the risk of trouble of treating patients with a PCWP of 18 mm Hg because inhaled NO increases the wedge pressure as taught by Loh et al. (see entire document). In summary, it remains the position of the Examiner, which is in alignment with the written opinion of the international search authority, that it is simply not inventive to 'inform' a medical provider that a neonate with LVD is at risk of adverse/serious adverse*

> *events from iNO therapy when the art already has established that fact and the ordinary artisan is alerted to this fact. If the patient has LVD then they are at risk of adverse and/or serious adverse events from iNO therapy and it is not inventive to further identify other secondary conditions/risk factors associated with LVD and provide further warnings for secondary conditions/risk factors that are separate and independent from the first condition/risk factor but nevertheless associated with LVD to the medical provider. Screening for conditions that predispose the patient to adverse/serious adverse effects from medical treatment is obvious given the teachings above.*" (emphasis in original)

It is inaccurate to represent Loh et al as "*serving as a guidepost for alerting the artisan to adverse events from inhaled NO,*" as Loh et al. reported no adverse events during administration of inhaled NO for 10 minutes to 19 stable patients with advanced heart failure. Rather, Loh et al. speculated that a finding of an elevation in PCWP in a subgroup of such patients could pose an increased risk of pulmonary edema in adults with congestive heart failure due to ischemic or dilated cardiomyopathy. As discussed above, extrapolation of that theoretical risk to neonates and children with different forms of heart disease, different cardiovascular hemodynamics, and different pulmonary vasculature physiology, pathophysiology and pathology was not obvious , as evidenced by the fact that the members of the INOT22 Screening Committee (including Dr. Wessel) who designed the INOT22 study protocol, the approximately 18 Institutional Review Boards and/or Independent Ethics Committee, and 5 National Health Authorities (FDA and national Health Authority for United Kingdom, France, Netherlands and Spain) who reviewed and approved the INOT22 study protocol prior to its initiation, all failed to predict that any untoward effects would be caused by the administration of inhaled NO within a pediatric patient population having left ventricular dysfunction who are not dependent on right-to-left shunting of blood.. Only after being informed of the present invention did the FDA mandate a change to the drug labeling for inhaled NO to include a new warning (separate and distinct from the pre-existing contraindication pertaining to neonates dependent on right-to-left shunting of blood) concerning the use of inhaled NO in patients with pre-existing left ventricular dysfunction.

27. On page 12 of the Office Action the Examiner states:

> *Respectfully, the instantly claimed method steps are in the realm of common sense and not in the realm of invention because it is already known in the art that patients with pre-existing LVD are at risk of adverse effects from iNO. It is obvious to the ordinary artisan that if the neonate has LVD with or without any number of conditions/risk factors, then in order to avoid the risk of adverse or serious adverse events associated with iNO, to then exclude the neonate from iNO therapy. In other words, given the art as a whole, determination of further conditions/risk factors that would exclude the neonate from iNO therapy is obvious given the teachings in the art as discussed above which direct the artisan to screen neonates about to undergo treatment with NO by inhalation and to exclude those with LVD from such treatment. In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a). From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary."*

The arguments by which this conclusion is supported are both medically and scientifically unsound. To summarize, the teaching of Atz & Wessel is inaccurately portrayed by the Examiner due to his confusion of the known risk of systemic vascular collapse if inhaled NO is administered to neonates dependent upon right-to-left shunting of blood, and the opposite case of adults where inhaled NO may be less effective than in children. The Examiner misconstrues Kinsella et al.'s clinical trial inclusion/exclusion criteria as a teaching of risk associated with inhaled NO administration, rather than as a routine practical measure in the design of clinical trials to minimize confounding factors and heterogeneity in the study population. Lastly, the Examiner grossly over-interprets the hemodynamic findings of Loh et al. in adults with ischemic or dilated cardiomyopathy and congestive heart failure (a disease process differing in etiology, physiology, pathophysiology and pathology from childhood congenital heart disease) as "*a guidepost to the artisan*" regarding the use of inhaled NO in children and neonates with pulmonary hypertension and left ventricular dysfunction, but not dependent on right-to-left shunting of blood. These inaccurate and erroneous interpretations of all three supporting publications cited by the Examiner lead the Examiner to draw incorrect conclusions regarding what is or is not taught or suggested by the prior art.

28. On June 28, 2011, I met with Dr. David L. Wessel, the chair of the INOT22 Steering Committee and the senior author of *Atz & Wessel (Seminars in Perinatology 1997, 21(5), pp 441-455*. During our discussion, I informed Dr. Wessel of the 12/820,866 and 12/820,980 patent applications, and the fact that in both pending patent applications, the Examiner was citing Atz & Wessel to allege that it would have been obvious to predict the adverse events and outcomes of the INOT22 study that lead to the inventions claimed in 12/820,866 and 12/820,980.

29. Dr. Wessel disagreed with the Examiner's allegation and found it ironic that his own publication would be cited to suggest the obviousness of the unexpected outcomes of the INOT22 study, when Dr. Wessel himself, the senior author of Atz & Wessel, failed to predict that neonatal and child patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at increased risk of adverse events when administered inhaled NO. A copy of a June 29, 2011 letter from Dr. Wessel to me stating this opinion is attached hereto as Exhibit 2.

30. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '359 patent.

Dated: 7/7/11

Douglas A. Greene, M.D.

EXHIBIT 1

(curriculum vitae)

CURRICULUM VITAE

PERSONAL DATA

Name:	Douglas Alan Greene, M.D.

EDUCATION

High School	Columbia High School, South Orange, NJ, 1962
Undergraduate	Princeton University, Princeton, NJ, BA Biology(cum laude), 1962-1966
Graduate/Professional	Johns Hopkins School of Medicine, Baltimore, MD, M.D., 1966-1970

POSTDOCTORAL TRAINING

Medical Internship:	Department of Medicine, Johns Hopkins, Baltimore, MD, 1970-1971
Medical Residency:	Department of Medicine, Johns Hopkins, Baltimore, MD, 1971-1972
Fellowship:	Medical Fellowship, Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, 1970-1972
	Post-doctoral Research Fellow, Diabetes, George S. Cox Medical Research Institute; Hospital of the University of Pennsylvania, Philadelphia, PA (Dr. Albert I. Winegrad, preceptor), 1972-1975
	Medical Fellowship, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA, 1972-1975

NON-ACADEMIC EMPLOYMENT

2000-2003 Executive Vice President, Clinical Sciences and Product Development (CSPD), Merck Research Laboratories, Rahway, New Jersey, and Corporate Officer, Merck, Inc. Supervised and directly managed all clinical research, regulatory affairs, clinical and non-clinical quality assurance and pharmaco-vigilance at Merck Research Laboratories.

2003-2006 Vice President, Head Corporate Regulatory Development, Sanofi-Aventis, Bridgewater, NJ. Overseeing all aspects of corporate regulatory development of all pre-clinical and clinical development projects/life-cycle products in Research & Development.

2006-2009 Senior Vice President, Chief Medical Officer, Sanofi-Aventis, Bridgewater, NJ. Overseeing medical, regulatory, pharmocovigilance, risk management, education and medical communications for US region, Member US Executive Committee, Member Committee Operational de Development, International Clinical Development.

2009-present Senior Vice President, Senior Scientific Advisor, Sanofi-Aventis, Bridgewater, New Jersey. Member Corporate Portfolio Valuation Process and Drug Development Committees. The position at the interface between the Research and Development and Pharmaceutical Operations is responsible for providing key scientific and medical guidance for sanofi-aventis' scientific strategy within U.S. and global contexts to enhance the quality and effectiveness of the company's research and product portfolio, including assessment and guidance of internal R&D product pipeline and franchise portfolio and external commercial and academic innovation opportunities.

ACADEMIC APPOINTMENTS

1975-1980	Assistant Professor of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania
1980-1986	Associate Professor of Medicine, Director, General Clinical Research Center and Diabetes Research Laboratories, University of Pittsburgh, School of Medicine
1986-2000	Professor of Internal Medicine, Director, Michigan Diabetes Research and Training Center, University of Michigan School of Medicine
1991-2000	Chief, Division of Endocrinology & Metabolism, University of Michigan School of Medicine
2000-Present	Adjunct Professor, Internal Medicine, Division of Endocrinology & Metabolism, University of Michigan, School of Medicine

SELECTED SCIENTIFIC ACTIVITIES

1988-1994	Chairman, Endocrinologic and Metabolic Drug Advisory Board, Food and Drug Administration, Washington D.C (Chair, 1990-1994)
1994-2000	Chairman, Merck Scientific Board of Advisors

SELECTED SCIENTIFIC PRIZES AND AWARDS

1986	First Annual Raymond A. and Robert L. Kroc Lecturer, Eisenhower Medical Center, Palm Springs, California
1987	Moore Award, The American Association of Neuropathologists, Seattle, Washington
1987	Carol Sinicki Manuscript Award (The Diabetes Educator), American Association of Diabetes Educators, Chicago, Illinois
1988	Kellion Lecture, International Diabetes Federation, Sydney, Australia
1989	Banting and Best Lecture, Toronto General Hospital, Toronto, Canada
1994	Charles H. Best Lecturer, Toronto Diabetes Association, Toronto, Canada
1996	Invited Speaker, Seventy-fifth Anniversary Celebrating the Discovery of Insulin, Toronto, Canada
1996	First Alan Robinson Lecturer, University of Pittsburgh
1998	Outstanding Foreign Investigator Award, Japan Society of Diabetic Complications

SELECTED BIBLIOGRAPHY

Peer-Reviewed Publications (Selected from over 170 peer-reviewed articles):

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2. Winegrad AI, Greene DA: Diabetic polyneuropathy: The importance of insulin deficiency, hyperglycemia and alterations in myoinositol metabolism in its pathogenesis. *N. Engl. J. Med.* 295:1416-1420, 1976.

3. Greene DA, Lattimer SA: Sodium- and energy dependent uptake of myo-inositol by rabbit peripheral nerve. Competitive inhibition by glucose and lack of an insulin effect. *J. Clin. Invest.* 70:1009-1018, 1982.

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EXHIBIT 2

(June 29, 2011, letter from Dr. David Wessel to Dr. Douglas Greene)

8191933.2

Children's National Medical Center

David L. Wessel, MD
Senior Vice President
Center for Hospital-Based Specialties
Ikaria® Distinguished Professor of
Critical Care Medicine

June 29, 2011

Douglas Greene, M.D.,
Executive Vice President and Head of Research & Development
Ikaria, Inc.
Perryville III Corporate Park
53 Frontage Road, 3rd Floor
PO Box 9001
Hampton, NJ 08827-9001

RE: **USSN 12/820,866 and 12/820,980**
Atz et al., Seminars in Perinatology 1997,21(5), pp 441-455

Dear Doug:

In 2005, I chaired the Steering Committee of the Sponsor, INO Therapeutics LLC (INOT), to establish, design and oversee the INOT22 Study. Presently, I am Chief, Division of Critical Care Medicine and Senior Vice President, Children's National Medical Center, Washington, D.C.[1]

In addition to being the Chair of the INOT22 Steering Committee, I also am the senior author of *Atz et al., Seminars in Perinatology 1997,21(5), pp 441-455* (Atz et al.).

At the time of the design of the INOT22 Study protocol, neither myself, the other Steering Committee members, nor the study Sponsor appreciated or anticipated that a child with left ventricular dysfunction who is not dependent on right-to-left shunting of blood would be at additional risk when treated with inhaled nitric oxide (INO). This is the reason such children were not originally excluded from the INOT22 Study entry criteria.

Neither the Atz et al. article that I co-authored, nor the medical literature or medical experience of which I was aware at the time, predict this risk. Instead, Atz et al describes two distinct, independent precautions with respect to the use of INO. First, with respect to adults, Atz et al. stated that INO may be more effective in newborns than in older patients, and noted that it

[1] In the interest of full disclosure, I formerly served as a consult for INO Therapeutics LLC. I currently serve without remuneration as a member of the Ikaria Scientific Board of Advisors. In 2010 I was appointed by my institution as the Ikaria Distinguished Professor of Critical Care Medicine.

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Children's National Medical Center

should be used with caution in adults with ischemic cardiomyopathy in whom a risk of pulmonary edema is a consideration (see page 452, left column). Second, with respect to neonates, we stated the well-known contraindication (currently found in the INOMAX® prescribing information) that INO should not be used in newborns dependent upon right-to-left shunting of blood across a patent ductus arterious to avoid circulatory collapse. What we did not disclose or predict was that neonatal patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at greater risk of adverse events.

It is ironic that my own publication would be cited to suggest that it would have been obvious to predict the adverse events and outcomes of the INOT22 Study when I, the senior author of Atz et al., failed to anticipate or predict these unexpected outcomes at the time I participated in drafting the original INOT22 Study protocol. If so, I would have been acting either negligently or intentionally to harm babies, and I most certainly was not. Furthermore, to my knowledge, none of the other members of the INOT22 Steering Committee who assisted me in designing the study, nor the approximately 18 Institutional Review Boards and 2 National Health Authorities who reviewed and approved the study prior to its initiation, predicted the adverse events in children with left ventricular dysfunction who are not dependent on right-to-left shunting of blood.

In summary, although it was known that neonates whose systemic circulation was dependent on right-to-left shunt should not receive INO, and it had been reported that adults with pre-existing left ventricular dysfunction (from coronary artery disease) may be at risk when provided INO, it was unanticipated and surprising that children with left ventricular dysfunction who are not dependent on right-to-left shunting would be at increased risk of adverse events when administered INO.

Sincerely,

David L. Wessel, M.D.

EXHIBIT C

Exhibit C

USSN: 12/820,866

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/820,866
Confirmation Number	2913
Filing Date	22-JUN-2010
Title of Application	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION
First Named Inventor	JAMES S. BALDASSARRE
Assignee	IKARIA, INC.
Group Art Unit	1616
Examiner	ARNOLD, ERNST V.
Attorney Docket Number	I001-0002USC1

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DAVID L. WESSEL, M.D.
UNDER 37 C.F.R. § 1.132

I, David L. Wessel, do hereby declare the following:

1. I currently hold the position of Senior Vice President, The Center for Hospital-based Specialties, at Children's National Medical Center in Washington, D.C., where I am also the Division Chief of Critical Care Medicine. I am also the Ikaria Distinguished Professor of Critical Care Medicine. A copy of my *curriculum vitae* is attached as **Exhibit 1.**

2. I received a bachelor's degree (B.S.) in physics from the College of William and Mary in 1972, a bachelor's degree (B.A.) in physiology from Oxford University in 1974, a doctoral degree (*cum laude*) in medicine (M.D.) from the Yale University School of Medicine in 1978, and a master's degree (M.A.) in physiology from Oxford University in 1983.

3. Following my graduation from Yale, the majority of my time as a practicing physician was spent in academic medicine, where I focused on pediatric cardiology. From 1978-1981, I performed an internship in pediatrics followed by a clinical fellowship at the Yale University School of Medicine. From 1981-1985, I was a fellow in pediatric anesthesiology at

Harvard Medical School, where I later became an instructor (1985), assistant professor (1987), associate professor (1994), and ultimately professor (2002), all in the area of pediatrics. In 2011, I will become a professor of pediatrics at the George Washington University School of Medicine and Health Sciences in Washington, DC.

4. In addition to my academic experience, I have extensive experience in the pharmaceutical industry as a member of scientific advisory boards, advisory panels or steering committees for companies such as Pfizer, Johnson & Johnson, Eli Lilly, Bristol-Myers Squibb, Sanofi-Avenits, and INO Therapeutics.[1]

5. In 2005, I chaired the Steering Committee of the Sponsor, INO Therapeutics LLC (INOT), to establish, design and oversee the INOT22 Study. In addition to being the Chair of the INOT22 Steering Committee, I also am the senior author of Atz and Wessel, *Seminars in Perinatology* 1997, 21(5), pp. 441-455 (Atz et al.).

6. At the time of the design of the INOT22 Study protocol, neither I, the other Steering Committee members, nor the study Sponsor appreciated or anticipated that a child with left ventricular dysfunction who is not dependent on right-to-left shunting of blood would be at additional risk when treated with inhaled nitric oxide (iNO). This is the reason such children were not originally excluded from the INOT22 Study entry criteria.

7. Neither the Atz et al. article that I co-authored, nor the medical literature or medical experience of which I was aware at the time, predict this risk. Instead, Atz et al. describes two distinct, independent precautions with respect to the use of iNO. First, with respect to adults, Atz et al. stated that iNO may be more effective in newborns than in older patients, and noted that it should be used with caution in adults with ischemic cardiomyopathy in whom a risk of pulmonary edema is a consideration (see page 452, left column). Second, with respect to neonates, we stated the well-known contraindication (currently found in the INOMAX®

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1 In the interest of full disclosure, I formerly served as a consultant for INO Therapeutics LLC. I currently serve without remuneration as a member of the Ikaria Scientific Board of Advisors. In 2010, I was appointed by my institution as the Ikaria Distinguished Professor of Critical Care Medicine.

prescribing information) that iNO should not be used in newborns dependent upon right-to-left shunting of blood across a patent ductus arteriosus to avoid circulatory collapse. What we did not disclose or predict was that neonatal patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at greater risk of adverse events.

8. It is ironic that my own publication would be cited to suggest that it would have been obvious to predict the adverse events and outcomes of the INOT22 Study when I, the senior author of Atz et al., failed to anticipate or predict these unexpected outcomes at the time I participated in drafting the original INOT22 Study protocol. If so, I would have been acting either negligently or intentionally to harm babies, and I most certainly was not. Furthermore, to my knowledge, none of the other members of the INOT22 Steering Committee who assisted me in designing the study, nor the approximately 18 Institutional Review Boards and 2 National Health Authorities who reviewed and approved the study prior to its initiation, predicted the adverse events in children with left ventricular dysfunction who are not dependent on right-to-left shunting of blood.

9. In summary, although it was known that neonates dependent on right-to-left shunt should not receive iNO and it had been reported that adults with pre-existing left ventricular dysfunction may be at risk when provided iNO, it was unanticipated and surprising that children with left ventricular dysfunction who are not dependent on right-to-left shunting would be at increased risk of adverse events when administered iNO.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '359 patent.

Dated: July 15 2011

David L. Wessel, M.D.

Applicant : James S. Baldassarre et al.
Serial No. : 12/820,866
Filed : June 22, 2010

Attorney Docket No. 26047-0003002

EXHIBIT 1

CURRICULUM VITAE

1) PERSONAL DATA

Date prepared:	April 2011
Name:	David Lloyd Wessel
Home address:	3251 Prospect St. NW, Suite 404 Washington, D.C. 20007
Home phone:	202-342-0908
Office Address:	Children's National Medical Center 111 Michigan Ave, NW Suite 3W-100 Washington, DC 20007 TEL: 202 476 5047 FAX: 202 476-5868
E-Mail Address:	dwessel@childrensnational.org
Place of Birth:	Newton, Iowa U.S.A.
Citizenship:	United States

2) EDUCATION:

1972	B.S.	College of William and Mary (Physics), Williamsburg, VA
1974	B.A.	Oxford University (Physiology), Oxford, England
1978	M.D.	Yale University School of Medicine (Medicine), New Haven, CT
1983	M.A.	Oxford University (Physiology), Oxford, England

POSTDOCTORAL TRAINING:

Internship and Residencies:

1978-79	Intern in Pediatrics, Yale-New Haven Hospital, New Haven, CT
1979-80	Resident in Pediatrics, Yale-New Haven Hospital, New Haven, CT
1981-83	Resident in Anesthesia, Massachusetts General Hospital, Boston, MA

Fellowships:

1980-81	Fellow in Pediatric Cardiology and Intensive Care, Yale-New Haven Hospital, New Haven, CT
1983-84	Fellow in Pediatric Cardiology, Children's Hospital, Boston, MA
1984-85	Fellow in Anesthesia and Intensive Care, Children's Hospital, Boston, MA

3) EMPLOYMENT

CHILDREN'S HOSPITAL, BOSTON

1985-87	Assistant in Anesthesia
1985-88	Assistant in Cardiology
1987-00	Associate in Anesthesia
1988-89	Associate in Cardiology
1988-07	Associate in Cardiovascular Surgery
1988-02	Chief, Cardiovascular Intensive Care Unit
1989-07	Senior Associate in Cardiology
1995-02	Division Chief
2000-07	Senior Associate in Anesthesia
2002-03	Honorary Consultant, Royal Brompton Hospital, London, U.K.

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON, DC

2007-	Interim Chief, Division of Critical Care Medicine
2007-09	Executive Director, Center for Hospital Based Specialties
2009-	Senior Vice President, Center for Hospital Based Specialties
2010-	IKARIA Distinguished Professor of Critical Care Medicine, Children's National Medical Center, Washington, DC

ACADEMIC APPOINTMENTS:

1980-81	Fellow in Pediatrics (Cardiology), Yale School of Medicine, New Haven, CT
1981-83	Clinical Fellow in Anaesthesia, Harvard Medical School, Boston, MA
1983-84	Clinical Fellow in Pediatrics, Harvard Medical School, Boston, MA
1984-85	Clinical Fellow in Anaesthesia, Harvard Medical School, Boston, MA
1985-86	Instructor in Anaesthesia, Harvard Medical School, Boston, MA
1987-93	Assistant Professor of Anaesthesia (Pediatrics), Harvard Medical School, Boston,
1987-94	Assistant Professor of Pediatrics (Anaesthesia), Harvard Medical School, Boston, MA
1994-99	Associate Professor of Pediatrics, Harvard Medical School, Boston, MA
1999-02	Associate Professor of Pediatrics (Anaesthesia), Harvard Medical School, Boston, MA
2002-03	Visiting Professor Imperial College, University of London, London UK (4/02-4/03)
2002-07	Professor of Pediatrics (Anaesthesia), Harvard Medical School, Boston, MA
2011-	Professor of Pediatrics, George Washington University School of Medicine and Health Sciences, Washington, DC (pending)

4) LICENSURE AND CERTIFICATION:

1979	National Board of Medical Examiners
1985-07	Massachusetts License Registration
1985	American Board of Pediatrics (Permanent)
1985	American Board of Pediatrics, Sub-board of Pediatric Cardiology (Permanent)
1986	American Board of Anesthesiology (Permanent)
1987	American Board of Pediatrics, Sub-board of Critical Care (Re-certified 1996, 2004, 2010)

5) PROFESSIONAL SOCIETIES & HONORS:

1982-	American Society of Anesthesiologists
1982-2007	Massachusetts Medical Society
1986-	American Academy of Pediatrics
1987-	Society of Critical Care Medicine
1987-	American Society of Critical Care Anesthesiologists
1987-	Society of Pediatric Anesthesia
1989-	American Heart Association (Fellow)
1991-	Society of Cardiovascular Anesthesiologists
1995-	Society of Pediatric Research
1999-	Pediatric Cardiac Intensive Care Society - President 2000-2004; Vice President, Development 2010-

AWARDS, HONORS AND NAMED LECTURES:

1968	Maytag Scholar (industry sponsored competitive college scholarship)
1971	Phi Beta Kappa
1971	Omicron Delta Kappa
1971	National Physics Honor Society (President)
1972	General Honors (William and Mary)
1972	Drapers' Scholar (Oxford)
1972	Mathematics Honor Society
1974	Balliol College Prize (Oxford)
1974	First Class Honours (Oxford)
1978	Cum Laude (Yale)
1978	Alpha Omega Alpha Honor Medical Society
1978	Harry S. Greene Prize (Yale)

1994 Katkov-Lundeen Memorial Lecture, Minneapolis Children's Hospital, Minneapolis, MN
1994 Saul Usher Memorial Lecture, Montreal Children's Hospital, Montreal, Canada
1994 Farouk Idriss Memorial Lecture, Children's Memorial Hospital, Chicago, IL
1995 A. W. Conn Lecture, Hospital for Sick Children, Toronto, Canada
1995 DiCerbo Foundation Lectureship in Pediatric Critical Care, North Shore University Hospital, New York, NY
1996 Teaching Award, Pediatric Cardiology, Children's Hospital Boston
1997- Listed, *Best Doctors in America*, continuously since inception
1999 29th Annual Jennifer B. Lalin Lecture, Babies Hospital, Columbia University College of Physicians and Surgeons, New York, NY
2000 Tenth Anniversary Lecture, Taiwan Pediatric Association, Critical Care Sub Committee, Kaohsiung Veterans General Hospital, Taipei, Taiwan
2001 Recipient, Papas Gift Award for Outstanding Clinical Care ($25,000 to Children's Hospital Boston)
2002 M.A. (Honorary) Harvard University, Cambridge, MA
2004 Keynote Address, Opening Ceremony, Annual Meeting of the European Society of Pediatric and Neonatal Intensive Care, London, United Kingdom
2004 Leadership & Mentor Award: "In recognition of your contributions toward improving children's heart health," *The Fifth International Symposium on Pediatric Cardiac Intensive Care*co-sponsored by the Pediatric Cardiac Intensive Care Society and the Texas Children's Heart Center
2005 Jared Ellsworth Memorial Lecture, Rainbow Babies and Children's Hospital, Cleveland, Ohio
2006 Eddie Farrell Memorial Lecture, Massachusetts Society of Respiratory Care
2007 Robert A. Boxer, M.D. Memorial Lecture,Schneider Children's Hospital LIJ, North Shore
2010 John J. Downes, Jr.,M.D. Lecture, Cardiology 2010, Orlando, FL. Sponsored by Children's Hospital Philadelphia.
2010 Outstanding Research Award in Pediatric Cardiology(Council on Cardiovascular Disease in the Young), AHA Scientific Sessions, Chicago, IL
2010 Anthony Chang Honorary (Inaugural) Lecture. Pediatric Cardiac Intensive Care Society.

6) ADMINISTRATIVE DUTIES & UNIVERSITY ACTIVITIES

HOSPITAL AND HEALTH CARE ORGANIZATION SERVICE RESPONSIBILITIES:

CHILDREN'S HOSPITAL, BOSTON
1985-91 Attending Physician and Associate Director, Multidisciplinary Intensive Care Unit
1985-07 Attending Physician in Cardiology (Intensive Care)
1985-07 Attending Physician in Anesthesia (Cardiac)
1985-07 Associate in Cardiovascular Surgery (teaching)

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON, DC
2007- Attending Physician in Critical Care Medicine, Cardiology, Cardiac Anesthesia
2007- Member, Children's National Heart Institute

MAJOR ADMINISTRATIVE RESPONSIBILITIES:

CHILDREN'S HOSPITAL, BOSTON
1988-02 Director, Cardiac Intensive Care Unit
1990 Associate Director, Critical Care Pediatrics Training Program
1993-02 Treasurer, Board of Directors, Boston Children's Heart Foundation including investigative and forensic accounting responsibilities surrounding departed chairman (1993-96)
1997-98 Board of Directors, Children's Hospital Physicians' Organization, Boston, MA
1998-03 Physician Leadership Council, Children's Hospital, Boston, MA
1999-02 Medical Director, Pharmacy, Children's Hospital, Boston, MA
2000-02 Clinical Sponsor, Critical Care Clinical Information System, Children's Hospital, Boston, MA
2003-04 Interdisciplinary Peer Review Assignments and Presentation of Critical Events to JCAHO
2004-05 Board of Directors, Boston Children's Heart Foundation
2004-05 Physician Leadership Council, Children's Hospital, Boston, MA

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON DC

2007-	Accountable executive for clinical Center of Excellence; $200M revenue, more than 700 full time employees. Includes divisions and departments of critical care medicine (both cardiac and pediatric ICI neonatology; hospitalist medicine (inpatient general pediatrics); emergency medicine; radiology; respira care services (respiratory therapy); infectious disease, hospital infection control and epidemiology; endocrinology and the diabetes care complex; transport medicine, fetal and transitional medicine, ECM
2007-	Leadership Council
2007-	Children's Hospital Foundation Board of Directors
2007-	Critical Care Committee (Co-Chair)
2007-	Executive Committee of the Medical Staff
2007-	Executive Directors Council (Senior Vice President Council 2008-)
2007-	Hospital Based Specialties (HBS) Leadership Committee (Chair)
2007-	HBS Campaign Council (Chair)
2007-	Strategic Planning Council
2007-	Interim Chief, Division of Critical Care Medicine
2008-	Healthcare Review Committee (risk management financial governance)
2009-	Steering Committee Strategic Planning Council (2010-15)

MAJOR COMMITTEE ASSIGNMENTS:

HARVARD MEDICAL SCHOOL

1996-98	Futility of Care Task Force, Harvard Medical School
1999	Search Committee, Chief of Pediatric Pulmonary Medicine, Children's Hospital, Boston, MA
2005-07	Ad Hoc Evaluation Committee for Professorial Promotion

CHILDREN'S HOSPITAL, BOSTON

1988-93	Multidisciplinary Intensive Care Committee
1989-90	Chairman, Hospital Task Force on Sedation
1990-92	Hospital HMO Committee
1991-92	Medical Staff Quality Improvement Committee
1991-93	Department Quality Improvement Officer
1991	Hospital Review Committee for Department of Clinical Laboratories
1992	Chairman, Nominating Committee, Medical Staff Association
1992-99	Chairman, Special Care Units Committee
1992	Hospital Search Committee for Director of Clinical Laboratories
1992	Physician Advisory Committee on Computers
1992	Operations Improvement Committee
1993	Hospital Search Committee for MICU Director
1993-01	Cardiovascular Program, Quality Improvement Committee
1996-98	Product Standardization Council
1998-01	Planning and execution committee for ICU electronic clinical information system
1998-01	Clinical Oversight Committee for Transport
2000	Nominating Committee, Physician's Organization
2000-02	Chairman, Pharmacy and Therapeutics Committee
2000-02	Hospital Task Force on Clinical Building and New Construction
2000-06	ICU Committee
2004-05	Committee on Pension Investments, Physicians' Organization
2004-07	Quality and Outcomes Measurement, Physicians' Organization
2005-07	Program for Patient Safety and Quality Implementation Committee
2006	Hospital Search Committee for Non-invasive Cardiology Division Chief
2006-07	Hospital Peer Review Panel
2006-07	Physician Profile Task Force

DEPARTMENT OF CARDIOLOGY, CHILDREN'S HOSPITAL, BOSTON

1988-01	Fellowship Selection Committee
1998-02	Audit and Finance Committee
1998-02	Computing Committee
2004-05	Audit and Finance Committee

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON DC

2007-09	Facilities Leadership Committee
2007-	Growth Management/ CARE
2007-	NICU Steering Committee
2007-	Quality and Clinical Effectiveness Committee
2007-	Quality and Safety Council
2007-	Information Technology Oversight Committee
2007-09	CTI Clinical Advisory Council (electronic medical record)
2007-	Task Force on Access/Referral
2007-08	Hospital Search Committee for Cardiology Division Chief
2007-	Safety Transformation Advisory Council
2009-	Executive Oversight Committee (post graduate education)
2009-	Physicians Advisory Committee (third party payor contracts)
2011-	Physician Productivity Committee (Chair)
2011-	Internal Advisory Board, GWU / CNMC, for NIH funded CTSI Award (Chair)

NATIONAL & INTERNATIONAL

1995	Clinical Trials Review Committee (*Ad hoc* reviewer), National Institutes of Health
1995-98	Invited Speaker, Cardio-renal Advisory Panel, U.S. Food and Drug Administration
2004-06	Task Force ACC AHA AAP: Requirements for Pediatric Cardiac Critical Care Training
2005-06	Multi-Societal Committee (PCICS/EACTS/STS) Complications in Pediatric and Congenital Cardiac Surgery Project
2008-	National Institute of Allergy and Infectious Disease Transplant Data and Safety Monitoring Board (DSMB) - Member
2010	FDA Invited Speaker, Continuing Education Series
2010	International Liaison Committee on Resuscitation (ILCOR): 2010 Consensus Statement and Treatment Recommendations.
2011	Joint American Heart Association (AHA) – American Thoracic Society Expert Guidelines Statement on Pediatric Pulmonary Hypertension.

INDUSTRY

1994-97	Scientific Advisory Board on Nitric Oxide, Ohmeda Pharmaceuticals
1998-02	Curriculum Development Committee, INO Therapeutics
1999-01	Steering Committee, Prophylactic use of Primacor® in pediatric patients at high risk of developing low cardiac output syndrome following cardiac surgery. PRIMACORP study-Prophylactic intravenous use of milrinone after cardiac operation in pediatrics. Sanofi-Synthelabo Inc.
2001-06	Chairman, Advisory Panel INOTherapeutics
2001-02	Scientific Advisory Board AGA-Linde
2001-03	Protocol Planning Committee (PDE V inhibitors) Pfizer
2001-09	Scientific Advisory Board for pulmonary hypertension research development, Pfizer
2003	Steering Committee for Multicenter Trial on Diagnostic Use of Inhaled Nitric Oxide
2005-07	Steering Committee for Multicenter Trial on Use of Nesiritide in Children, SCIOS (Johnson & Johnson)
2005-07	Advisory Committee on Iloprost and Treatment of Pulmonary Hypertension in Children, Cotherix
2005-07	Advisory Board, Eli Lily Vardenafil for Pediatric Pulmonary Hypertension
2006-	Steering Committee (Chairman) for Multicenter Trial on Use of Clopidogrel in Children (CLARINET), Bristol-Myers Squibb & Sanofi-Aventis
2009	Advisory Panel, Nesiritide Use in Pediatric Cardiovascular patients, Johnson & Johnson

COMMUNITY SERVICE RELATED TO PROFESSIONAL WORK:

1994-97 Lecturer, Human Body Curriculum, Wellesley Public School System, Wellesley, MA
1996 Hospital Spokesman, Boston/Filenes' Holiday Festival
1996 Campaign for William & Mary, 25th Anniversary Committee
2000-02 Hospital Spokesman, Capital Campaign and Children's Hospital Boston Fundraising, including keynot speaker, 2000
2007- Multiple CNMC Fundraising and Community Benefit Events presentations to Emeritus and Lady Visitc Boards, etc.
2008 Speaker, CNMC Corporate Leadership Council "What's Up, Doc?" Breakfast, World Bank, Washingtor D.C.

EDITORIAL BOARDS/REVIEW COMMITTEES:
Ad Hoc Reviewer:

Acta Pediatrica
American Journal of Cardiology, American Journal of Physiology
American Review of Respiratory Diseases and Critical Care
Anesthesia & Analgesia, Anesthesiology
Annals of Thoracic Surgery
Archives of Diseases of Childhood
Cardiovascular and Interventional Radiology
Chest
Circulation
Critical Care Medicine
European Heart Journal
Future Cardiology
Journal of Intensive Care Medicine
Journal of Pediatrics
Journal of Thoracic and Cardiovascular Surgery
Mayo Clinic Proceedings
Pediatrics, Pediatric Cardiology, Pediatric Critical Care Medicine, Pediatric Research
Proceedings of the National Academy of Science

Invited consultant, to review and make recommendations to institutional programs for pediatric cardiovascular care (national and international)

Asked by Children's Hospital Boston to chair ad hoc committees reviewing sentinel events, other critical incidents and report the hospital's analysis and action to the Hospital's Board of Trustees, JCAHO, etc.

7) EDUCATIONAL ACHIEVEMENTS

REPORT OF TEACHING

1. LOCAL CONTRIBUTIONS
   a) **MEDICAL SCHOOL**

Yale University School of Medicine, New Haven, CT

1975-76 Program leader, Cardiovascular physiology core lectures in Physician's Associate Program
Designed lecture series for new PA program; 20 hours/year

Harvard Medical School, Boston, MA

1983-98 Instructor, Cardiovascular Physiology Animal Laboratory, Harvard Medical School
Approximately 60 medical students; one day per year

1985-89 Cardiovascular Pathophysiology, Laboratory section on congenital heart disease
Approximately 30 medical students; half day per year

1985-89 PGY clerkship in Pediatrics

Lecturer in Critical Care (Multidisciplinary ICU)
2 medical students each lecture; 12 hours/year

b) GRADUATE MEDICAL EDUCATION (LOCAL)

1985-89 Didactic seminars on cardiovascular pathophysiology for pediatric critical care fellows and rotating residents
Lecture once per week, 1 hour, 6 trainees per lecture

1986-93 Developed and taught core curriculum: introduction to anesthesia and critical care for cardiologists
Lecture once per week, 1 hour, six weeks, 20 fellows and junior faculty. Preparation, 40 hours per year

1985-89 Co-developed tutorials on congenital heart disease and supervised core staff (3 tutors) for instruction of cardiology and cardiac ICU fellows during ICU rotation
Lectures three mornings per week, 1/2 hour, 3-4 fellows; preparation, 2 hours per week

1985-07 CICU attending rounds
3 pediatric residents (1985-1989), 4-8 fellows and senior surgical residents; 18 hours/week, 16-40 weeks/year (varies with year)

1990-96 Chiefs' Ward Rounds
3 medical students, 3 pediatric residents, 1 cardiology fellow; monthly 12 hous/year

1996-07 Didactic lectures to cardiology fellows teaching program
20 fellows 3 times per year

2002-03 Didactic lectures (monthly) to trainees at Royal Brompton Hospital, London

c) BOSTON INVITED TEACHING PRESENTATIONS (SELECTED)

1984 Anesthesia Grand Rounds, Children's Hospital, Boston, MA
1991 Anesthesia Grand Rounds, Children's Hospital, Boston, MA
1992 Surgical Grand Rounds, Children's Hospital, Boston, MA
1992 Medical Grand Rounds, Children's Hospital, Boston, MA
1994 Anesthesia Grand Rounds, Children's Hospital, Boston, MA
1996 Anesthesia Grand Rounds, Massachusetts General Hospital, Boston, MA
1996 PICU Teaching Sessions, Massachusetts General Hospital, Boston, MA
1997 Surgical Grand Rounds, Children's Hospital, Boston, MA
1997 Medical Grand Rounds, Children's Hospital, Boston, MA
1998 Anesthesia Grand Rounds, Children's Hospital, Boston, MA
2003 Grand Rounds and teaching rounds, Royal Brompton Hospital, London, UK
2004 Neonatology Clinical Working Group, Children's Hospital, Boston, MA
2004 Department of Respiratory Therapy Clinical Working Group, Children's Hospital, Boston, MA
2005 Department of Cardiology, Didactic Series, Children's Hospital Boston, Boston, MA

d) WASHINGTON DC AREA INVITED TEACHING PRESENTATIONS (SELECTED)

- Chief Rounds Monthly to ICU & Cardiology fellows and staff (15-20 physicians, 2hrs/month), CNMC, D(
- ICU Attending Rounds, Children's National Medical Center, DC
- Clinical Research Presentation to ICU/Cardiology Fellows 2 times per year, Children's National Medica Center, DC
- Grand Rounds, Children's National Medical Center, DC
- Grand Rounds, Mary Washington Hospital, VA
- Grand Rounds, Anne Arundel Medical Center, MD
- Teaching Rounds, Division of Critical Care Medicine, National Institutes of Health

e) CONTINUING MEDICAL EDUCATION (LOCAL)

1988 Lecturer
Harvard Medical School, Continuing Education Course in Pediatric Anesthesia
"Anesthesia for Congenital Heart Disease"

1990 Lecturer
Harvard Medical School Continuing Education Course in Pediatric Anesthesia
"Common Congenital Cardiac Lesions"

1989 Moderator
Harvard Medical School, Continuing Education Course in Pediatric Cardiovascular Disease

1993 Lecturer
Symposium on Brain Injury and Cardiac Surgery, Harvard Medical School, Boston, MA
"Choreoathetosis After Cardiopulmonary Bypass"

1996 Lecturer
Harvard Medical School Continuing Education Course in Pediatric Anesthesia
"New Vasoactive Drugs"

1998 Co-director, First Annual Course: Frontiers in the Diagnosis and Management of Congenital Heart Disease, Children's Hospital, Boston, Harvard Medical School, Boston, MA

1999 Co-director, Second Annual Course: Frontiers in the Diagnosis and Management of Congenital Heart Disease, Children's Hospital, Boston, Newport, Rhode Island

2001 Co-director, Third Annual Course: Frontiers in the Diagnosis and Management of Congenital Heart Disease, Children's Hospital, Boston, Newport, Rhode Island

f) ADVISORY AND SUPERVISORY RESPONSIBILITIES (LOCAL)

1987- Responsible for clinical supervision and educational component of crifical care for cardiology fellows in a large pediatric cardiology training program (two months each year for each of 18 fellows spread over 2-3 years of training).

1990-02 Responsible as mentor for clinical, educational and clinical research activities of2-3 senior clinical fellows each year.

1985- Shared responsibilities for cardiovascular education and clinical supervision of pediatric critical care fellows in the CICU (3-5 months per year for 5-6 fellows spread over 2-3 years of training).

1985-02 Shared responsibilities for critical care educational component of pediatric cardiovascular surgical training program (10 surgical residents each year rotating for 6 months each).

1987-02 Responsible for medical education and clinical advisory tasks for conthuing education seminars for 80 critical care nurses.

g) LEADERSHIP ROLE (LOCAL)

1998-01 Program Co-Director
Annual Course, "Frontiers in Diagnosis and Management of Congenital Heart Disease" Shared responsibility for organizing and executing post graduate course attended by 200 pediatric cardiologists cardiovascular surgeons and nurses from the US and abroad.

h) **NAMES OF SELECTED TRAINEES AND/OR FORMER CICU STAFF WHO HAVE CURRENT LEADERSHIP POSITIONS**

1985-88 Gil Wernovsky, MD, FACC *†§
Director of Program Development
Former Director, Cardiac Intensive Care Unit
The Children's Hospital of Philadelphia
Professor of Pediatrics
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

1988-89 Ling Chen, MD *
Director, Cardiac Intensive Care Unit
Shanghai Children's Medical Center
Shanghai, China

1989-92 Pierre C. Wong, MD *†
Cardiology Medical Director, Transplantation
Children's Hospital of Los Angeles
Los Angeles, California

1989-92 Stephen J. Roth, MD, MPH *†
Director, Cardiac Intensive Care Unit
Lucile Packard Children's Hospital
Associate Professor of Pediatrics
Stanford University School of Medicine
Palo Alto, California

1989-92 Nancy Bridges, MD
Chief, Transplantation Immunology Branch, Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Disease
Bethesda, Maryland

1990-92 Howard A. Zucker, MD, FACC*
Deputy Director of the World Health Organization
Geneva, Switzerland

1990-93 Kevin B. Churchwell, MD
Chief Executive Officer (CEO) for Nemours/Alfred I. duPont Hospital for Children
Wilmington, DE.

1990-93 Anthony C. Chang, MD *†§
Medical Director, CHOC Children's Heart Institute
Children's Hospital Orange County
Orange, California

1991-94 Ian Adatia, MB, ChB, MRCP (UK), FRCP (C) *†
Director, Pediatric Cardiac Critical and Intermediate Care Program,
Director, Pediatric Pulmonary Hypertension Clinic,
Stollery Children's Hospital,
Professor of Pediatrics
University of Alberta
Edmonton, Alberta, Canada

1992-96 Andrew M. Atz, MD *†§
Director, Pediatric Cardiac Intensive Care Unit
The Children's Heart Program
Associate Professor of Pediatrics
Medical University of South Carolina
Charleston, South Carolina

1992-96 David P. Nelson, MD, PhD *
Director, Cardiac Intensive Care
Cincinnati Children's Hospital Medical Center
Professor of Pediatrics
Cincinnati, Ohio

1992-97 Sarah Tabbutt, MD, PhD *
Director, Pediatric Cardiac Intensive Care Unit
UCSF Children's Hospital
San Francisco, California

1994-97 Ricardo A. Muñoz, MD *†§
Director, Pediatric Cardiac Intensive Care
Director, Global Business and Telemedicine
Children's Hospital Pittsburgh
Associate Professor of Pediatrics
University of Pittsburgh
Pittsburgh, Pennsylvania

1994-99 Melvin C. Almodovar, MD *†§
Medical Director, Cardiac Intensive Care Unit
Boston Children's Hospital
Assistant Professor
Harvard Medical School
Boston, Massachusetts

1995-96 Brendan O'Hare, MD *
Consultant in Anesthesia and Critical Care
Our Lady's Hospital for Sick Children
Crumlin, Dublin, Ireland

1995-96 Steven Schwartz, MD
Director of Cardiac Intensive Care
Hospital for Sick Children
Assistant Professor of Pediatrics
University of Toronto
Toronto, Ontario, Canada

1996-97 Alain Fraisse, MD *†
Chief, Clinical Pediatric Cardiology
Hopital D'Enfants de la Timone
Professer of Pediatrics
Universitaire de Marseille
Marseille, France

1997-98 Guillermo Palacio, MD
Director Pediatric Cardiac Intensive Care Unit
Fundacion Cardio Infantil
Bogota, Colombia

1997-98 Mary B. Taylor, MD *
Director, Pediatric Cardiac Critical Care
Cardiology and Critical Care
Vanderbilt Children's Hospital
Associate Professor of Pediatrics
Vanderbilt University Medical Center
Nashville, Tennessee

1997-99 Rajiv Chaturvedi, MB BChir, MRCP (UK), MD *
Pediatric Cardiology
Hospital for Sick Children
Assistant Professor
University of Toronto
Toronto, Ontario, Canada

1998-01 Ravi Thiagarajan, M.D.* † §
Director, Cardiac ECMO Program
Children's Hospital Boston
Associate Professor of Pediatrics
Harvard Medical School
Boston, Massachusetts

1998-02 Peter C. Laussen, MBBS §
Chief, Division of Cardiovascular Intensive Care
D.D. Hansen Chair in Pediatric Anesthesia
Senior Associate in Cardiology
Children's Hospital Boston
Professor of Anesthesia
Harvard Medical School
Boston, Massachusetts

1998-99 Mary P. Mullen, MD, PhD *§
Director, Pulmonary Hypertension Program
Assistant in Cardiology
Children's Hospital Boston
Assistant Professor in Pediatrics
Harvard Medical School
Boston, Massachusetts

1999 Janet M. Simsic, M.D.*
Director, Pediatric Cardiac Intensive Care Unit
Nationwide Children's Hospital
Columbus, Ohio

2000 Erica A. Kirsch, MD*
Director of Pediatric ECMO Program
Associate Professor of Pediatrics
University of Missouri-Kansas City School of Medicine
Kansas City, Missouri

2003-05 Margarita Burmester, MBBS* †
Consultant in Pediatric Intensive Care
Royal Brompton Hospital
Imperial College
London, United Kingdom

* Clinical Trainees
† Research Trainees
§ Faculty in CICU, Children's Hospital Boston during my tenure as Chief

TEACHING AND EDUCATIONAL LEADERSHIP ROLES (LOCAL AND INTERNATIONAL)

1987 Critical Care Consultant for Project Hope and the Cardiac htensive Care Unit, Xin Hua, Shanghai, China. Developed teaching program for critical care and supervised clinical training of physicians during 2-6month exchange programs.

1996- Abstract and Program Reviewer for many National and International Societies including SPR, AHA, ACC, PCICS, World Congress

2000 Invited faculty and cardiovascular program curriculum track convener III International Congress of Pediatric Intensive Care, Montreal, Canada.

2002 Scientific Programme, Coordinator
The Third Special Topics in Paediatric Cardiac Intensive Care, The Failing Myocardium
Royal Brompton Hospital, Imperial College, London, United Kingdom

2003 Invited faculty and cardiovascular program curriculum track convener IV International Congress of Pediatric Intensive Care, Boston, MA

2004 Discussant Leader and Co-author (after Tom Kulik) on Critical Care Training Guidelines in Cardiology (SCCM, PCICS, AHA, ACC)

2005 Scientific Program Committee Pediatric Cardiac Intensive Care Symposium 2005 (PCICS 2005), Miami, FL

2006 Planning Committee, First International Conference on Childhood Pulmonary Vascular Disease, San Francisco, CA 2007

2008 Critical Care Consultant, University of Mississippi Medical Center, Jackson, MS

TEACHING AWARD(S) RECEIVED

1996 Faculty Teaching Award, Dept. Cardiology, Children's Hospital, Harvard Medical School
2010 Top rated faculty teacher for division of critical care medicine in trainee survey

MAJOR CURRICULUM OFFERING, TEACHING CASES OR INNOVATIVE EDUCATIONAL PROGRAMS DEVELOPED

1990-02 Developed a senior clinical fellowship training program for cardiac intensive care with short term training experience available through formal training program relationships with the MICU, Children's Hospital; PICU, Massachusetts General Hospital; Neonatology, Children's Hospital; Neonatology, University of Vermont. Long term (6-36 month) training program applicants accepted (2-3 per year) from candidates in advanced levels of fellowship training from national and international programs.

1988-90 In collaboration with the Cardiovascular Nursing Director, developed, reviewed and edited algorithms for care, nursing practice and clinical practice guidelines and quality improvement manuals for the Cardiovascular Program, Children's Hospital, Bosbn.

2004 In collaboration with Pediatric Cardiac Intensive Care Society and the Training Program Directors for Pediatric Cardiology, coauthored (with T. Kulik and others) the report to the Joint Committee on Training Programs (AHA/ACC) on training requirements in critical care for pediatric cardiology trainees.

2008 As interim division chief of critical care medicine at Children's National Medical Center, I implemented and supervised a reorganization of the fellowship training program, its leadership and aspects of its curriculum

8) CONSULTANT APPOINTMENTS

VISITING PROFESSORSHIP:

1986 Visiting Professor
"Critical Care of the Child with Congenital Heart Disease"
Department of Cardiology, Children's National Medical Center, Washington, D.C.

1993 Visiting Professor,
"Perioperative Care of the Neonate with Congenital Heart Disease"
University of Southern California, Children's Hospital of Los Angeles

1993 Visiting Professor
"Nitric Oxide and ECMO Therapies for Persistent Pulmonary Hypetension of the Newborn"
Schneider Children's Hospital, Albert Einstein College of Medicine, New York, NY

1994 Visiting Professor
"Perioperative Care of the Critically Ill Neonate with Congenital Heart Disease; Perioperative Management of Low Cardiac Output"
Medical University of South Carolina, Charleston, SC

1994 Visiting Professor
"Inhaled Nitric Oxide in the Treatment of Children with Congenital Heart Disease"
Dennison Young Memorial Symposium, Montefiore Medical Center, New York, NY

1994 Visiting Professor
"Care of the Critically Ill Neonate"
Minneapolis Children's Hospital, Minneapolis, MN

1994 Visiting Professor
"Therapeutic Applications of Inhaled Nitric Oxide"
Children's Memorial Hospital, Chicago, IL

1994 Visiting Professor
Grand Rounds: "Treatment of Pulmonary Hypertension"
Montreal Children's Hospital, Montreal, Canada

1995 Visiting Professor
"Multidisciplinary Management of Complex Congenital Heart Disease"
Anesthesia and Critical Care Grand Rounds, Hospital for Sick Children
University of Toronto, Toronto, Canada

1995 Visiting Professor
"Controversy in Critical Care: New Views of Simple Gases (O_2, CO_2, H_2 and NO)"
Anesthesia Grand Rounds, Children's Hospital of Philadelphia, Philadelphia, PA

1995 Visiting Professor
"Nitric Oxide: Magic and Medicine"
Medical College of Georgia, Augusta, GA

1995 Visiting Professor
"Controversy in Critical Care: New Views of Simple Gases"
Children's Hospital of Pittsburgh, Dept of Surgery, University of Pittsburgh,Pittsburgh, PA

1995 Visiting Professor
"Perioperative Care of the Newborn with Congenital Heart Disease"
Division of Pediatric Cardiology, Yale University School of Medicine, New Haven, CT

1997 Visiting Professor
"Perioperative Care in the Child with Congenital Heart Disease"
Pediatric Grand Rounds, Vanderbilt Children's Hospital, Nashville, TN

2000 Visiting Professor
"Newborns with Heart Disease: Extending the Limits of Intervention"
Columbia-Presbyterian Medical Center, Babies Hospital, New York, NY

2003 Visiting Professor
"Treatment of Low Cardiac Output"
Cardiovascular Rounds, Hospital for Sick Children, Great Ormand Street,
London, United Kingdom

2005 Visiting Professor
Multiple lectures, University of Pittsburgh, Department of Critical Care Medicine, University of Pittsburgh Medical
Center and the Children's Hospital of Pittsburgh

2005 Visiting Professor
" Progress and problems in the treatment of critical heart disease"
Ellsworth Memorial Lecture, Pediatric Grand Rounds, Rainbow Babies & Children's Hospital,
Cleveland, OH

2006 Visiting Professor
"Navigating a career in Medicine". Health Careers Club, College of William & Mary,
Williamsburg, VA

2009 Visiting Professor
"The Challenges of Postoperative Care of the Child with CHD"
Pediatric Grand Rounds, Vanderbilt Children's Hospital, Nashville, TN

9) PRESENTATIONS

NATIONAL

1990 Seminar Moderator
"Cardiovascular Disease"
Fourth Pediatric Critical Care Colloquium, Waterville, NH

1991 Invited Lecture
"Perioperative Management of Congenital Heart Disease"
Annual Meeting, Society of Pediatric Anesthesia, San Francisco, CA

1992 Workshop Faculty
"Anesthesia for Congenital Heart Disease"
Annual Meeting of the Society of Cardiovascular Anesthesiologists, Bosbn, MA

1992 Invited Lectures
"Perioperative Management & Decision making in the Neonate with Congenital Heart Disease"
Critical Care Pediatrics Symposium, Arnold Palmer Hospital, Orlando, FL

1992 Invited Lectures
Multiple topics on Critical Care of Children with Heart Disease and
"Treatment of Pulmonary Hypertension with Inhaled Nitric Oxide"
First World Congress of Pediatric Critical Care, Baltimore, MD

1992 Anesthesia Grand Rounds
"Postoperative Care of the Child with CongenitalHeart Disease"
Maine Medical Center, Portland, ME

1992 Invited Faculty
"Postoperative Management of the Open Heart Surgery Patient"
Society of Critical Care Medicine, Pediatric Critical Care Clinical Review Series,
San Antonio, TX

1993 NIH Invited Lecture
"Nitric Oxide in Congenital Heart Disease"
National Institutes of Health Workshop: The effects of Nitric Oxide on the Lung, Bethesda, MD

1993 NIH Invited Lecture
"Indications for NO in the Newborn with Heart Disease"
National Institutes of Health Workshop on Nitric Oxide and the Perinatal Period, Bethesda, MD

1993 Symposium
"Nitric Oxide Gas in the Evaluation and Management of Pulmonary Hypertension"
Annual Meeting of the American College of Cardiology, Anaheim, CA

1993 Invited Lecture
"New Strategies for Treating Pulmonary Hypertension"
Annual Meeting, American Academy of Pediatrics, Washington, DC

1993 Invited Lecture
"Use of Inhaled Nitric Oxide for the Acute Treatment of Pulmonary Hypertension in Patients with Congenital Heart Disease" Annual Meeting, American Heart Association, Atlanta, GA

1993 NIH Workshop Lecture
"Nitric Oxide in the Perinatal Period" National Institutes of Health, Bethesda, MD

1993 Invited Lecture
"Inhaled Nitric Oxide for the Treatment of Persistent Pulmonary Hypertension of the Newborn"
Fourth Annual New England ECMO Symposium, Children's Hospital, Boston, MA

1993 Symposium
"Vasodilator Therapy and Inhaled Nitric Oxide in Children" Infant Hearts and Lungs Transplantation and Alternative Strategies.
Children's Hospital of Los Angeles, Long Beach, CA

1994 Symposium
"Update on Nitric Oxide"
Annual Meeting, Society of Critical Care Medicine, Orlando, FL

1994 Symposium
"Nitric Oxide Gas in the Evaluation and Management of Pulmonary Hypertension"
Annual Meeting of the American College of Cardiology, Atlanta, GA

1994 Invited Lecture
"Nitric Oxide for Pulmonary Hypertension"
Post Graduate Course on Congenital Heart Disease
American Association of Thoracic Surgery, New York, NY

1994 Plenary Session
"Inhaled Nitric Oxide for the Treatment of Pulmonary Hypertension in Children"
International Conference on Biochemistry and Molecular Biology of Nitric Oxide, University of California, Los Angeles, CA

1994 Guest Faculty
"Nitric Oxide in the Treatment of Pulmonary Hypertension in Congenital Heart Disease"
Pediatric Cardiology-The Failing Heart Conference, Given Biomedical Institute, University of Colorado, Aspen, CO

1994 Invited Lecture
"Perioperative Use of Inhaled Nitric Oxide"
Annual Meeting of the American Academy of Pediatrics, Dallas, TX

1994 Invited Faculty
"Serious Heart Disease of the Neonate: Management"
American Academy of Pediatrics Neoprep Course, St. Louis, MO

1994 Invited Faculty
"Perioperative Care of the Critically Ill Child with Congenital Heart Disease"
Society of Critical Care Medicine, Pediatric Critical Care Clinical Review Series, San Francisco, CA

1995 Invited Lecture
"Pulmonary Hypertension and Nitric Oxide"
Annual Meeting, American College of Cardiology, New Orleans, LA

1995 Invited Lecture
"Current Therapeutic Applications of Inhaled Nitric Oxide"
International Business Communications, Nitric Oxide Conference, Philadelphia, PA

1995 Invited Lecture
"Choreoathetosis After Cardiopulmonary Bypass"
Annual Meeting, American Society of Extra-Corporeal Technology, Boston, MA

1995 Invited Lecture
"Nitric Oxide for Perioperative Management of Congenital Heart Disease"
Annual Meeting of the American College of Surgeons, New Orleans, LA

1995 Invited Lecture
Controversy in Critical Care: New Views of Simple Gases
DiCerbo Foundation Lectureship in Pediatric Critical Care, North Shore University Hospital, New York, NY

1995 Dinner Speaker
"Diagnostic and Therapeutic Applications of Inhaled Nitric Oxide"
Annual Dinner Meeting, New York Society of Pediatric Critical Care Medicine, New York, NY

1995 Pediatric Grand Rounds
"Controversy in Critical Care: New Views of Simple Gases"
Cornell University Medical Center, New York, NY

1995 FDA Invited Lecture
"Inhaled Nitric Oxide for the Treatment of Persistent Pulmonary Hypertension of the Newborn"
Open Meeting, Cardiovascular and Renal Drugs Advisory Committee, United States Food & Drug Administration, Bethesda, MD

1995 FDA Invited Discussant
"Use of Inhaled Nitric Oxide in Pediatrics"
Division of Cardiorenal Drug Products, U.S. Food & Drug Administration Rockville, MD

1996 Invited Lecture
"Persistent Pulmonary Hypertension and Alveolar/Capillary Dysplasia"
Pediatric Grand Rounds, Elliot Hospital, Manchester, NH

1996 Invited Lecture
"Clinical Use of Inhaled Nitric Oxide"
International Business Communications Nitric Oxide Conference, Philadelphia, PA

1996 Seminar Speaker
"Postoperative Management of Pulmonary Hypertension in Pediatric Patients with Congenital or Acquired Heart Disease" Annual Meeting, American College of Cardiology, Orlando, FL

1996 Invited Lecture
"Inhaled Nitric Oxide-Clinical Experience"
First International Meeting on Pediatric Cardiac Intensive Care, Miami, FL

1996 Invited Lecture
"Pre and Postoperative Manipulation of the Vascular Resistance"
Annual Meeting, American Heart Association, New Orleans, LA

1996 Invited Lecture
"Current Concepts in Neonatology"
Section on Perinatology, American Academy of Pediatrics and the Joint Program in Neonatology, Harvard Medical School, Boston, MA

1997 Seminar
"Medical Management of Perioperative Pulmonary Hypertension"
Annual Meeting, Society of Critical Care Medicine, San Diego, CA

1997 Invited Lecture
"Nitric Oxide and the Treatment of Postoperative Pulmonary Hypertension"
Second World Congress of Pediatric Cardiology and Cardiac Surgery, Honolulu, Hawaii

1997 Invited Lecture
"Inhaled Nitric Oxide for the Treatment of Persistent Pulmonary Hypertension of the Newborn"
Open Meeting, Division of Cardiorenal Drugs, United States Food & Drug Administration, Bethesda, MD

1997 Invited Lecture
"Perioperative Care of the Child with Congenital Heart Disease: New Treatment Strategies for Pulmonary Hypertension"
Cardiothoracic Anesthesia Meeting, Washington University, St. Louis, MO

1997 Plenary Session
"Advances and Controversies in Cardiac Management"
Tenth Annual Pediatric Critical Care Colloquium, Hot Springs, AR

1997 Invited Faculty
"Critical Care of the Child with Congenital Heart Disease" (moderator lecturer, judge)
Second International Symposium on Pediatric Cardiac Intensive Care, Palm Beach, FL

1997 Invited Speaker
"Nitric Oxide in Neonatal Care"
Topics in Neonatal and Respiratory Care, Brigham & Women's Hospital, Boston, MA

1998 Invited Lectures
"Cardiac Surgery in Neonates: Morbidity and Mortality"
Charleston Symposium on Congenital Heart Disease, Medical University of SouthCarolina, Charleston, SC

1998 Symposium
"Advances in ICU Management for Congenital Heart Disease"
Annual Meeting, American College of Cardiology, Atlanta, GA

1998 Invited Lecture
"Intensive Care After Neonatal Cardiac Surgery: State-of-the-Art"
First Annual Course on Frontiers in Diagnosis and Management of Congenital Heart Disease, Boston, MA

1999 Invited Faculty
"Myocardial Support for Low Cardiac Output"
Society of Critical Care Medicine
Current Concepts in Pediatric Critical Care Course, San Francisco, CA

1999 Invited Lecture
"Nitric Oxide and the Treatment of Pulmonary Hypertension"
Oral Presentation Moderator, Walk Rounds with the Professor
28th Scientific Symposium, Society of Critical Care Medicine, San Francisco, CA

1999 Symposium
"The Airway, Mechanical Ventilation and Cardiopulmonary Interaction"
Annual Meeting, American Heart Association, Atlanta, GA

1999 Invited Speaker
"Nitric Oxide and New Therapies"
Third International Symposium on Pediatric Cardiac Intensive Care, Miami, FL

2000 Invited Faculty
"Nitric Oxide in the Perioperative Management of CHD"
Cardiology Y2K, Annual Update on Pediatric Cardiovascular Disease, Orlando, FL

2000 Symposium
"Intensive Care Unit Management After Surgery for Sngle Ventricle HLHS Syndrome"
Annual Meeting, American College of Cardiology, Anaheim, CA

2000 Invited Lecture
"Perioperative Care of the Premature Newborn with Congenital Heart Disease"
Castañeda Society Meeting, Boston, MA

2000 Invited Faculty
"Perioperative Care of the Premature Newborn with Congenital Heart Disease"
Tenth Charleston Symposium on Congenital Heart Disease, Charleston, SC

2001 Invited Faculty
"Clinical Research"
The Changing Face of Pediatric Cardiology 1950-2000: A Tribute to Alexander S. Nadas, M.D.
The Cardiovascular Program at Children's Hospital, Boston, MA

2001 Invited Faculty
"Cardiopulmonary Support in the Pediatric Cardiac Intensive Care Unit"
Third Course on Frontiers in Diagnosis and Management of Corgenital Heart Disease, Newport, RI

2001 Invited Faculty
Diverse Topics
Fourth International Symposium on Pediatric Cardiac Intensive Care, Palm Beach, FL

2002 Invited Speaker
"Sildenafil for Treatment of Pulmonary Hypertension"
ECMO Meeting, Children's National Medical Center, Keystone, Colorado

2002 Invited Speaker
"Novel Pediatric Applications of Commonly Used Adult Drugs"
Back to our Future: Establishing Safety and Evidence in Pediatric Research
Duke University, FDA & Industry, Washington, DC

2002 Invited Lecturer
"The Future of Inhaled Nitric Oxide for Children with Congenital Heart Disease"
CME Course in Hematology, Northwestern University Medical School
Chicago, Illinois

2002 Invited Faculty
"Manipulating Vascular Resistance in the Newborn: Is it Feasible?"
3rd International Pediatric Cardiovascular Symposium, Atlanta, Georgia

2002 Invited Speaker
"Viagra for Pulmonary Hypertension"
Hot Topics in Neonatology, Washington, DC

2003 Plenary Speaker
"Changes in Worldwide Activity and Mortality in Cardiac Intensive Care"
Debate: "Cardiac Patients Need Their Own ICU"
Symposium Chairman: "New Strategies in Treatment of Pulmonary Hypertension"
4th World Congress of Pediatric Intensive Care, Boston, Massachusetts

2004 Invited Faculty
"Pharmacologic Management of Low Cardiac Output Syndrome After Congenital Heart Surgery" Current Concepts in Pediatric Critical Care Medicine Course
Society for Critical Care Medicine, Orlando, Florida

2004 Invited Faculty
"Structure of a Training Program in Pediatric Cardiac Intensive Care"
33rd Annual Meeting of the Society for Critical Care Medicine, Orlando, Florida

2004 Invited Faculty
"Reconciling FDA, Academic, and Industry Objectives in Pediatric ClinicalTrials"
Cardiology 2004, Orlando, Florida (Children's Hospital of Philadelphia)

2004 Invited Speaker
"Cardiac and Central Nervous System Interactions
15th Annual Pediatric Critical Care Colloquium, New York City, New York

2004 Invited Faculty
"Advances in the Management of Pulmonary Hypertension"
"Physician Perspective on Electronic Billing:
Congenital Cardiovascular Surgery Symposium, San Diego, California

2004 Invited Participant in "How To" Session
"How to Evaluate and Manage Pediatric Patients with Pulmonary Hypertension"
American Heart Association, Scientific Sessions 2004, New Orleans, LA

2004 Invited Faculty, Special Session
"Twenty Year Retrospective: The Early Years and Later"
Pediatric Cardiac Intensive Care Symposium, Miami, FL

2004 Invited Faculty
"Nitric Oxide and the Intensive Care Setting"
Pediatric Cardiac Intensive Care Symposium, Miami, FL

2004 Invited Faculty
"How to Design and Conduct Drug Trials"
Pediatric Cardiac Intensive Care Symposium, Miami, FL

2005 Invited Faculty
"Therapies to Enhance the Effect of Inhaled Nitric Oxide"
Symposium on New Directions in Nitric Oxide Therapy, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas

2005 Invited Speaker
"Pulmonary Hypertension: Approaches to Management", 21st Annual Fetus and Newborn Conference, Boston, MA

2005 Invited Moderator
"Low Birth Weight Neonates with Congenital Heart Disease", Pediatric Cardiac Intensive Care Symposium 2005 (PCICS 2005), Miami, FL

2005 Invited Faculty
Consensus Report on Treatment of Myocarditis. Pediatric Cardiac Intensive Care Symposium 2005 (PCICS 2005), Miami, FL

2006 Invited Faculty
"Challenges in Industry Sponsored Trials" and "Management of PVR in the Neonate" Ninth Annual Update on Pediatric Cardiovascular Disease (Children's Hospital of Philadelphia), Scottsdale, AZ

2006 Invited Speaker
Eddie Farrell Memorial Lecture, Massachusetts Society of Respiratory Care, Sturbridge, MA

2006 Invited Faculty
Second International Conference on Heart Failure in Children and Young Adults Children's Hospital Orange County, Laguna Niguel, CA

2007 Invited Speaker
"Pulmonary Vascular Alterations in CHD" & "Drug Treatment for Pulmonary Hypertension". First International Conference on Childhood Pulmonary Vascular Disease, San Francisco, CA

2008 Invited Speaker
"Cardiac Critical Care: What's New and What Matters "STS Congenital Surgical Symposium, Ft. Lauderdale, Fl.

2008 Invited Speaker
Session Chair "Anticipating the Growing ACHD Population"
Update on Pediatric Cardiovascular Disease - New and Evolving Concepts and Practices, Speaker: "Considerations for Caring for Adult Patients in a Pediatric ICU" & "Current Status of Inpatient Therapy"
Scottsdale, AZ

2008 Invited Speaker
Forum Moderator: "Inhaled Nitric Oxide in the OR"
ASA 2008 Annual Meeting, Orlando, FL

2008 Invited Speaker
"Postoperative Management and Outcome of the Term vs. Premature Newborn with Congenital Heart Disease"
Management of Congenital Heart Disease in the Fetus & NeonateSymposium, Washington, DC

2008 Invited Speaker
"Pulmonary Hypertension"
NPCNA Annual Fall Conference, Innovation and Inquiry in Pediatric Cardiology Nursing Washington, DC

2008 Invited Speaker
"Critical Treatment Strategies for Acute Pulmonary Hypertension in Infants and Children cGMP-related Drugs »
PCICS Annual Symposium 2008, Miami, FL

2009 Invited Speaker
Session Moderator. "Cardiac Surgery"
38th Annual Critical Care Congress of the Society of Critical Care Medicine, Nashville, TN

2009 Invited Speaker
Session Moderator. "Cardiac ECMO: State-of-the-Art"
The 25th Annual CNMC Symposium: ECMO & The Advanced Therapies for Respiratory Failure
Keystone, CO

2009 Invited Speaker
Session Moderator. "Pulmonary Vascular Alterations in Congenital Heart Disease"
The 2nd International Neonatal and Childhood Pulmonary Vascular Disease Conference
San Francisco, CA

2009 Invited Speaker
"Advances in Cardiac Intensive Care"
9th Annual Cardiac Research Symposium – A.I. DuPont Hospital for Children, Nemours Symposia, Wilmington, DE

2010 Invited Speaker
"Cardiac Intensive Care: Celebrating Successes, Meeting Challenges"
3rd Annual John J. Downes Lecture in Pediatric Anesthesia and Critical Care Medicine
Orlando, FL

2010 Invited Speaker
"A Randomized Trial of Clopidogrel to Reduce Mortality and Shunt-Related Morbidity in Infants Palliated with a Systemic to Pulmonary Artery Shunt
Outstanding Research Awards (Council on Cardiovascular Disease in the Young)
AHA Scientific Sessions, Chicago, IL

2011 Invited Speaker
"Working with the FDA & Industry in Designing Pediatric Trials"
The 27th Annual CNMC Symposium: ECMO & The Advanced Therapies for Respiratory Failure
Keystone, CO

2011 Invited Speaker
"Resuscitation of the Patient with Pulmonary Hypertension"
4th International Neonatal and Childhood Pulmonary Vascular Disease
San Francisco, CA

INTERNATIONAL

1986 Invited Lecture
"Recent Advances in the Intensive Treatment of Neonates with Congenital Heart Disease,"
A Week with the Experts, Ospedale Pediatrico Bambino Gesu, Rome, Italy

1988 Invited Lecture
"Perioperative Care of the Patient with HLHS"
European Congress on Hypoplastic Left Heart Syndrome, Ospedale Pediatrico Bambino Gesu, Rome, Italy

1990 Invited Lecture
"Perioperative Care of the Neonate with Congenital Heart Disease"
Pediatric Critical Care Conference, Hospital for Sick Children, University of Toronto, Toronto Canada

1991 Invited Lecture
"Perioperative Intensive Care of the Child with Congenital Heart Disease"
First International Pediatric Intensive Care Congress, Buenos Aires, Argentina

1993 Invited Faculty
"Regulation of the Pulmonary Circulation: Therapeutic Implications"
First European Postgraduate Course in Neonatal and Pediatric Intensive Care, Berne, Switzerland

1993 Invited Faculty
"Pulmonary Hypertension: Pathophysiologic and Therapeutic Implications in Post Surgical Patients" Third International Meeting on Pediatric Intensive Care, University of Padova, Italy

1993 Invited Lecture
"Nitric Oxide to Test Pulmonary Vascular Reactivity to Control Hypertensive Crises and as a Potential Chronic Therapy" Canadian Cardiovascular Society, Vancouver, Canada

1993 Invited Lecture
"Nitric Oxide Inhalation after Correction of Congenital Heart Defects"
International Conference on ARDS, Tutzing, Germany

1994 Plenary Presentation
"Perioperative Care of the Neonate"
Cardiac Surgery Today: State of the Art, Onassis Medical Center, Athens, Greece

1995 Invited Faculty
"Nitric Oxide in the Treatment of Congenital Heart Disease"
Annual Meeting of the Austrian Society for Lung Diseases, Gmunden, Austria

1995 Plenary Speaker
"Inhaled Nitric Oxide for Perioperative Management of Congenital Heart Disease"
The VII Brazilian Congress of Intensive Care Medicine, Recife, Brazil

1996 Invited Lecture
"Nitric Oxide in Pulmonary Hypertension after Surgery for Congenital Heart Defects"
Annual Meeting, European Society of Cardiology, Birmingham, United Kingdom

1997 Symposium
"The Failing Heart—Pediatric Aspects"
The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada

1997 Plenary Session
"Inhaled Nitric Oxide"
XXX Brazilian Pediatrics Congress and International Pediatric Symposium, Rio de Janeiro, Brazil

1998 Invited Faculty
Multiple lectures and workshops
Pediatric Cardiac Intensive Care at the European Heart House
European Society of Cardiology, Nice, France

1998 Invited Faculty
Lectures on Congenital Heart Disease
Argentine Congress of Cardiology, Buenos Aires, Argentina

1999 Invited Lecture
"Critical Aortic Stenosis in the Neonate"
Second Postgraduate Course on Congenital and Acquired Heart Disease, Modena, Italy

1999 Invited Lecture
"Pathophysiology and Treatment of Pulmonary Hypertension
Lund University Hospital, Lund, Sweden

1999 Invited Lecture
"Pulmonary Hypertension and Mechanical Support in Children with Heart Disease"
Lindgren Children's Hospital at the Karolinska Institute, Stockholm, Sweden

1999 Plenary Speaker
"Frontiers in Pediatric Intensive Care"
Annual Meeting, Society of Anesthesia and Critical Care, Gothenburg, Sweden

1999 Invited Faculty
"ICU Management of Two Stage Arterial switch"
"The Role of Nitric Oxide in the Cardiac Patient"
The First Hispano Latin American Course, Diagnosis and Management of Congenital Heart Disease, San Juan, Puerto Rico

1999 Invited Lecture
"Current Concepts in Post-operative Management"
"ECMO in the New Millennium"
Symposium on Pediatric Cardiology, Cordoba, Argentna

1999 Invited Speaker
"Inhaled Nitric Oxide"
"Perioperative Care of the Newborn"
The First Sino-American Symposium: New Developments in the Care of Children with Congenital Heart Disease, Shanghai Children's Medical Center, Shanghai, China

2000 Invited Faculty and Track Convener
"Issues in Perioperative Care" and multiple lectures
The Third International Symposium on Pediatric Cardiac Intensive Care, Montreal, Canada

2000 Invited Lecture
"Endothelial Cell Function During Cardiopulmonary Bypass"
5th World Congress on Trauma, Shock, Inflammation and Sepsis, Munich, Germany

2000 Invited Lecture
"Inhaled Nitric Oxide Therapy in Children after Cardiac Surgery"
American Thoracic Society, 96th International Conference, Toronto, Canada

2000 Invited Lecture
"Pulmonary Hypertension and its Impact on Hemodynamics"
Special Topics in Pediatric Cardiac Intensive Care Symposium, Royal Brompton & Harefield NHS Trust, London, United Kingdom

2000 Invited Faculty
"Critical Care and Congenital Heart Disease"- diverse topics
Pediatric FCCS Course, Taipei, Taiwan

2000 Invited Lecture
"Advances in Perioperative Care of the Child with Congenital Heart Disease"
Tenth Anniversary Lecture, Kaohsiung Veterans General Hospital, Taiwan

2000 Invited Lecture
"Postoperative Care of the Child with AV Septal Defect"
European Cardiovascular Surgery's Postgraduate Course, Frankfurt, Germany

2000 Seminar
"Postoperative Care of Patients with Hypoplastic Left Heart Syndrome"
European Association of Cardio-Thoracic Surgery, Annual Meeting, Frankfurt, Germany

2000 Plenary Lecture
"Diagnosis and Treatment of Pulmonary Hypertension"
XIX Pan American Congress of Pediatrics, Montevideo, Uruguay

2000 Invited Lecture
"Postoperative Management of the Child with D-Transposition of the Great Arteries"
"Diagnosis and Management of Pulmonary Arterial Hypertension"
I Pediatric Cardiology Symposium, Dr. Aldo Castañeda, Guatemala City, Guatemala

2001 Invited Faculty
"Pulmonary Hypertension and Nitric Oxide"
"Assessing and Managing Premature Newborns for Surgical and Catheter Intervention"
Harvard Winter Course in Congenital Heart Management, Dubai, United Arab Emirates

2001 Invited Lecture
"Brain Protection During CPB"
V European Postgraduate Course in Neonatal and Pediatric Intensive Care, Bern, Switzerland

2001 Invited Lecture
"Strategic Management of the Patient after Surgery"
Third World Congress of Pediatric Cardiology and Cardiac Surgery, Toronto, Canada

2001 Invited Faculty
Special Topics in Paediatric Cardiac Intensive Care – 2001, The Challenging Neonate,
The Royal Brompton Hospital & The National Heart & Lung Institute, London, England

2002 Moderator
European Consensus Meeting on Inhaled Nitric Oxide
European Society of Pediatric and Neonatal Intensive Care, Rome, Italy

2002 Invited Faculty
"Assessment of Myocardial Function in the ICU"
"Postoperative Management After Staged Repair of HLHS"
"ECMO Management of the Single Ventricle Circulation"
New Era in Congenital Heart Management
Universidad Complutense Madrid and Real Colegio Complutense en Harvard, The Heart Institute Hospital, Universitario "12 de Octubre", Madrid, Spain

2002 Guest Lecturer
"Failing Hearts: The Paediatric Problem and Current Treatments"
"Inhaled Nitric Oxide and Pulmonary Vasodilators for the Failing Right Heart"
"Routine ECMO for Resuscitation"
The Third Special Topics in Paediatric Cardiac Intensive Care, The Failing Myocardium
Royal Brompton Hospital, Imperial College, London, United Kingdom

2003 Invited Faculty
"Support for the Failing Ventricle"
"Management of Pulmonary Hypertension: From the OR to the Home"
Debate: "Early Extubation Is the Best Defense Against Postoperative Complications"
First Asia Pacific Symposium on Pediatric Cardiac Intensive Care, Phuket, Thailand

2003 Guest Lecturer
"Pharmacologic Management of Pulmonary Hypertension" and Other Topics
IX Curso de Actualización en Cardiología Pediátrica, Madrid, Spain

2003 Guest Lecturer
"Recent Advances in the Use of Inhaled Nitric Oxide in Patients with Congenital Heart Disease". Inhaled Nitric Oxygen Symposium for Neonatologists. Madrid, Spain

2003 Invited Participant
Third World Symposium on Pulmonary Arterial Hypetension (WHO). Venice, Italy

2003 Special Guest Lecturer
"Indications for Inhaled Nitric Oxide in the Neonatal and Postoperative Care of Critically Ill Children".
Annual Meeting of the German Society of Pediatric Cardiology. Weimar, Germany

2003 Invited Speaker
"Predicting and Treating Low Cardiac Output in the Postoperative Patient"
Annual Meeting of the European Association of Cardiothoracic Surgeons. Vienna, Austria

2003 Invited Faculty
"The Paperless ICU"
"Pulmonary and Systemic Vasodilators"
"Genetic Basis for Heterotaxy"
Harvard Medical International, Children's Hospital Boston Course in Congenital Heart Disease
Abu Dhabi, United Arab Emirates

2003 Invited Speaker
"Extracorporeal Membrane Oxygenation for Cardiopulmonary Resuscitation in Children"
Hammersmith Hospital Workshop on Perfusion. London, United Kingdom

2004 Keynote Speaker
"Pulmonary Hypertension Therapy—Now and in the Future"
Pulmonary Hypertension in Early Life, St. Guys and St. Thomas' Hospital
London, United Kingdom

2004 Keynote Address
"Pulmonary Hypertension: State of the Art"
Opening Ceremony, Annual Meeting of the European Society of Pediatric and Neonatal Intensive Care, London, United Kingdom

2005 Plenary Speaker
"Recent Advances in Heart Failure and Pulmonary Hypertension", The Fourth World Congress of Pediatric Cardiology and Cardiac Surgery, Buenos Aires, Argentina.

2005 Invited Speaker
Controversy Session: "Inhaled Iloprost Is the Best Pulmonary Vasodilator?",The Fourth World Congress of Pediatric Cardiology and Cardiac Surgery, Buenos Aires, Argentina.

2005 Invited Speaker
Chair, Oral Presentations: "Cardiac Intensive Care", The Fourth World Congress of Pediatric Cardiology and Cardiac Surgery, Buenos Aires, Argentina.

2006 Invited Faculty
"Outcomes of Heart Failure in the ICU: Mechanisms of Postoperative Dysfunction." Congress of Ventricular Dysfunction in Childhood, OPBG Cardiovascular International. Rome, Italy

2007 Invited Speaker
Multiple Oral Presentations and Panel Chair
Fifth World Congress on Pediatric Critical Care, Geneva, Switzerland

2007 Invited Speaker
"Cuidado perioperatorio del recién nacido con enfermedad cardiaca congénita",VI Annual Colombian Critical Care Congress, Medellín, Colombia

2007 Invited Speaker
"Postoperative Treatment of Pulmonary Hypertension,"& "Postoperative Care of Hypoplastic Left Heart: Comparing Norwood with BT Shunt vs. Sano from Birth through the Fontan."
International Cardiology Meeting, Avignon, France

2008 Invited Faculty
Plenary Lecture: "Pediatric Cardiac Intensive Care: Past, Present and Future"; "Dedicated Training Pathways in Pediatric Cardiac Intensive Care" & "How to plan a Research Study in ICU"
PCICS Europe Symposium, Monte Carlo, Monaco

2009 Invited Speaker
"Acute Heart Failure Pathophysiology", Treatment of Postoperative Acute Cardiac Failure", "Mechanical Support of Acute Cardiac Failure"
International Pediatric Cardiology Conference
Cartagena, Colombia

2010 Invited Faculty
Session Moderator: Pulmonary Hypertension, Right Ventricular Function and Congenital Heart Disease
3rd International Conference Neonatal and Childhood Pulmonary Vascular Disease
Banff, Alberta, Canada

2010 Invited Speaker
"Intraoperative Care and Perioperative Management for Transpostion"
The World Society for Pediatric and Congenital Heart Surgery
Antigua, Guatemala

10) GRANTS AWARDED

FUNDING INFORMATION

1987-89 The effects of ventilation on pulmonary vascular resistance in infants following cardiopulmonary bypass. Principal Investigator, American Society of Anesthesiologists Research Starter Grant.

1988-91 Infant heart surgery: CNS sequelae of circulatory arrest. Co-Investigator, National Institutes of Health. Grant No. HL41786.

1993-96 Ischemic neonatal brain injury: clinical and basic science. Co-investigator, National Institutes of Health. Grant No. P20 NS32570

1994-96 Inhaled nitric oxide for the treatment of pulmonary hypertension and acute respiratory failure in children. Principal Investigator, Clinical Research Grant-in-Aid Award, Children's Hospital, Boston, Massachusetts. Grant No. CH 89430.

1994-99 Pathogenesis of brain injury in infant heart surgery. Clinical advisor / mentor to Dr. Adre J. DuPlessis, National Institutes of Health. Grant No. K08 NS01721

1996-99 Dose response of inhaled nitric oxide in congenital heart disease. Principal Investigator, U.S. Food and Drug Administration. Grant No. FD R-001316.

1997-99 Neurodevelopmental follow up of patients with PPHN in a randomized trial of nitric oxide. Principal Investigator, Industry Sponsored.

1997-00 Echocardiographic assessment of right ventricular function in patients with pulmonary hypertension. Sponsor for Dr. Ricardo Munoz (MCAP), National Institutes of Health Grant No. M01 RR02172.

2000-01 Prophylactic use of Primacor® in pediatric patients at high risk of developing low cardiac output syndrome following cardiac surgery. Principal Investigator, Industry Sponsored.

2004-06 Principal Investigator (Boston) on three industry sponsored trials of sildenafil for treatment of pediatric pulmonary hypertension (see below).

2004-08 A Randomized, Double-Blind, Placebo Controlled, Dose Ranging, Parallel Group Study of Oral Sildenafil in the Treatment of Children, Aged 1-16 Years, With Pulmonary Hypertension. Principal Investigator, Industry Sponsored

2004-08 Multicenter, Long-Term Extension Study to Assess Safety of Oral Sildenafil in the Treatment of Subjects Who Have Completed Study A1481131. Principal Investigator, Industry Sponsored

2004-06 7-Day, Open-Label, Multicenter, Pharmacokinetic Study (Part 1) of IV Sildenafil in the Treatment of Neonates With Persistent Pulmonary Hypertension of the Newborn (PPHN) or Hypoxic Respiratory Failure and at Risk for PPHN. Principal Investigator, Industry Sponsored

2006-08 Pilot Study of the Effects of Nesiritide on Hemodynamics and Urine Output Following Cardiopulmonary Bypass in Children. Co-investigator and mentor (John M. Costello); American Heart Association.

2006-10 Multinational Trial on the Efficacy and Safety of Clopidogrel in Infants with Cyanotic Congenital Heart Disease Palliated with a Systemic to Pulmonary Shunt (CLARINET). (Chair, Steering Committee, Institutional Co-investigator). Industry Sponsored (Sanofi-Aventis).

2009-14 Collaborative Pediatric Critical Care Research Network (CPCCRN). NIH-NICHD U10410HD049981. Principal Investigator; 20% effort. Base award over 5 years $925,000 direct costs plus annual awards for protocol funds (e.g. 2010 = $200,000)

- Critical Pertussis in US Children. Protocol #001
- The Critical Illness Stress-induced Immune Suppression Prevention Trial (CRISIS). Protocol #003
- Development of a Quantitative Functional Status Scale (FSS) for Pediatric Patients. Protocol #004
- Therapeutic Hypothermia after Pediatric Cardiac ArrestTrials (THAPCA). Protocol #010
- Cortisol Quantification Investigation. Protocol #012
- Measuring Opioid Tolerance Induces by Fentanyl (or Other Opioids). Protocol #026
- Physician's Perspectives on the Physician-Parent Follow-Up Conference.
- Pediatric Intensive Care Unit Bereavement Study
- CPCCRN Asthma Study

REPORT OF CURRENT RESEARCH ACTIVITIES

1. My primary current research activity involves designing and executing national and international pediatric clinical trials:

2. Safety and efficacy of type V phosphodiesterase inhibitors in children as selective pulmonary vasodilators and to augment vasodilatory potential of nitric oxide and attenuate rebound pulmonary hypertension. I was the overall primary scientific advisor in the development and execution of international multicenter randomized trials on type V inhibitors in pediatrics, Industry sponsored.Final publications in press.

3. Outcome studies evaluating ventilator management, inotropic agents, mechanical support of the circulation and new strategies in the critical care managementand perioperative care of
   a) premature newborns with congenital heart disease
   b) newborns after reparative surgery involving the right ventricle
   c) extracorporeal membrane oxygenation resuscitation of children with congenital heart disease.

4. Multinational Trial on the Efficacy and Safety of Clopidogrel in Infants with Cyanotic Congenital Heart Disease Palliated with a Systemic to Pulmonary Shunt(CLARINET). (Chair, Steering Committee, Institutional Co-investigator). Industry Sponsored.

5. I am the Principal Investigator (CNMC) and steering committee member for the NIH funded clinical research network with multiple active protocols listed above.

11) PUBLICATIONS

PAPERS IN REFEREED JOURNALS

1. Hickey PR, Hansen DD, Wessel DL, Lang P, Jonas RA. Pulmonary and systemic hemodynamic responses to fentanyl in infants. Anesth Analg 1985;64:483-6.

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16. Chang AC, Kulik TJ, Hickey P, Wessel DL. Real-time gas exchange measurement of oxygen consumption in neonates and infants after cardiac surgery. Crit Care Med 1993;21:1287-1295.

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CHAPTERS, REVIEWS:

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EXHIBIT D

Exhibit D

Acute pulmonary hypertension in infants and children: cGMP-related drugs

Alain Fraisse, MD, PhD; David L. Wessel, MD

Pharmacologic strategies to reduce pulmonary vascular tone and to treat pulmonary hypertension originally aimed to enrich vascular smooth muscle cyclic adenosine monophosphate levels. Alternatively, increasing cyclic guanosine monophosphate (cGMP) also reduces pulmonary vascular tone. Inhaled nitric oxide is extremely efficacious in increasing cGMP and selectively reducing mean pulmonary arterial pressure in pediatric cardiac patients. It is considered standard treatment in most centers. However, not all patients respond to inhaled nitric oxide and withdrawal is sometimes problematic. This has prompted investigation of alternative methods to increase intracellular vascular smooth muscle cGMP. Phosphodiesterase type 5 is particularly abundant in the lung vasculature of patients with severe pulmonary hypertension. Its inhibition with the sildenafil class of drugs is now commonplace. Drugs that affect cGMP metabolism in children with acute pulmonary hypertension are the subject of this review and consensus statement. Oral sildenafil is recommended in postoperative pulmonary hypertension after failed withdrawal of inhaled NO (class I, level of evidence B). The effectiveness of prolonged treatment with sildenafil in documented postoperative pulmonary hypertension is not well established (class IIb, level of evidence C). Sildenafil is indicated in idiopathic pulmonary hypertension, although data have been extrapolated mainly from adult trial (class I, level of evidence A, extrapolated). Recently, completed pediatric trials have seemed to support this recommendation. Longer-acting and intravenous forms of phosphodiesterase type 5 inhibitors, brain natriuretic peptides, and direct soluble guanylate cyclise activators all have appeal, but there is insufficient experience in children with acute pulmonary hypertensive disorders for recommendations on treatment. (Pediatr Crit Care Med 2010; 11[Suppl.]:S37–S40)

Key Words: inhaled nitric oxide; sildenafil; congenital heart disease; postoperative pulmonary hypertension.

In children with pulmonary arterial hypertension (PAH), endothelial dysfunction results in an imbalance of endogenous vasoconstrictors (e.g., endothelin-1) and vasodilators (e.g., nitric oxide [NO]), leading to vascular constriction, *in situ* thrombosis, and vascular remodeling (1–3). Postoperative PAH and endothelial dysfunction are further exacerbated by the effects of cardiopulmonary bypass.

Strategies to reduce pulmonary vascular tone aim to enrich vascular smooth muscle cyclic adenosine monophosphate levels through β agonists (isoproterenol) or with phosphodiesterase type III inhibitors (e.g., milrinone). Alternatively, increasing cyclic guanosine monophosphate (cGMP) with nitro-vasodilators (sodium nitroprusside, nitroglycerin, inhaled NO) also reduces pulmonary vascular tone. Inhaled NO is extremely efficacious in selectively reducing mean pulmonary arterial pressure (PAP) in cardiac patients and is considered standard treatment in most centers. However, not all patients respond to inhaled NO. Its application is limited as it is cumbersome and expensive to consider administering chronically and there is a withdrawal response seen in some postoperative patients. Withdrawal of inhaled NO can lead to significant rebound PAH.

Sildenafil and other phosphodiesterase type 5 (PDE5) inhibitors may play a role in the management of PAH as an alternative or adjunct to current therapies by preferentially inhibiting PDE5. Sildenafil acts by inhibiting the breakdown of cGMP through PDE5, an enzyme that metabolizes intracellular cGMP to inactive 5′-GMP. Other cGMP-related drugs may act through direct guanylate cyclase activation.

Pharmacology of Sildenafil

PDE5 is particularly abundant in the lung vasculature of patients with severe PAH. The main pharmacologic mechanism by which sildenafil achieves its clinical effect is by preferential inhibition of PDE5 that is present in penile tissue, platelets, skeletal muscle, and vascular and visceral smooth muscle, thereby slowing the degradation of cGMP, resulting in lower levels of intracellular calcium and relaxation of vascular smooth muscle. In PAH, this results ultimately in a reduction of PAP and pulmonary vascular resistance (3). However, other factors may play a significant role, such as atrial natriuretic peptide and NO up-regulation (4). One potential contraindication for sildenafil therapy is postcapillary hypertension. When left atrial pressure is elevated, sildenafil could worsen heart failure by increasing pulmonary blood flow through its vasodilator effect, as has been reported with inhaled NO. Although with sildenafil, this might be counterbalanced by its peripheral vasodilator properties (5). Furthermore, sildenafil may be an important regulator for contraction and stress remodeling pathways. Studies in surgical specimens and in rat hypertrophied right ventricular myocardium demonstrated that PDE5 is markedly upregulated there. Consequently, administration of PDE5 inhibitors increases right

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ventricular inotropy and decreases right ventricular afterload, making them potentially ideal for the treatment of diseases affecting the right ventricle like PAH (6).

Clinical Studies With Oral Sildenafil in Adult PAH

Four randomized, controlled trials have been performed to evaluate sildenafil in patients with "chronic" PAH (7–10), with inclusion of few pediatric patients in one (10). They all reported positive results, primarily based on improvement with exercise, using the 6-min walk test. Following the results of the pivotal study from Galiè and colleagues, the U.S. Food and Drug Administration approved oral sildenafil for therapy for PAH (7). More recently, combination therapy was evaluated in a double-blind, randomized trial in which either oral sildenafil or placebo was given to patients already receiving intravenous epoprostenol. The primary end point (6-min walk test) significantly improved in treated patients relative to placebo, along with secondary end points (hemodynamics, quality of life, and time to clinical worsening) (11).

Experience is very limited in adults with the use of sildenafil in acute PAH after cardiac surgery. Beside anecdotal case reports of oral sildenafil in cardiac surgical patients, only one small, retrospective study demonstrated significantly decreased mean PAP and pulmonary vascular resistances in eight postoperative patients after mitral valve surgery or left ventricular assist device placement (12).

Indications and Clinical Applications of Oral Sildenafil in Pediatric PAH

The first human use of sildenafil for the purpose of treating PAH was more than a decade ago in infants with postoperative PAH after failure to wean inhaled NO. The administration of sildenafil blunted rebound PAH during inhaled NO withdrawal (13). From this first experience, there have been growing anecdotal evidence and widespread adoption of the use of sildenafil to treat PAH in pediatric patients. Studies in support of chronic administration of oral sildenafil in children are only now appearing. In a 12-month open-label, clinical trial, Humpl and colleagues demonstrated significant improvement with hemodynamics and exercise capacity (6-min walk test) in 14 children with idiopathic or secondary PAH (14). More recently, results of a large, prospective, double-blind, placebo-controlled trial in children have been announced (R. J. Barst and D. L. Wessel, personal communication). Improvement in exercise capacity and secondary outcome variables was observed.

In acute PAH, the use of oral sildenafil has been studied during the early postoperative period, mainly to prevent rebound PAH during inhaled NO withdrawal (13, 15). Of particular interest is the prospective, randomized, double-blind, placebo-controlled study of Namachivayam and colleagues. They demonstrated in 15 postoperative infants and children who were receiving inhaled NO after cardiac surgery that a single dose of enteral sildenafil effectively prevented the development of rebound PAH after NO withdrawal, as compared with 14 children allocated to placebo. Sildenafil also reduced the subsequent duration of mechanical ventilation (15). This raises potential interest in the prophylactic administration of sildenafil in such patients with elevated pulmonary vascular resistance and failure to wean inhaled NO. This concept of the prophylactic use of sildenafil to facilitate weaning from NO was further enhanced by Lee and colleagues, who succeeded with oral sildenafil in withdrawing inhaled NO in seven postoperative cardiac children with PAH who had previously failed attempts at inhaled NO weaning (16). In this study, the sildenafil was continued for an average duration of 28 days.

Beside postoperative PAH, sildenafil has been studied in persistent pulmonary hypertension of the newborn, another acute form of PAH. In a placebo-controlled, randomized study in infants >35.5 wks' gestation and <3 days old with severe persistent pulmonary hypertension of the newborn and oxygenation index >25, sildenafil was given at a dose of 1 mg/kg. Oxygenation index improved in all infants within 6 hrs to 30 hrs. All the patients demonstrated a steady improvement in pulse oxygen saturation over time, and none had noticeable effect on blood pressure (17).

Currently, the optimal dose of oral sildenafil in children remains undetermined, but is likely to be in the range of 0.3–1.0 mg/kg three times per day. Bioavailability in a postoperative child may be significantly impaired. Few serious adverse events have been reported in patients on sildenafil, most frequently dizziness, tachycardia, erythema, and drowsiness (18). Of concern is the report of cases of nonarteritic anterior ischemic optic neuropathy in adult patients using sildenafil for erectile dysfunction. This suggests a possible causal relationship with sildenafil, although such population with erectile dysfunction also often presents with generalized endothelial disease, which also constitutes a risk factor for nonarteritic anterior ischemic optic neuropathy. In children, a single case of ischemic optic neuropathy was reported (19). In pediatric PAH, no significant effect on systemic arterial and central venous pressures was seen after incremental doses of 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, and 2.0 mg/kg (20). Even accidental ingestions of adult pills of Viagra (Pfizer, New York, NY) did not result in significant nor sustained hemodynamic compromise (21).

Guidelines are as follows:

1. Sildenafil is recommended in postoperative PAH after failed withdrawal of inhaled NO (class I, level of evidence B). There are several case reports and small cohort studies (13, 16) as well as one small prospective, randomized, double-blind, placebo-controlled study in 30 patients (15).
2. The effectiveness of prolonged treatment with sildenafil in documented postoperative PAH is not well established (class IIb, level of evidence C). There are limited data on prolonged use of sildenafil in such indication. In the study by Lee and colleagues, sildenafil was continued for an average duration of 28 days (16). Sildenafil may be reasonable for more prolonged perioperative treatment if PAH is hemodynamically significant. Preliminary review of a large, randomized, pediatric trial suggested a good safety profile and potential mid-term benefit. This will likely raise the class of evidence to IIa.
3. Sildenafil is indicated in idiopathic PAH, although data are extrapolated mainly from adult trials (7–10) (class I, level of evidence A, extrapolated). Completed pediatric trials seem to support this recommendation, but final review and publication are pending.

Intravenous Sildenafil

When sildenafil is administered enterally, its bioavailability is only about 40% in healthy subjects (22). In critically ill, postoperative children with even more unpredictable enteral absoption, the intravenous form of sildenafil seems more appropriate. Several preliminary studies in children with intravenous sildenafil have reported encouraging results to lower PAP and pulmonary vascular resistances after cardiac surgery or during cardiac catheterization (23–25). In a recent work investigating the pharmacologic properties of three different doses of intravenous sildenafil on postoperative PAH, the use of a bolus followed by maintenance dose for a maximal duration of 72 hrs was specifically designed for treating PAH in the early postoperative course. Beside the ability for the three doses of intravenous sildenafil to decrease PAP effectively, patients experienced a shorter time to extubation and a shorter intensive care unit length of stay compared with placebo (25). This preliminary and underpowered study cannot be used for recommendations regarding this unapproved form of the drug.

Whereas the majority of animal and human studies on intravenous sildenafil did not document any clinically significant hemodynamic and respiratory side effects (25–28), Schulze-Neick and colleagues reported significant intrapulmonary shunting in postoperative children with PAH after cardiac surgery, although no patient experienced significant hypoxemia (23). In another study, systemic hypotension and impaired oxygenation were observed after 0.35 mg/kg IV of sildenafil in postoperative infants at risk but not suffering from PAH (24). In a dose-finding trial of intravenous sildenafil for newborns with persistent pulmonary hypertension of the newborn, the drug was associated with improved oxygenation and, in some patients, may have prevented the need for standard therapy (inhaled NO) (29).

Second-Generation PDE Inhibitors (Tadalafil, Vardenafil)

With a longer plasma half-life and a more specific and potent PDE inhibition, the new PDE inhibitors are of potential interest in heart failure. To date, no studies in children have been completed and published. In an animal model of persistent pulmonary hypertension of the newborn, tadalafil improves oxygenation (30).

Direct Soluble Guanylate Cyclase Activators

The limitation of NO donors, such as nitroprusside, includes development of tolerance and lack of selectivity for the pulmonary circulation. This has prompted investigation into a new promising class of compounds that directly activate soluble guanylate cyclase. The so-called BAY compounds (e.g., cinaciquat) have been shown to selectively activate the oxidized/heme free enzyme, causing marked vasodilation in diseased organs. Phase II trials are ongoing and no experience in children has been reported.

Nesiritide

The natriuretic hormone system is an important regulator of neurohormonal activation, cardiac diastolic function, and fluid balance, as well as vascular tone. Furthermore, brain natriuretic peptide seems to be a useful marker to monitor disease severity in pediatric PAH (31). Nesiritide (synthetic B-type natriuretic peptide) may have a hemodynamic profile that is comparable with milrinone as a rather nonspecific pulmonary vasodilator. It reduces PAP in adults and improves diuresis and fluid balance in children after congenital surgery but no study has been conducted in acute PAH children (32).

Conclusion

Over the last decade, oral sildenafil has played a growing role in the treatment of acute PAH, emerging as an effective first-line therapeutic agent. Selective pulmonary vasodilation and antiremodeling properties played an important role in its clinical efficacy, whereas very few serious adverse events were associated with its administration in children. Future well-designed trials are needed to clarify the efficacy of sildenafil in acute PAH. Other cGMP-related agents are of potential interest but they require more specific studies to provide information on their therapeutic use in acute PAH.

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EXHIBIT E

Exhibit E

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)	
Application Serial Number	12/820,866
Confirmation Number	2913
Filing Date	June 22, 2010
Title of Application	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor	James S. Baldassarre
Assignee	Ikaria, Inc.
Group Art Unit	1613
Examiner	Arnold, Ernst V.
Attorney Docket Number	I001-0002USC1

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
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DECLARATION OF JAMES S. BALDASSARRE, M.D.
UNDER 37 C.F.R. § 1.132

I, James S. Baldassarre, declare the following:

1. I currently hold the position of Vice President of Clinical Research at Ikaria, Inc. ("Ikaria"), the assignee of U.S. Patent Application No. 12/820,866. My *curriculum vitae* is attached as Exhibit 1.

2. I have over 20 years of experience as a physician, and over fifteen years of experience directing clinical research in the pharmaceutical industry.

3. Ikaria markets pharmaceutical grade nitric oxide (NO) gas under the brand name INOMAX® (nitric oxide) for inhalation. INOMAX® was approved by the U.S. Food and Drug Administration ("FDA") in December 1999, for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure (HRF) associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (ECMO).

4. In May 2004, INO Therapeutics LLC[1] initiated a clinical trial, entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing", and designated the INOT22 trial, to compare the utility and side effects of oxygen (O_2), nitric oxide (iNO) and a combination of iNO and O_2 for determining pulmonary reactivity.

5. The INOT22 study was to be an open, prospective, randomized, multi-center, controlled diagnostic trial, with an expected total enrollment of a minimum of 150 patients, in approximately 18 study sites over approximately 2 years.

6. The expected patient population for enrollment into the INOT22 trial were subjects between the ages of four (4) weeks and eighteen (18) years undergoing diagnostic right heart catheterization scheduled to include acute pulmonary vasodilation testing to assess pulmonary vasoreactivity. The expected population were subjects with idiopathic pulmonary arterial hypertension, congenital heart disease (with or without intravascular shunt) with pulmonary hypertension and cardiomyopathies.

7. The INOT22 study was established and designed by the study sponsor, INO Therapeutics LLC (INO), and a Steering Committee comprising internationally recognized experts in the field of pediatric heart and lung disease, whose members would assist INO to develop the INOT22 protocol, monitor the progress of the trial, and provide recommendations to INO on changes in the procedures and conduct of the trial.

8. The Steering Committee consisted of:

a. David L. Wessel, MD, presently Division Chief, Pediatric Critical Care Medicine at Children's National Medical Center, Washington, DC (co-author of Atz., et al., Seminars in Perinatology);[2]

[1] INO Therapeutics LLC is a wholly owned subsidiary of Ikaria, Inc., and holder of the NDA for INOMAX.
[2] Cited in pending Office Action.

b. Robyn J. Barst, MD, presently Professor Emeritus of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York; and

c. Duncan J. Macrae, MD, presently Director, Pediatric Intensive Care, Royal Brompton Hospital, London, U.K. (lead author of Macrae, et al., Intensive Care Medicine, 2004)[3]

9. The original INOT22 protocol designed by INO and the Steering Committee contained the following inclusion and exclusion criteria:

Inclusion Criteria

The patient must meet the following criteria:

1. *Have any one of the three disease categories:*

   a. *Idiopathic Pulmonary Arterial Hypertension*

      i. *PAPm >25mmHg at rest, PCWP ≤ 15mmHg, and PVRI >3 u· m^2 or diagnosed clinically with no previous catheterization.*

   b. *CHD with pulmonary hypertension repaired and unrepaired,*

      i. *PAPm >25mmHg at rest, and PVRI >3 u· m^2 or diagnosed clinically with no previous catheterization*

   c. *Cardiomyopathy*

      i. *PAPm >25mmHg at rest, and PVRI >3 u•m^2 or diagnosed clinically with no previous catheterization.*

2. *Scheduled to undergo right heart catheterization to assess pulmonary vasoreactivity by acute pulmonary vasodilation testing.*

3. *Males or females, ages 4 weeks to 18 years, inclusive.*

[3] Cited in pending Office Action.

4. Signed IRB/IEC approved informed consent (and assent if applicable).

Exclusion Criteria

The patient will be excluded from enrollment if any of the following are true:

1. Focal pulmonary infiltrates on chest radiograph.

2. Diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.

3. Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.

4. Pregnant (urine HCG +).

10. The INOT22 investigational plan and study protocol was further reviewed, and approved by the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the participating study institutions, including review by the principal investigator within each study institution.

11. At no time did any member of the Steering Committee, nor any member of an IRB, IEC, or individual principal investigator, appreciate, recognize or otherwise suggest that the exclusion criteria be amended to exclude study subjects with pre-existing left ventricular dysfunction (LVD), due to an anticipated or predicted risk of adverse events or serious adverse events arising from the use of INO in patients with pre-existing LVD, and/or elevated pulmonary capillary wedge pressure. Nor was it, in my expert opinion, common sense to any expert in this field of medicine to exclude neonates, near-term neonates or children diagnosed with pre-existing LVD to be excluded from having INO administered for diagnostic or treatment purposes.

12. After initiation and enrollment of the first 24 subjects in INOT22, there were 5 serious adverse events (SAEs) – a rate much higher than expected by INO and

the Steering Committee based on prior clinical experience. These were all cardiovascular events, and included pulmonary edema, cardiac arrest and hypotension (low blood pressure).

13. Thereafter, in February 2005, INO and the Steering Committee convened to review the unexpected SAEs described above, and upon review and discussion, expressed concern that the unexpected SAEs may be due to the administration of INO in subjects having pre-existing LVD. Accordingly, based upon a review of the cases, the exclusion criteria of the INOT22 protocol was amended to thereafter exclude subjects with pre-existing LVD. For the purpose of the study, the exclusion criteria was amended to exclude subjects from enrollment if the subjects demonstrated an elevated pulmonary capillary wedge pressure (PCWP), defined within the study as subjects having a PCWP greater than 20 mmHg. All study sites were notified immediately. The amended exclusion criteria (see point 5.) was as follows:

Exclusion Criteria

The patient will be excluded from enrollment if any of the following are true:

1. *Focal pulmonary infiltrates on chest radiograph.*
2. *Diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.*
3. *Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.*
4. *Pregnant (urine HCG +)*
5. *Baseline PCWP > 20 mmHg*

14. Upon conclusion of the INOT22 study and completion of the final study report, INO noted that subsequent to excluding patients with pre-existing LVD, the rate of serious adverse events (including serious adverse events associated with heart failure) was significantly reduced. There were 5 SAEs amongst the first 24 subjects

prior to the additional exclusion criteria, but only 2 SAEs amongst the last 80 subjects in the study after the additional exclusion. Furthermore, there were 2 SAEs amongst the 4 subjects with evidence of pre-existing left ventricular dysfunction, but only 5 SAEs amongst the 120 subjects without evidence of left ventricular dysfunction.

15. Based upon this unexpected finding, on February, 25, 2009, INO submitted a labeling supplement to the FDA seeking to amend the prescribing information for INOMAX to include a warning statement for physicians such that the use of INO in patients with pre-existing LVD could cause serious adverse events, such as pulmonary edema.

16. On August 28, 2009, the FDA approved the INO labeling supplement and included (i) a statement in the Warnings and Precautions section of the INOMAX prescribing information that states "Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema", and (ii) new section 5.4 of the INOMAX prescribing information that states "Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema)."

17. Based upon my review of the medical literature of record in this patent application and pending Office Action, none of the prior art suggests, appreciates or otherwise recognizes that exclusion of neonates, near-term neonates or children with LV dysfunction from administration of INO for diagnostic or treatment purposes would reduce the risk of adverse events and/or serious adverse events, as such terminology is well understood in the medical arts.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from this patent application.

Dated: Sept 29 2010

James S. Baldassarre, M.D.

CURRICULUM VITAE

James S. Baldassarre, MD

HOME ADDRESS:	145 Pebble Woods Dr Doylestown, Pa 18901	**PHONE:** 215-348-2835
PERSONAL:	Married (Susan Cohen-Baldassarre) Children Alyssa (18), Julia (16) and Andrew (10) Citizenship: USA	
BUSINESS ADDRESS:	Ikaria/ INO Therapeutics 6 Route 1173 Clinton, NJ 08809	**PHONE:** 908-238-6363

EDUCATION:	S.U.N.Y. Downstate Medical Center Brooklyn, NY 1986 - M.D.
	S.U.N.Y., Binghamton, NY 1982 - Biology, B.S.

EMPLOYMENT:

2007- present	**Ikaria (INO Therapeutics)** VP, Clinical Research
2009-present	Project Team Leader: IK 5001
2008-2010	Project Team Leader: INOmax®
2003- 2007	**INO Therapeutics** Senior Director, Clinical Research
2003	**Johnson & Johnson Pharmaceutical Research and Development LLC** Compound Development Team Leader/Clinical Leader-REGRANEX®
2001-2003	**Johnson & Johnson Pharmaceutical Research and Development LLC** Senior Director, Operations Team Management
1999-2001	**Janssen Research Foundation** Director of Clinical Research Italy/Greece
1997 -1999	**Janssen-Cilag Limited, UK** Head of Clinical Research and Senior Medical Advisor
1993 - 1997	**R.W. Johnson Pharmaceutical Research Institute** Spring House, PA 1995-1997 Associate Director, Clinical Research 1993-1995 Assistant Director, Clinical Research
1992 - 1993	**Presbyterian Medical Center** Philadelphia, PA Attending Physician, Division of Infectious Diseases
1986 - 1993	**Medical College of Pennsylvania** Philadelphia, PA 1990-1993 Fellow, Division of Infectious Diseases 1989-1990 Medical Director (half time)

1986-1989 Internship/Residency Internal Medicine

1989 - 1990 **Philadelphia Department of Health**
Philadelphia, PA
Medical Director, Sexually Transmitted Diseases Clinic (half time)

ACADEMIC APPOINTMENT:

John Radcliffe Hospital, Oxford, UK

1999-2000 Honorary SHO, Dept of Clinical Pharmacology

Medical College of Pennsylvania, Philadelphia, USA

1994 - Clinical Assistant Professor, Department of Medicine

1991 - 1993 Instructor in Medicine

CERTIFICATION:

Diplomat, A.B.I.M.
Internal Medioine, 1989
Infectious Diseases, 1992
Limited GMC registration, 1999

EMPLOYMENT-RELATED ACTIVITIES/COMMITTEES:

RWJ-PRI Continuous Process Improvement Committee	1995-1996
Johnson & Johnson Signature of Quality submission	1997 and 1999
JJ PRD New Product Development Committee Implementation Team	2002-2003
Ikaria Opportunity Review Team	2007-present

PUBLICATIONS:

1. Levison M E and Baldassarre J S: Intra-Abdominal Infections. *Current Practice of Medicine* 1993.

2. Baldassarre J S and Abrutyn E: Antibiotic-Resistant Streptococcus pneumoniae. Infectious Disease Practice 1993; 17 (9).

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4. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Time to Reassess Treatment Strategies. *Modern Med* 1992; 60:12 86-91.

5. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Keys to Making the Diagnosis. *Modern Med* 1992; 60: 11 42-58.

6. Baldassarre J S, Ingerman M J, Nansteel J, and Santoro J: Development of Listeria Meningitis during Vancomycin Therapy: A Case Report. *J Infect Dis* 1991; 164: 221-222.

7. Baldassarre J S, Update on the Management of Sexually Transmitted Diseases. *Phila Med* 1991; 87-5 230-233.

8. Baldassarre J S and Kaye D: Special Problems in Urinary Tract Infection in the Elderly. *Med Clin North Am* 1991; 75:2 375-390.

9. Baldassarre J S, Johnson CC and Levison M E: Peritonitis: Update on Pathophysiology, Clinical Manifestations and Management. *Clinical Infectious Diseases* 1997; 24(6); 1035-47.

10. Baldassarre JS and Levison ME: Intra-abdominal Infections *Current Practice of Medicine* 1999, vol 2 (4)591-605

11. Baldassarre JS and Pledger GW Clinical Trial Design for New Antiepileptic Drugs: Determination of Dose and Titration Schedules *Rev Contemp Pharmacother* 1999; 10

12. E. Potapov, D. Meyer, M. Swaminathan, M. Ramsay, A. El Banayosy, C. Diehl et al. Use of Inhaled Nitric Oxide After Left Ventricular Assist Device Placement: Results of a Prospective, Randomized, Double-Blind, Multicenter, Placebo-Controlled Trial *J Heart Lung Transplant* 2010 accepted

13. Mercier JC, Hummler H, Durrmeyer X, Sanchez-Luna M, Carnielli V, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, Baldassarre J; EUNO Study Group. Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial. *Lancet.* 2010 Jul 31;376(9738):346-54.

14. Barst RJ, Agnoletti G, Fraisse A, Baldassarre J, Wessel DL; NO Diagnostic Study Group. Vasodilator testing with nitric oxide and/or oxygen in pediatric pulmonary hypertension *Pediatr Cardiol.* 2010 Jul;31(5):598-606.

Book Chapters

Baldassarre J S and Kaye D: Principles and Overview of Antibiotic Use in Infective Endocarditis. In: Kaye D (ed) *Infective Endocarditis* 2nd ed. New York: Raven Press, 1992; 169-190.

Abstracts

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2. Sutherland J and Baldassarre JS : Mediastinal Adenopathy in a Patient with AIDS. American College of Physicians Regional Scientific Meetings, October 2, 1992.

3. Baldassarre J S and Stull T L: Characterization of Aminopeptidase (AP) Activity in Haemophilus ducreyi. American College of Physicians Regional Scientific Meetings, October 3, 1992.

4. Fontinella E. Dorfman M, Baldassarre J, Kaye D and Murasko D: Immune Response to Influenza Immunization in an Elderly Community Dwelling Africa American Population. FASEB J 1991 5: A1373 Abst 5814.

5. Doose DR, Walker SA, Baldassarre J. The effect of food on the oral bioavailability of topiramate from an investigational paediatric sprinkle formulation. Epilepsia 1997; 38(suppl 3):147.

6. Glauser TA, Olberding L, Clark P, Reife R, Baldassarre J, Conover D. Topiramate monotherapy substitution in children with partial epilepsy. Epilepsia 1996; 37(suppl 4):98.

7. JC Mercier, H. Hummler, X Durrmeyer, M. Sanchez-Luna, V Carnielli, D Field, A. Greenough, B. Van Overmeire, B Jonsson, M Hallman, J Baldassarre, for the EUNO Study Group. The effects of inhaled nitric oxide on the development of bronchopulmonary dysplasia (BPD) in preterm infants: the 'EUNO' multicentre randomised clinical trial. European Academy of Pediatrics; Nice, France October 2008

8. RJ Barst, G Agnoletti, A Fraisse, J Baldassarre, DL Wessel. Nitric Oxide in Combination with Oxygen Versus Either Oxygen Alone or Nitric Oxide Alone for Acute Vasodilator Testing in Children with Pulmonary Hypertension: A Multicenter, Randomized Study. Pediatric Academic Societies Scientific Meeting, Baltimore Md; May 2009 [3861.195]

9. EV Potapov; D Meyer; M Swaminathan; M Ramsay; A El Banayosy; C Diehl; B Veynovich; ID Gregoric; J Baldassarre; M J Zucker; R Hetzer Use of Inhaled Nitric Oxide After Left Ventricular

Assist Device Placement: Results of a Prospective, Randomized, Double-Blind, Multicenter, Placebo-Controlled Trial. American Heart Association Scientific Sessions Orlando, Fl; Nov 2009 [3663]

EXHIBIT F

Exhibit F

USSN: 12/820,866

UNITED STATES PATENT AND TRADEMARK OFFICE	
Application Serial Number	12/820,866
Confirmation Number	2913
Filing Date	22-JUN-2010
Title of Application	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION
First Named Inventor	JAMES S. BALDASSARRE
Assignee	IKARIA, INC.
Group Art Unit	1616
Examiner	ARNOLD, ERNST V.
Attorney Docket Number	I001-0002USC1

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JAMES S. BALDASSARRRE, M.D.
UNDER 37 C.F.R. § 1.132

I, James S. Baldassarre, do hereby declare the following:

1. I currently hold the position of Vice President of Clinical Research at INO Therapeutics LLC ("INO"), which is a wholly-owned subsidiary of Ikaria, Inc. A copy of my *curriculum vitae* is attached as **Exhibit 1.**

2. I have over 20 years of experience as a physician and over fifteen years of experience directing clinical research in the pharmaceutical industry.

3. In 2004, I was the Medical Monitor responsible for the design and execution of the INOT22 study.

4. The INOT22 study, entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilatory Testing", was a randomized, multi-center study having an expected

enrollment of 150 patients, aged four weeks to 18 years, in approximately 18 study sites over approximately 2 years.

5. The INOT22 study was established and designed by the study sponsor, INO Therapeutics LLC and a Steering Committee comprising international recognized experts in the field of pediatric heart and lung disease, whose members would assist INO to develop the INOT22 protocol, monitor the progress of the trial, and provide recommendations to INO on changes in the procedures and conduct of the trial

6. The Steering Committee consisted of:

a. David L. Wessel, MD, presently Senior Vice President, The Center for Hospital based Specialties, and Division Chief, Pediatric Critical Care Medicine at Children's National Medical Center, Washington, DC;
b. Robyn J. Barst, MD, presently Professor Emeritus of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York; and
c. Duncan J. Macrae, MD, presently Director, Pediatric Intensive Care, Royal Brompton Hospital, London, UK.

7. The original INOT22 study protocol designed by INO and the Steering Committee did not exclude study patients with pre-existing left ventricular dysfunction who were not dependent on right-to-left shunting of blood.

8. After the INOT22 study protocol design, but prior to study initiation and enrollment, the original INOT22 study protocol was reviewed by an Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the 18 participating study institutions, including review by the principal investigator within each study institution. In addition, prior to study initiation and enrollment, the original INOT22 study protocol was reviewed by the US Food and

Drug Administration (FDA) and separately reviewed by each national Health Authority (European equivalent to FDA) within the four European countries participating in the INOT22 trial (United Kingdom, France, Netherlands and Spain). In addition, INO regularly requested input and scientific guidance on clinical trials from its own Scientific Advisory Board At no time did any member of the Steering Committee, INOT, an IRB, IEC, individual principal investigator, Advisory Board member, FDA or European Health Authority appreciate, recognize or otherwise suggest that subjects with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunt should be excluded from the INOT22 study or that such subjects would be anticipated or predicted to have an increased risk of adverse events or serious adverse events arising from the administration to them of inhaled nitric oxide.

9. Under FDA regulations, an IRB is an appropriately constituted group that has been formally designated to review and monitor biomedical research involving human subjects. In accordance with FDA regulations, an IRB has the authority to approve, require modifications in (to secure approval), or disapprove research. This group review serves an important role in the protection of the rights and welfare of human research subjects. The purpose of IRB review is to assure, both in advance and by periodic review, that appropriate steps are taken to protect the rights and welfare of humans participating as subjects in the research. To accomplish this purpose, IRBs use a group process to review research protocols to ensure protection of the rights and welfare of human subjects of research. An IRB must have at least five members and each member must have enough experience, expertise and diversity to make an informed decision on whether the research is ethical, informed consent if sufficient and the appropriate safeguards have been put in place (see 21 CFR Part 56).

10. In Europe, an Ethics Committee is an independent body in a Member State consisting of healthcare professionals and non-medical members whose responsibility is to protect the rights, safety and well being of human subjects involved in a clinical trial and to provide public assurance of that protection by expressing an opinion on a proposed clinical trial protocol, the suitability of the investigators and adequacy of facilities involved in a trial (see Directive 2001/20/EC).

11. In total, at least 115 individuals experienced in, and responsible for, the review of clinical trial protocols for patient safety, in addition to the FDA and four European Health Authorities reviewed the original INOT22 protocol prior to initiating the INOT22 study. Again, not a single individual or authority suggested, predicted or raised a concern about an increased risk associated with the use of inhaled nitric oxide in study subjects with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunt.

12. On the contrary, it was only after unexpected serious adverse events (including at least one death) occurred during the course of the INOT22 study that the study protocol was amended to exclude study subjects with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunt. In particular, the exclusion criteria of the INOT22 study was amended to exclude subjects having an elevated pulmonary capillary wedge pressure greater than 20 mm Hg.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '359 patent.

Dated: 7 July 2011

James S. Baldassarre, M.D.

CURRICULUM VITAE

James S. Baldassarre, MD

HOME ADDRESS:	145 Pebble Woods Dr Doylestown, Pa 18901	**PHONE:** 215-348-2835

PERSONAL:	Married (Susan Cohen-Baldassarre) Children Alyssa (18), Julia (16) and Andrew (10) Citizenship: USA

BUSINESS ADDRESS:	Ikaria/ INO Therapeutics 6 Route 1173 Clinton, NJ 08809	**PHONE:** 908-238-6363

EDUCATION:	S.U.N.Y. Downstate Medical Center Brooklyn, NY 1986 - M.D.
	S.U.N.Y., Binghamton, NY 1982 - Biology, B.S.

EMPLOYMENT:

2007- present	**Ikaria (INO Therapeutics)** VP, Clinical Research
2009-present	Project Team Leader: IK 5001
2008-2010	Project Team Leader: INOmax®
2003- 2007	**INO Therapeutics** Senior Director, Clinical Research
2003	**Johnson & Johnson Pharmaceutical Research and Development LLC** Compound Development Team Leader/Clinical Leader-REGRANEX®
2001-2003	**Johnson & Johnson Pharmaceutical Research and Development LLC** Senior Director, Operations Team Management
1999-2001	**Janssen Research Foundation** Director of Clinical Research Italy/Greece
1997 -1999	**Janssen-Cilag Limited, UK** Head of Clinical Research and Senior Medical Advisor
1993 - 1997	**R.W. Johnson Pharmaceutical Research Institute** Spring House, PA 1995-1997 Associate Director, Clinical Research 1993-1995 Assistant Director, Clinical Research
1992 - 1993	**Presbyterian Medical Center** Philadelphia, PA Attending Physician, Division of Infectious Diseases
1986 - 1993	**Medical College of Pennsylvania** Philadelphia, PA 1990-1993 Fellow, Division of Infectious Diseases 1989-1990 Medical Director (half time)

1986-1989 Internship/Residency Internal Medicine

1989 - 1990 **Philadelphia Department of Health**
Philadelphia, PA
Medical Director, Sexually Transmitted Diseases Clinic (half time)

ACADEMIC APPOINTMENT :

John Radcliffe Hospital, Oxford, UK

1999-2000 Honorary SHO, Dept of Clinical Pharmacology

Medical College of Pennsylvania, Philadelphia, USA

1994 - Clinical Assistant Professor, Department of Medicine

1991 - 1993 Instructor in Medicine

CERTIFICATION:

Diplomat, A.B.I.M.
Internal Medicine, 1989
Infectious Diseases, 1992
Limited GMC registration, 1999

EMPLOYMENT-RELATED ACTIVITIES/COMMITTEES:

RWJ-PRI Continuous Process Improvement Committee	1995-1996
Johnson & Johnson Signature of Quality submission	1997 and 1999
JJ PRD New Product Development Committee Implementation Team	2002-2003
Ikaria Opportunity Review Team	2007-present

PUBLICATIONS:

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5. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Keys to Making the Diagnosis. *Modern Med* 1992; 60: 11 42-58.
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13. Mercier JC, Hummler H, Durrmeyer X, Sanchez-Luna M, Carnielli V, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, Baldassarre J; EUNO Study Group. Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial. *Lancet.* 2010 Jul 31;376(9738):346-54.

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Book Chapters

Baldassarre J S and Kaye D: Principles and Overview of Antibiotic Use in Infective Endocarditis. In: Kaye D (ed) *Infective Endocarditis* 2nd ed. New York: Raven Press, 1992; 169-190.

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2. Sutherland J and Baldassarre JS : Mediastinal Adenopathy in a Patient with AIDS. American College of Physicians Regional Scientific Meetings, October 2, 1992.

3. Baldassarre J S and Stull T L: Characterization of Aminopeptidase (AP) Activity in Haemophilus ducreyi. American College of Physicians Regional Scientific Meetings, October 3, 1992.

4. Fontinella E, Dorfman M, Baldassarre J, Kaye D and Murasko D: Immune Response to Influenza Immunization in an Elderly Community Dwelling Africa American Population. FASEB J 1991 5: A1373 Abst 5814.

5. Doose DR, Walker SA, Baldassarre J. The effect of food on the oral bioavailability of topiramate from an investigational paediatric sprinkle formulation. Epilepsia 1997; 38(suppl 3):147.

6. Glauser TA, Olberding L, Clark P, Reife R, Baldassarre J, Conover D. Topiramate monotherapy substitution in children with partial epilepsy. Epilepsia 1996; 37(suppl 4):98.

7. JC Mercier, H. Hummler, X Durrmeyer, M. Sanchez-Luna, V Carnielli, D Field, A. Greenough, B. Van Overmeire, B Jonsson, M Hallman, J Baldassarre, for the EUNO Study Group. The effects of inhaled nitric oxide on the development of bronchopulmonary dysplasia (BPD) in preterm infants: the 'EUNO' multicentre randomised clinical trial. European Academy of Pediatrics; Nice, France October 2008

8. RJ Barst, G Agnoletti, A Fraisse, J Baldassarre, DL Wessel. Nitric Oxide in Combination with Oxygen Versus Either Oxygen Alone or Nitric Oxide Alone for Acute Vasodilator Testing in Children with Pulmonary Hypertension: A Multicenter, Randomized Study. Pediatric Academic Societies Scientific Meeting, Baltimore Md; May 2009 [3861.195]

9. EV Potapov; D Meyer; M Swaminathan; M Ramsay; A El Banayosy; C Diehl; B Veynovich; ID Gregoric; J Baldassarre; M J Zucker; R Hetzer Use of Inhaled Nitric Oxide After Left Ventricular

Assist Device Placement: Results of a Prospective, Randomized, Double-Blind, Multicenter, Placebo-Controlled Trial, American Heart Association Scientific Sessions Orlando, Fl; Nov 2009 [3663]

EXHIBIT G

Exhibit G

INOmax® (nitric oxide) for inhalation

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INOmax safely and effectively. See full prescribing information for INOmax.

INOmax (nitric oxide) for inhalation
Initial U.S. Approval: 1999

RECENT MAJOR CHANGES

Warnings and Precautions, Heart Failure (5.4) 8/2009

INDICATIONS AND USAGE

INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (1.1).

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration (1.1).

Utilize additional therapies to maximize oxygen delivery (1.1).

DOSAGE AND ADMINISTRATION

Dosage: The recommended dose of INOmax is 20 ppm, maintained for up to 14 days or until the underlying oxygen desaturation has resolved (2.1).

Administration:

- INOmax must be delivered via a system which does not cause generation of excessive inhaled nitrogen dioxide (2.2).
- Do not discontinue INOmax abruptly (2.2).

DOSAGE FORMS AND STRENGTHS

INOmax (nitric oxide) is a gas available in 100 ppm and 800 ppm concentrations.

CONTRAINDICATIONS

Neonates known to be dependent on right-to-left shunting of blood (4).

WARNINGS AND PRECAUTIONS

Rebound: Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure (5.1).

Methemoglobinemia: Methemoglobin increases with the dose of nitric oxide; following discontinuation or reduction of nitric oxide, methemoglobin levels return to baseline over a period of hours (5.2).

Elevated NO_2 Levels: NO_2 levels should be monitored (5.3).

Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).

ADVERSE REACTIONS

Methemoglobinemia and elevated NO_2 levels are dose dependent adverse events. Worsening oxygenation and increasing pulmonary artery pressure occur if INOmax is discontinued abruptly. Other adverse reactions that occurred in more than 5% of patients receiving INOmax in the CINRGI study were: thrombocytopenia, hypokalemia, bilirubinemia, atelectasis, and hypotension (6).

To report SUSPECTED ADVERSE REACTIONS, contact INO Therapeutics at 1-877-566-9466 and http://www.inomax.com/ or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Nitric oxide donor agents: Nitric oxide donor compounds, such as prilocaine, sodium nitroprusside, and nitroglycerin, when administered as oral, parenteral, or topical formulations, may have an additive effect with INOmax on the risk of developing methemoglobinemia (7).

Revised: August 2009

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoxic Respiratory Failure

INOmax® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies *[see Clinical Studies (14)]*.

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage

Term and near-term neonates with hypoxic respiratory failure

The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

2.2 Administration

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

Do not discontinue INOmax abruptly, as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

3 DOSAGE FORMS AND STRENGTHS

Nitric oxide is a gas available in 100 ppm and 800 ppm concentrations.

4 CONTRAINDICATIONS

INOmax is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.

5 WARNINGS AND PRECAUTIONS

5.1 Rebound

Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

5.2 Methemoglobinemia

Methemoglobinemia increases with the dose of nitric oxide. In clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide, the methemoglobin levels returned to baseline over a period of hours.

5.3 Elevated NO_2 Levels

In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

5.4 Heart Failure

Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from the clinical studies does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

6.1 Clinical Trials Experience

Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse reactions that occurred in at least 5% of patients receiving INOmax in the CINRGI study with event rates >5% and greater than placebo event rates. None of the differences in these adverse reactions were statistically significant when inhaled nitric oxide patients were compared to patients receiving placebo.

Table 1:
Adverse Reactions in the CINRGI Study

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 *(10%)*	13 *(13%)*
Withdrawal	9 *(10%)*	12 *(12%)*
Atelectasis	8 *(9%)*	9 *(9%)*
Hematuria	5 *(6%)*	8 *(8%)*
Hyperglycemia	6 *(7%)*	8 *(8%)*
Sepsis	2 *(2%)*	7 *(7%)*
Infection	3 *(3%)*	6 *(6%)*
Stridor	3 *(3%)*	5 *(5%)*
Cellulitis	0 *(0%)*	5 *(5%)*

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of INOmax. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to estimate their frequency reliably or to establish a causal relationship to drug exposure. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

7 DRUG INTERACTIONS

No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

8.2 Labor and Delivery

The effect of INOmax on labor and delivery in humans is unknown.

8.3 Nursing Mothers

Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

8.4 Pediatric Use

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

8.5 Geriatric Use

Nitric oxide is not indicated for use in the adult population.

10 OVERDOSAGE

Overdosage with INOmax will be manifest by elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

11 DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:

$$\cdot\ddot{N}=\ddot{O}:$$

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide selectively dilates the pulmonary vasculature, and because of efficient scavenging by hemoglobin, has minimal effect on the systemic vasculature.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

12.2 Pharmacodynamics

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

12.3 Pharmacokinetics

The pharmacokinetics of nitric oxide has been studied in adults.

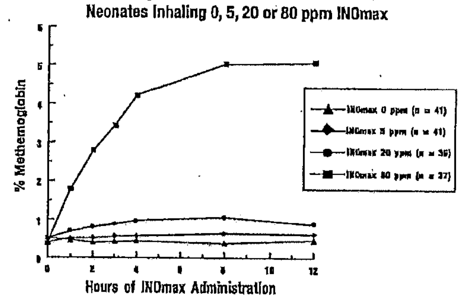
12.4 Pharmacokinetics: Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

12.5 Pharmacokinetics: Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1:
Methemoglobin Concentration – Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groupe, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 35% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients, but one patient did not exceed 7% until 40 hours.

12.6 Pharmacokinetics: Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

14 CLINICAL STUDIES

14.1 Treatment of Hypoxic Respiratory Failure (HRF)

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI= mean airway pressure in cm H_2O × fraction of inspired oxygen concentration [FIO_2]× 100 divided by systemic arterial concentration in mm Hg [PaO_2]) and increases PaO_2 *[see Clinical Pharmacology (12.1)]*.

NINOS Study

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 2.

Table 2:
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups *[see Adverse Reactions (6.1)]*. Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

CINRGI Study

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 3.

Table 3:
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO*,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 58%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups *[see Adverse Reactions (6.1)]*.

14.2 Ineffective in Adult Respiratory Distress Syndrome (ARDS)

ARDS Study

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FIO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

16 HOW SUPPLIED/STORAGE AND HANDLING

INOmax (nitric oxide) is available in the following sizes:

Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)
Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure

The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

 SPC-0303 V:4.0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	James S. Baldassarre et al.	Art Unit	:	1613
Serial No.	:	12/821,041	Examiner	:	Ernst V. Arnold
Filed	:	June 22, 2010	Conf. No.	:	3219
Title	:	METHODS OF TREATING TERM AND NEAR-TERM NEONATES HAVING HYPOXIC RESPIRATORY FAILURE ASSOCIATED WITH CLINICAL OR ECHOCARDIOGRAPHIC EVIDENCE OF PULMONARY HYPERTENSION			

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT UNDER 37 CFR 1.57(f)

The Amendment in Reply to the Final Office Action dated June 27, 2011, filed on even date herewith amends the specification at paragraph [0020] to include language from the INOmax® prescribing information current as of the priority date of this application. (A copy of that INOmax® prescribing information is attached hereto as Exhibit 1.) This language was expressly incorporated by reference by the last sentence of paragraph [0020] of the original specification, in accordance with the requirements of 37 CFR 1.57(b).

In particular, paragraph [0020] was amended as follows:

[0020] INOmax® (nitric oxide) for inhalation was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999. Nitric oxide, the active substance in INOmax®, is a selective pulmonary vasodilator that increases the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from the lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios. INOmax® significantly improves oxygenation, reduces the need for extracorporeal oxygenation and is indicated to be used in conjunction with ventilatory support and other appropriate agents. The current

FDA-approved prescribing information for INOmax® is incorporated herein by reference in its entirety. The CONTRAINDICATIONS section of the prescribing information for INOmax® states that INOmax® should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood.

The amendment described above is appropriately made within the time period set by the Office for responding to the current rejection. To the extent that the claims pending in the case following entry of the Amendment of even date herewith include language based upon the text incorporated by reference from the INOmax® prescribing information, the claims use language based upon the portion of the INOmax® prescribing information that has now been inserted into paragraph [0020].

In accordance with 37 CFR 1.57(f), applicant hereby states that the material inserted into paragraph [0020] of the specification in the Amendment filed on even date herewith (and described above) is material that was previously incorporated by reference in the original specification. The amendment to paragraph [0020] contains no new matter.

If any fees are due for this filing, please apply them to Deposit Account 06-1050, referencing attorney docket no. 26047-0003005.

Respectfully submitted,

Date: January 6, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22766174.doc

EXHIBIT 1

Exhibit 1

INOmax® (nitric oxide) for inhalation
100 and 800 ppm (parts per million)

DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:

•N̈ = Ö:

CLINICAL PHARMACOLOGY

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide produces pulmonary vasodilation.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

PHARMACOKINETICS

The pharmacokinetics of nitric oxide has been studied in adults.

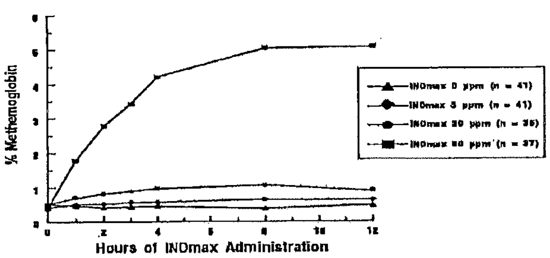
Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1
Methemoglobin Concentration - Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groups, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 35% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients; but one patient did not exceed 7% until 40 hours.

Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

CLINICAL TRIALS

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI= mean airway pressure in cm H_2O x fraction of inspired oxygen concentration [FIO_2] x 100 divided by systemic arterial concentration in mm Hg [PaO_2]) and increases PaO_2 (See CLINICAL PHARMACOLOGY).

NINOS study

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 1.

Table 1
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups (See ADVERSE REACTIONS). Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

CINRGI study

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 2.

Table 2
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO*,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 58%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups (See ADVERSE REACTIONS).

ARDS study

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FIO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

INDICATIONS
INOmax, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

CONTRAINDICATIONS
INOmax should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood.

PRECAUTIONS

Rebound
Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

Methemoglobinemia
Methemoglobinemia increases with the dose of nitric oxide. In the clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide the methemoglobin levels returned to baseline over a period of hours.

Elevated NO_2 Levels
In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

Drug Interactions
No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

Carcinogenesis, Mutagenesis, Impairment of Fertility
No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

Pregnancy: Category C
Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

Nursing Mothers
Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

Pediatric Use
Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

ADVERSE REACTIONS
Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse events with an incidence of at least 5% on INOmax in the CINRGI study, and that were more common on INOmax than on placebo.

ADVERSE EVENTS IN THE CINRGI TRIAL

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 *(10%)*	13 *(13%)*
Withdrawal	9 *(10%)*	12 *(12%)*
Atelectasis	8 *(9%)*	9 *(9%)*
Hematuria	5 *(6%)*	8 *(8%)*
Hyperglycemia	6 *(7%)*	8 *(8%)*
Sepsis	2 *(2%)*	7 *(7%)*
Infection	3 *(3%)*	6 *(6%)*
Stridor	3 *(3%)*	5 *(5%)*
Cellulitis	0 *(0%)*	5 *(5%)*

OVERDOSAGE
Overdosage with INOmax will be manifest by elevations in methemoglobin and NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

POST-MARKETING EXPERIENCE
The following adverse events have been reported as part of the post-marketing surveillance. These events have not been reported above. Given the nature of spontaneously reported post-marketing surveillance data, it is impossible to determine the actual incidence of the events or definitively establish their causal relationship to the drug. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

DOSAGE AND ADMINISTRATION

Dosage
The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

Administration
Additional therapies should be used to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies (see CLINICAL TRIALS).

INOmax should be administered with monitoring for PaO_2, methemoglobin, and NO_2.

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

The INOmax dose should not be discontinued abruptly as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

HOW SUPPLIED
INOmax (nitric oxide) is available in the following sizes:

Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)
Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure
The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

CAUTION
Federal law prohibits dispensing without a prescription.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

 SPC-0303 V:3.0

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Lisa Gray
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Request for Prioritized Examination	1817	1	4800	4800
Pages:				
Claims:				
Miscellaneous-Filing:				
Publ. Fee- early, voluntary, or normal	1504	1	300	300
Processing Fee, except for Provis. apps	1808	1	130	130
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Request for continued examination	1801	1	930	930
		Total in USD ($)		**6160**

Electronic Acknowledgement Receipt

EFS ID:	11781400
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Denise Siede
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	06-JAN-2012
Filing Date:	22-JUN-2010
Time Stamp:	18:08:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$6160
RAM confirmation Number	5373
Deposit Account	061050
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	TrackOne Request	260470003005RequestPriortzdExam.pdf	136674 22901afe3c6b6b7b595b3f9753058268d50f06a5	no	1
Warnings:					
Information:					
2	Request for Continued Examination (RCE)	260470003005RCE.pdf	121860 960094b751fa6b1177030c89db4b049988b90289	no	1
Warnings:					
This is not a USPTO supplied RCE SB30 form.					
Information:					
3		260470003005Reply.pdf	8528318 1f02027b2a34fa082a7829ddedeff85700906f71	yes	155

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment Submitted/Entered with Filing of CPA/RCE	1	1
Specification	2	2
Claims	3	5
Applicant Arguments/Remarks Made in an Amendment	6	155

Warnings:					
Information:					
4	Miscellaneous Incoming Letter	260470003005Statement.pdf	393263 3e5a6a51bbbc8c5fc86a33f93b1b701264d832d8	no	5
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	37375 8a94c0b9eb2661a646bcff55e00ee62fa09331b2	no	2
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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Document code: WFEE

United States Patent and Trademark Office
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HDENDY ADJ #00000001 Mailroom Dt: 01/06/2012
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Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 01/18/2012

HDENDY ADJ #00000002 Mailroom Dt: 01/06/2012
Seq No: 5373 Sales Acctg Dt: 01/09/2012 061050 12821041
01 FC : 1817 4800.00 CR

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number: 12/821,041
Filing Date: 06/22/2010
☐ To be Mailed

APPLICATION AS FILED – PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY ☒ RATE ($)	SMALL ENTITY FEE ($)		OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
			TOTAL			TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED – PART II

AMENDMENT

01/06/2012	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)		OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 15	Minus	** 20	= 0	X $30 =	0	OR	X $ =	
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $125 =	0	OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE	0	OR	TOTAL ADD'L FEE	

AMENDMENT

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/MARY PEOPLES/

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 01/18/2012

HDENDY SALE #00000001 Mailroom Dt: 01/09/2012 061050 12821041
01 FC : 2801 465.00 DA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	James S. Baldassarre et al.	Art Unit	:	1613
Serial No.	:	12/821,041	Examiner	:	Ernst V. Arnold
Filed	:	June 22, 2010	Conf. No.	:	3219
Title	:	METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE			

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR REFUND

This application has small entity status. On January 6, 2012, in a filing made via the Electronic Filing System (EFS), Applicants inadvertently paid large entity fees for a Request for Prioritized Examination ($4800) and Request for Continued Examination ($930), by way of Deposit Account authorization to Fish & Richardson's Deposit Account No. 06-1050. These two fees were thus overpaid by a total of $2865.

Pursuant to 37 CFR § 1.26, Applicants respectfully request that the excess payment be refunded in the form of a credit of $2865 to Deposit Account No. 06-1050, referencing attorney docket number 26047-0003005.

Respectfully submitted,

Date: January 10, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22770415.doc

CERTIFICATE OF (A) MAILING BY FIRST CLASS MAIL OR (B) TRANSMISSION
I hereby certify under 37 CFR §1.8(a) that this correspondence is either (A) addressed as set out in 37 CFR §1.1(a) and being deposited with the United States Postal Service as first class mail with sufficient postage, or (B) being transmitted by facsimile in accordance with 37 CFR § 1.6(d) or via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4), on the date indicated below.

January 10, 2012
Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

Electronic Acknowledgement Receipt

EFS ID:	11802876
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	10-JAN-2012
Filing Date:	22-JUN-2010
Time Stamp:	16:16:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Refund Request	requestrefund.pdf	49472 806c627c2fea5af6a00ae0bca01fe99f531476fd	no	1

Warnings:

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Total Files Size (in bytes):	49472

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	1	Barst et al., "Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension," Received: 14 September 2009 / Accepted: 19 January 2010 Springer Science + Business Media, LLC, 2010, 9 pages	☐
	2	Beggs et al., "Cardiac Failure in Children," 17th Expert Committee on the Selection and Use of Essential Medicines, Geneva, March 2009, 31 pages	☐
	3	Canadian Office Action mailed May 31, 2011 for Canadian patent application No. 2671029, a counterpart foreign application of US patent application No. 12/494,598	☐
	4	UTMB Respiratory Care Services, "Delivery of Inhaled Nitric Oxide Therapy through an Adult or Pediatric Nasal Cannula," (4 pages) July 2003	☐
	5	Douwes et al., "The Maze of Vasodilator Response Criteria," Published online: 26 November 2010, Pediatr Cardiol, (2011) 32: pp. 245-246	☐
	6	Fraisse et al., "Acute pulmonary hypertension in infants and children: cGMP-related drugs," Pediatric Crit Care Med 2010, Vol. 11, No. 2 (Suppl.), 4 pages	☐
	7	Fraisse et al., "Doppler echocardiographic predictors of outcome in newborns with persistent pulmonary hypertension," Cardiol. Young, Vol. 14, pages 277-283, 2004	☐
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	9	INOmax (nitric oxide) for inhalation 100 and 800 ppm (parts per million), drug label insert, 2007, 2 pages	☐
	10	Kay et al., "Congestive heart failure in pediatric patients," From the Department of Pediatrics, Duke University Medical Center, 2001, by Mosby, Inc., 6 pages	☐
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	12	Malloy, "Nitric Oxide Weaning, RT: For Decision Makers in Respiratory Care," http://rtmagazine.com/issues/articles/2000-12_05.asp, 3 pages, December 2000	☐
	13	Rosenberg, "Inhaled nitric oxide in the premature infant with severe hypoxemic respiratory failure: A time for caution," The Journal of Pediatrics, Vol. 133, pages 720-722, December 1998	☐
	14	Advances in Pulmonary Hypertension, Vol 7(4), pages 1-418, Winter 2008-2009 (entire issue)	☐
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	16	Lee & Hayes, Amendment in Reply to Office Action in US Serial No. 12/820,866, mailed June 8, 2011, filed July 8, 2011, 105 pages	☐
	17	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/820,866, mailed August 24, 2011, 27 pages	☐
	18	Fish & Richardson P.C., Brief on Appeal in US Serial No. 12/820,866, filed October 4, 2011, 211 pages	☐
	19	U.S. Examiner Ernst V. Arnold, Examiner Answer in US Serial No. 12/820,866, mailed November 1, 2011, 27 pages	☐
	20	Fish & Richardson P.C., Reply Brief in US Serial No. 12/820,866, filed December 16, 2011, 21 pages	☐
	21	U.S. Examiner Ernst V. Arnold, Non-Final Office Action for US Patent Application 12/820,980, mailed June 10, 2011, 30 pages	☐
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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	23	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/820,980, mailed September 9, 2011, 26 pages	☐
	24	Bates, "Inhaled Nitric Oxide: A Selective Pulmonary Vasodilator," 2004, 9 pages	☐
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	27	NIH CC: Critical Care Services, http://www.cc.nih.gov/ccmd/clinical_services.html; retrieved 3/10/2011, 3 pages	☐
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	Attorney Docket Number	26047-0003005

	34	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/821,020, mailed June 27, 2011, 29 pages	☐
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	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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Name/Print	Janis K. Fraser	Registration Number	34819

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International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	19-JAN-2012
Filing Date:	22-JUN-2010
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Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Lisa Gray
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EFS ID:	11781400
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Denise Siede
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Attorney Docket Number:	26047-0003005
Receipt Date:	06-JAN-2012
Filing Date:	22-JUN-2010
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Application Type:	Utility under 35 USC 111(a)

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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	1	U.S. Examiner Ernst V. Arnold, Office Action in U.S. Serial No. 12/821,020, mailed 01/31/2012, 30 pages.	☐
	2	Krohn, "Effect of inhaled nitric oxide on left ventricular and pulmonary vascular function," The Journal of Thoracic and Cardiovascular Surgery, Vol. 117(1), pages 196-196 (1999).	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-02-07
Name/Print	Janis K. Fraser	Registration Number	34819

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	12017292
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	07-FEB-2012
Filing Date:	22-JUN-2010
Time Stamp:	14:22:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	SB08260470003005.pdf	734937 abf77444af42073fed2af5de3d85abc2895e0255	no	4

Warnings:

Information:

This is not an USPTO supplied IDS fillable form					
2	Non Patent Literature	OA_12821020_013112.pdf	1234093 39bc3b13648b3297c703cba8aeb7c6bc84dfb487	no	30
Warnings:					
Information:					
3	Non Patent Literature	Krohn.pdf	970792 c8a338c71885c7c15688f309fe98dd0974c1886c	no	2
Warnings:					
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		Total Files Size (in bytes):	2939822		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

94169 7590 02/10/2012

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

MAIL DATE	DELIVERY MODE
02/10/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No.	Applicant(s)	
	12/821,041	BALDASSARRE ET AL.	
	Examiner	**Art Unit**	
	ERNST ARNOLD	1613	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE _3_ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on _06 January 2012_.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) _38-52_ is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) _38-52_ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _1/19/12, 2/7/12_.

4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 1/6/12 has been entered.

Claims 1-37 have been cancelled and claims 38-52 are new.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 1/19/12 was filed after the mailing date of the Office Action on 6/27/11. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Withdrawn rejections:

Applicant's amendments and arguments filed 1/6/12 are acknowledged and have been fully considered. Any rejection and/or objection not specifically addressed below is herein withdrawn. Applicant has cancelled all previously pending claims and rejections over those claims have been rendered moot. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set of rejections and/or objections presently being applied to the instant application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 38-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fraisse et al. (Cardiol Young 2004; 14: 277-283 IDS filed on 1/19/12) and Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455) and Kinsella et al. (The Lancet 1999, 354, 1061-1065) and Loh et al. (Circulation 1994, 90, 2780-2785) and Beghetti et al. (the Journal of Pediatrics 1997 page 844) and Ichinose et al. (Circulation 2004; 109:3106-3111: IDS filed on 1/19/12) and INOmax insert (IDS filed on 1/19/12).

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicants claims, for example:

38. (New) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, the method comprising:

(a) identifying a term or near-term neonate patient in need of inhaled nitric oxide treatment, wherein the patient is not known to be dependent on right-to-left shunting of blood;

(b) determining that the patient identified in (a) has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has pre-existing left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

Determination of the scope and content of the prior art

(MPEP 2141.01)

Fraisse et al. sought to identify the predictors of extracorporeal membrane oxygenation therapy, death and response to iNO by performing detailed diagnostic screening with Doppler echocardiographic **screening** of the patient, **neonates**, with suspected pulmonary hypertension (Abstract; page 278 Patients and methods). The non-invasive technique allows for measurement of ventricular function and estimates both the direction and degree of shunting including bi-directional shunting (page 277 right column; page 278, right column; and pages 279-280, Tables 1 and 2 and appropriate text). Fraisse et al. teach that right to left ductal shunting of blood was found to be an independent predictor of death (Abstract). Fraisse et al. teach that ***a left to right***

shunting of blood increases the risk of failing to respond to iNO including a patient with severe left ventricular dysfunction (Abstract and page 281 upper left column). Thus the patient is not known to be dependent on right to left shunting of blood and the patient had pre-existing left ventricular dysfunction before administration of iNO was performed. Furthermore, patients without LVD were provided the iNO therapy (see Tables 1 and 2).

Fraisse et al. teach that 44 neonates started iNO therapy at 40-80 ppm and the clinical data and hemodynamic characteristics are in Table 2 (page 280 right column).

Fraisse et al. teach on page 281:

A comprehensive echocardiographic examination is an integral element of the initial evaluation of newborns with persistent pulmonary hypertension, both in order to exclude structural congenital heart disease, and to assess cardiac function.[11] Echocardiography is also a valuable non-invasive method for evaluating the degree of pulmonary hypertension, the extrapulmonary shunt, and ventricular function.[3,5,8–12] In the present study, the majority of the patients had either normal, or only mildly depressed, left and right ventricular systolic function. Several factors can cause biventricular dysfunction in newborns with persistent pulmonary hypertension. These include pulmonary hypertension by itself, an alteration in the left ventricular geometry due to the pressure overloaded right ventricle, hypoxaemia causing generalised myocardial ischeamia, and metabolic acidemia.[13] As in our study, however, others have found significant depression of left ventricular function in less than one-fifth of patients with persistent pulmonary hypertension of the newborn.[8,11] Right ventricular dys-

And on page 282:

The results of our present study, however, indicate that an exclusively left-to-right shunt across the atrial septum increases the risk of failing to respond to nitric oxide, with an odds ratio of 7.46, and a p value equal to 0.028. Left-to-right shunting across the atrial septum is usual in newborns with a patent oval foramen and normally compliant ventricles. In persistent pulmonary hypertension of the newborn, a left-to-right atrial shunt associated with a predominantly left-to-right ductal shunt and a normal biventricular function may reflect intrapulmonary shunting. In this subgroup of patients, systemic oxygenation is significantly less improved by inhalation of nitric oxide.[10] Another potential pathophysiologic mechanism that underlies this finding may involve reduced left ventricular compliance, leading to increased left atrial pressure, with a resultant left-to-right shunt across the oval foramen. Decreased left ventricular compliance may occur in persistent pulmonary hypertension of the newborn due to adverse interaction between the ventricles, a leftward shift of the ventricular septum secondary to right ventricular hypertension, decreased left ventricular diastolic filling, and left ventricular systolic dysfunction due to decreased preload, hypoxaemia, and acidosis. Even when left ventricular systolic function is severely depressed in these patients, the right ventricle can maintain systemic output through the patent arterial duct. Selective pulmonary vasodilation with inhalation

> of nitric oxide in this circumstance may not give the desired clinical response, because the blood flowing across the duct is redistributed away from the systemic circulation towards the lungs, decreasing post-ductal systemic output, and increasing the left atrial pressure.[14]

And…

> are at increased risk of death. A pure left-to-right ductal shunt tends to be associated with greater need for extracorporeal membrane oxygenation, and should prompt cautious re-evaluation of the indication for further treatment aimed at increasing pulmonary vasodilation. The direction of flow across the atrial

The Examiner interprets “reduced left ventricular compliance” to be a dysfunction of the left ventricle such that compliance is reduced.

Atz et al. teach methods using inhaled nitric oxide in the **neonate**, which is a child, with cardiac disease, hence an **identified patient** in need of nitric oxide treatment, (title and Abstract) which intrinsically provides pharmaceutically acceptable NO gas for inhalation to a medical provider to provide to the patient. Atz et al. warn that sudden pulmonary vasodilation may produce **pulmonary edema** (page 452, left column). Atz et al. teach that: “Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension.” (page 452, left column). Since the patients have pulmonary hypertension as claimed in instant claim 25 then they also intrinsically have hypoxic respiratory failure in the absence of evidence to the contrary. It is irrelevant how the hypoxic respiratory failure is associated with clinical or echocardiographic evidence of pulmonary hypertension because the hypoxia is intimately tied to the pulmonary hypertension regardless of how it is evidenced. Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively ***right to left shunting*** at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse outcomes*** in this circumstance." (page 452, left column) (Examiner added emphasis). Therefore, it is known in the art that patients who had pre-existing LVD treated with NO for any duration may experience adverse outcomes. Artz et al. thus identify conditions in the patients which is screening of the patient. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.

To summarize, the methods disclosed by Atz et al. are interpreted to mean:

- identifying a patient eligible for NO treatment;
- diagnosing/identifying if the patient has left ventricular dysfunction;
- excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment.

Atz et al. teach neonates with pulmonary hypertension (Abstract and page 442, left column to right column) thus the hypertension is diagnosed in the patient population.

Kinsella et al. teach excluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and page 1062, Methods). Since left ventricular dysfunction is a congenital heart disease, as acknowledged by Applicant, (see specification [0028]), and it would be pre-existing, then the methods of Kinsella et al. intrinsically exclude this patient population from the method. The patients also had pulmonary hypertension which would be associated with the cardiac function (Abstract). Thus, one or more adverse events are reduced in the neonates excluded from the method. The neonate must breathe oxygen to survive. Furthermore, if the patients are already excluded then any further limitations on the treatment are truly irrelevant. The intended patient population is intrinsically at risk of one or more adverse events. Patients are intrinsically identified for nitric oxide inhalation treatment, diagnosed for congenital heart disease which intrinsically includes left ventricular dysfunction, and if the patient meets the criteria than treatment with NO is performed thereby reducing the risk of adverse events associated with the treatment. The neonate must breathe oxygen to survive.

Loh et al. teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract). Loh et al. clearly teaches that patients with pulmonary artery wedge pressure, which is synonymous with the instantly claimed pulmonary capillary wedge pressure, ***of greater than or equal to 18 mm Hg*** had a greater effect of inhaled NO due to the greater degree of reactive pulmonary hypertension present in such patients (page 2784, left column). Loh et al. state: "*Since the degree of reactive pulmonary hypertension is generally related to the severity of hemodynamic compromise in patients with LV failure, it might be anticipated that patients with more severe heart failure will* *have a more marked hemodynamic response to inhaled NO*." Loh et al. examined this prediction further and verified it (page 2784, left column). Loh et al. establishes that a pulmonary capillary wedge pressure (PCWP) of greater than 18 mg Hg serves as a guidepost for alerting the artisan to adverse events from inhaled NO. Thus, the art already teaches inhaled NO increases the wedge pressure as taught by Loh et al. (see entire document).

Beghetti et al. teach:

> A structurally normal heart with severe LV dysfunction and a bidirectional shunt through a patent ductus arteriosus does not suggest that systemic perfusion is duct dependent, inasmuch as the shunt is not exclusively unidirectional right to left. Bidirectional shunting usually is explained by a high, near systemic, total pulmonary vascular resistance resulting from maladaptation of the pulmonary circulation to the extrauterine life, and perhaps also by reflex pulmonary vasoconstriction induced by severe LV dysfunction.
>
> In patients with increased pulmonary venous pressure caused by LV dysfunction and elevated left atrial pressure, a decrease in pulmonary vascular resistance (induced by iNO) will lead to an increase in pulmonary venous return and hence to an increase in left atrial and LV filling pressure.[2,3] This increase may not be assumed by a failing left ventricle that is working on the flat portion of the Frank-Starling curve. Accordingly, we believe that, in the patient described, massive vasodilation induced by iNO resulted in further LV failure.
>
> Inhaled nitric oxide should be administered with caution to babies with LV dysfunction because pulmonary vasoconstriction may act as a protective mechanism of LV overfilling.

Let the Examiner reiterate: "***Inhaled nitric oxide should be administered with caution to babies with LV dysfunction because pulmonary vasoconstriction may act as a protective mechanism of LV overfilling***." (page 844).

Ichinose et al. teach inhalation of NO can increase left ventricle filling pressure in patients with severe left ventricle dysfunction and that it is important to be aware of the possibility that inhaled NO can produce pulmonary vasodilation and may overwhelm a failing left ventricle thereby producing **pulmonary edema** (page 3109 bottom left to top right columns).

INOmax insert provides a source of compressed blend of nitrogen and nitric oxide gas for inhalation therapy (see entire insert).

Summary of the art

Does the art teach administration of iNO to neonates? Yes, neonates or child, are administered inhaled nitric oxide therapy as taught by Atz, Fraisse, Ichinose and Beghetti.

Can neonates have left ventricular dysfunction? Yes, neonates can have left ventricle dysfunction which can be physically manifested in different forms as taught by Fraisse.

Is it known in the art that iNO therapy can cause pulmonary edema? Yes, the preponderance of art cited clearly indicates that inhaled NO can produce pulmonary vasodilation and can overwhelm a dysfunctional left ventricle resulting in pulmonary edema regardless of right to left shunting.

Is it known in the art that iNO causes an increase in pulmonary wedge pressure? Yes, the art teaches that iNO increases pulmonary wedge pressure as taught by Loh.

Does the art caution and warn the ordinary artisan that adverse events can occur in neonates with left ventricular dysfunction? Yes, the art cautions and warns of administering inhaled NO to babies with left ventricle dysfunction as taught by Atz, Beghetti and Ichinose because of adverse events which include pulmonary edema.

It cannot be concluded that Applicant discovered any of these concepts.

Ascertainment of the difference between the prior art and the claims

(MPEP 2141.02)

1. The difference between the instant application and Fraisse et al. is that Fraisse et al. do not expressly teach the step of excluding a neonate with LVD from a plurality of neonates from iNO therapy such that the risk of occurrence of pulmonary edema is reduced or informing the medical provider that iNO may cause pulmonary edema in a neonate with pre-existing LVD or wherein the neonate has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg but administering iNO therapy to a neonate that does not have pre-existing LVD but has congenital heart disease. This deficiency in Fraisse et al. is cured by the teachings of Kinsella et al., Loh et al. Fraisse, Ichinose and Beghetti.

2. The difference between the instant application and Fraisse et al. is that Fraisse et al. do not expressly teach the source of nitric oxide gas is a cylinder containing a compressed blend of nitric oxide and nitrogen. This deficiency in Fraisse et al. is cured by the teachings of the INOmax insert.

Finding of prima facie obviousness

Rational and Motivation (MPEP 2142-2143)

1. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Fraisse et al. and exclude a term or near term neonate with LVD from a plurality of neonates from iNO therapy such that the risk of occurrence of pulmonary edema is reduced or informing the medical provider that iNO may cause pulmonary edema in a neonate with pre-existing LVD or wherein the neonate has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg but administering iNO therapy to a neonate that does not have pre-existing LVD but has congenital heart disease**,** as suggested by Atz et al., Kinsella et al., Loh et al. Fraisse, Ichinose and Beghetti., and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because it is overwhelmingly known in the art that inhaled nitric oxide can cause pulmonary edema especially in those with a dysfunctional left ventricle. Consequently, it requires absolutely no inventive skill to exclude those patients, including term and near term neonates, with pre-existing left ventricle dysfunction from inhalation of nitric oxide gas in order to reduce the occurrence of pulmonary edema by informing the medical provider because iNO is known not only to increase pulmonary wedge pressure but also cause pulmonary edema. Indeed, Loh et al. provide the benchmark value for the wedge pressure as discussed above. Furthermore, allowing those patients without left ventricle dysfunction but having congenital heart disease to receive iNO therapy is part of the purpose of iNO therapy in the first place and requires absolutely no inventive skill to perform as evidenced by the numerous references cited by the Examiner in this rejection and the references made of record by the Examiner.

For the record, to reduce the risk of occurrence of any adverse side effect of any medical therapy the obvious choice is to exclude that patient from the medical therapy based on the sound logic that if the medical therapy is not administered then the adverse side effect cannot occur because of the medical therapy. In the instant case, the preponderance of art teaches and suggests that neonates with left ventricle dysfunction regardless of directional blood shunting are prone to adverse effects from iNO therapy. The artisan with that knowledge can then exclude or include such a patient for iNO therapy at their discretion knowing and expecting that an adverse event such as pulmonary edema can occur. As stated by Berghetti et al: "***Inhaled nitric oxide should be administered with caution to babies with LV dysfunction because pulmonary vasoconstriction may act as a protective mechanism of LV overfilling***." (page 844). The consequence of this is that the left ventricle overfills and the blood backs up to the lungs thereby increasing the pressure which forces fluid into the tissue and causes pulmonary edema. In other words, the art is already aware that patients with left ventricular dysfunction are at risk of pulmonary edema from iNO therapy and consequently it obvious to the ordinary artisan that this will occur regardless of the shunting of blood direction. As stated in paragraph 22 of Dr. Greene's Declaration: "*On analyzing the data from the study, the inventors concluded that a correlation did, in fact, exist between the severe adverse events that had occurred during the study and the left ventricular dysfunction of the patients that had suffered them*." It is the Examiner's position that all Applicant has done is confirmed what was already known in the art and that the data is worthy of publication but is obvious to the ordinary artisan given the art as a whole.

2. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Fraisse et al. with the nitric oxide source from INOmax and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because Fraisse et al. teach using inhaled nitric oxide but not the source and the INOmax insert provides a ready source of nitric oxide blended with nitrogen in a cylinder for use in inhalation therapy. It is then simply a matter of judicious selection of known sources of nitric oxide for inhalation therapy by the ordinary artisan.

In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a).

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Response to Arguments:

In light of the new ground of rejection and despite Applicant's 27 pages of argument, the Declaration of Dr. Greene and the informative recent interview conducted with Applicant's representative, Applicant's arguments are moot and the claims remain rejected as obvious. Respectfully, the totality of the art informs the artisan that patients, be they neonates, children or adults, with a failing left ventricle can be predisposed to pulmonary edema due to inhalation of NO gas therapy. It appears that Applicant has confirmed the teachings in the art and it remains obvious to exclude those patients with pre-existing left ventricular dysfunction from iNO therapy because of the risk of adverse effects such as pulmonary edema. In other words, the risk of pulmonary edema from iNO therapy is 0% if the patient does not receive iNO therapy.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

1. Claims 38-53 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 29-42 of copending Application No. 12/820980. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy by informing medical providers that iNO therapy may cause pulmonary edema.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

2. Claims 38-52 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 31-45 of copending Application No. 12/821020. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left

ventricular dysfunction from iNO therapy by informing medical providers that iNO therapy may cause pulmonary edema.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

3. Claims 38-52 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 28-42 of copending Application No. 12/820866. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy.

The copending application does not expressly teach informing the medical provider of the risk of pulmonary edema.

However, informing a medical provider of a possible risk is obvious in the medical arts.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

No claims are allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

- Henrichsen teaches that iNO caused systemic hypotension in a baby with primary pulmonary hypertension and left ventricular dysfunction and therefore NO should be administered with caution to such babies (Journal of Pediatrics 1996, 129(1) page 183).
- Krohn cites a paper that reporting 3 incidents where left atrial pressure increased and pulmonary edema appeared when subjects with left ventricular dysfunction inhaled NO therapeutically (Krohn The Journal of Thoracic and Cardiovascular Surgery 1999, 117(1) pages 195-196).
- Semigran teaches that inhaled NO causes an increase in left ventricular filling pressure by an unknown mechanism (Abstract of J Am Coll Cardiol 1994; 24: 982-988).
- Hayward (Cardiovascular Research 1999; 43:628-638) reports that massive pulmonary vasodilation may occasionally overwhelm the left ventricle if it is significantly impaired (page 633, bottom left to top right column) and that pulmonary edema during administration of iNO has been documented in patients with congestive heart failure (page 632, 4.2 right column).
- Bocchi teach that patients that inhaled 40 ppm or 80 ppm NO caused an increase in pulmonary wedge pressure and resulted in pulmonary edema which was

caused by the acute increment of blood return to the impaired left ventricle that caused the increase in wedge pressure and consequently pulmonary edema (pages 70-71 of The American Journal of Cardiology 1994, 74, pp: 70-72. 4 pages).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ERNST ARNOLD whose telephone number is (571)272-8509. The examiner can normally be reached on M-F 7:15-4:45.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Kwon can be reached on 571-272-0581. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

Notice of References Cited	Application/Control No. 12/821,041	Applicant(s)/Patent Under Reexamination BALDASSARRE ET AL.	
	Examiner ERNST ARNOLD	Art Unit 1613	Page 1 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
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	C	US-			
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	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Henrishsen (Journal of Pediatrics 1996, 129(1) page 183).
	V	Krohn (The Journal of Thoracic and Cardiovascular Surgery 1999, 117(1) pages 195-196).
	W	Semigren (Abstract of J Am Coll Cardiol 1994; 24: 982-988).
	X	Hayward (Cardiovascular Research 1999; 43:628-638)

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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	Examiner ERNST ARNOLD	Art Unit 1613	Page 2 of 2

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	I	US-			
	J	US-			
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	M	US-			

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	N					
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	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Bocchi (The American Journal of Cardiology 1994, 74, pp: 70-72. 4 pages).
	V	Beghetti et al. (the Journal of Pediatrics 1997 page 844).
	W	
	X	

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12821041	BALDASSARRE ET AL.
	Examiner	**Art Unit**
	ERNST V ARNOLD	1616

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
inventor name EAST/PALM	8/11/10	eva
EAST 424/718 text limted all databases	8/11/10	eva
google	8/10/10	eva
various disucssions with QAS Bennett Celsa and Jean Vollano concering proper incorporation by reference as well as patentabilityn	6/18/11	eva
updated IDS	2/9/12	eva
consultation QAS Jean Vollano	2/2/12	eva

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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	Art Unit		1613
	Examiner Name	Ernst V. Arnold	
	Attorney Docket Number		26047-0003005

	1	Barst et al., "Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension," Received: 14 September 2009 / Accepted: 19 January 2010 Springer Science + Business Media, LLC, 2010, 9 pages	☐
	2	Beggs et al., "Cardiac Failure in Children," 17th Expert Committee on the Selection and Use of Essential Medicines, Geneva, March 2009, 31 pages	☐
	3	Canadian Office Action mailed May 31, 2011 for Canadian patent application No. 2671029, a counterpart foreign application of US patent application No. 12/494,598	☐
	4	UTMB Respiratory Care Services, "Delivery of Inhaled Nitric Oxide Therapy through an Adult or Pediatric Nasal Cannula," (4 pages) July 2003	☐
	5	Douwes et al., "The Maze of Vasodilator Response Criteria," Published online: 26 November 2010, Pediatr Cardiol, (2011) 32: pp. 245-246	☐
	6	Fraisse et al., "Acute pulmonary hypertension in infants and children: cGMP-related drugs," Pediatric Crit Care Med 2010, Vol. 11, No. 2 (Suppl.), 4 pages	☐
	7	Fraisse et al., "Doppler echocardiographic predictors of outcome in newborns with persistent pulmonary hypertension," Cardiol. Young, Vol. 14, pages 277-283, 2004	☐
	8	Ichinose et al., "Inhaled Nitric Oxide, A Selective Pulmonary Vasodilator: Current Uses and Therapeutic Potential," Circulation, Vol. 109, pages 3106-3111, February 11, 2011	☐
	9	INOmax (nitric oxide) for inhalation 100 and 800 ppm (parts per million), drug label insert, 2007, 2 pages	☐
	10	Kay et al., "Congestive heart failure in pediatric patients," From the Department of Pediatrics, Duke University Medical Center, 2001, by Mosby, Inc., 6 pages	☐
	11	Kondun et al., "A Randomized Trial of Early Versus Standard Inhaled Nitric Oxide Therapy in Term and Near-Term Newborn Infants With Hypoxic Respiratory Failure," Pediatrics, Vol. 113, pages 559-564, 2004	☐

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	Attorney Docket Number	26047-0003005

	12	Malloy, "Nitric Oxide Weaning, RT: For Decision Makers in Respiratory Care," http://rtmagazine.com/issues/articles/2000-12_05.asp, 3 pages, December 2000	☐
	13	Rosenberg, "Inhaled nitric oxide in the premature infant with severe hypoxemic respiratory failure: A time for caution," The Journal of Pediatrics, Vol. 133, pages 720-722, December 1998	☐
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	15	U.S. Examiner Ernst V. Arnold, Non-final Office Action in US Serial No. 12/820,866, mailed June 8, 2011, 33 pages	☐
	16	Lee & Hayes, Amendment in Reply to Office Action in US Serial No. 12/820,866, mailed June 8, 2011, filed July 8, 2011, 105 pages	☐
	17	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/820,866, mailed August 24, 2011, 27 pages	☐
	18	Fish & Richardson P.C., Brief on Appeal in US Serial No. 12/820,866, filed October 4, 2011, 211 pages	☐
	19	U.S. Examiner Ernst V. Arnold, Examiner Answer in US Serial No. 12/820,866, mailed November 1, 2011, 27 pages	☐
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	31	Lee & Hayes, Replacement Reply Amendment in US Serial No. 12/821,020, mailed August 13, 2010, filed February 14, 2010, 18 pages	☐
	32	Lee & Hayes, Supplemental Reply Amendment in US Serial No. 12/821,020, filed April 12, 2011, 9 pages	☐
	33	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/821,020, mailed August 13, 2010, 32 pages	☐

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	Attorney Docket Number	26047-0003005

	34	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/821,020, mailed June 27, 2011, 29 pages	☐
	35	Fish & Richardson P.C., Supplement to the Reply Brief, US Serial No. 12/820,866, filed January 3, 2012, 3 pages	☐
	36	Fish & Richardson P.C., Amendment in Reply to Final Office Action, US Serial No. 12/821,020, mailed June 27, 2011, filed December 27, 2011, 153 pages	☐

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Examiner Signature	/Ernst Arnold/	Date Considered	02/07/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-01-19
Name/Print	Janis K. Fraser	Registration Number	34819

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

/E.A./	1	U.S. Examiner Ernst V. Arnold, Office Action in U.S. Serial No. 12/821,020, mailed 01/31/2012, 30 pages.	☐
/E.A./	2	Krohn, "Effect of inhaled nitric oxide on left ventricular and pulmonary vascular function," The Journal of Thoracic and Cardiovascular Surgery, Vol. 117(1), pages 196-196 (1999).	☐

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Examiner Signature	/Ernst Arnold/	Date Considered	02/09/2012

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number		12821041
	Filing Date		2010-06-22
	First Named Inventor	Baldassarre	
	Art Unit		1613
	Examiner Name	Ernst V. Arnold	
	Attorney Docket Number		26047-0003005

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-02-07
Name/Print	Janis K. Fraser	Registration Number	34819

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	1	Azeka, et al., "Effects of Low Doses Of Inhaled Nitric Oxide Combined with Oxygen for the Evaluation Of Pulmonary Vascular Reactivity in Patients with Pulmonary Hypertension," Pediatric Cardiol, Vol. 23, pages 20-26 (2002)	☐
	2	Barst et al., "Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension," Pediatr. Cardiol., Vol. 31, pages 598-606 (2010)	☐
	3	Beghetti et al., "Inhaled nitric oxide and congenital cardiac disease," Cardiol. Young, Vol. 11, pages 142-152 (2001).	☐
	4	Bichel et al., "Successful weaning from cardiopulmonary bypass after cardiac surgery using inhaled nitric oxide," Pediatric Anaesthesia, Vol. 7, pages 335-339 (1997)	☐
	5	Bin-Nun et al., "Role of iNO in the modulation of pulmonary vascular resistance," Journal of Perinatology, Vol. 28, pages S84-S92 (2008)	☐
	6	Dickstein et al., "A theoretic analysis of the effect of pulmonary vasodilation on pulmonary venous pressure: Implications for inhaled nitric oxide therapy," J Heart Lung Transplant, Vol. 15, pages 715-21 (1996)	☐
	7	Haddad et al., "Use of inhaled nitric oxide perioperatively and in intensive care patients," Anesthesiology, Vol. 92, pages 1821-1825 (2000)	☐
	8	Hayward et al., "Inhaled Nitric Oxide in Cardiac Failure: Vascular Versus Ventricular Effects," Journal of Cardiovascular Pharmacology," Vol. 27, pages 80-85 (1996)	☐
	9	Kieler-Jensen et al., "Inhaled nitric oxide in the evaluation of heart transplant candidates with elevated pulmonary vascular resistance," J Heart Lung Transplant, Vol. 13, pages 366-375 (1994)	☐
	10	Kulik, "Inhaled nitric oxide in the management of congenital heart disease," Current Opinion in Cardiology, Vol. 11, pages 75-80 (1996)	☐
	11	Madriago M.D. et al., "Heart Failure in Infants and Children," Pediatrics in Review, Fol. 31, pages 4-12 (2010)	☐

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	Application Number	12821041
	Filing Date	2010-06-22
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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	12	Steudel et al., "Inhaled nitric oxide," Anesthesiology, Vol. 91, pages 1090-1121 (1999)	☐
	13	Wessel et al., "Managing low cardiac output syndrome after congenital heart surgery," Crit. Care Med., Vol. 29(10) pages S220-S230 (2001)	☐
	14	U.S. Examiner Ernst V. Arnold, Interview Summary in U.S. Serial No. 12/821,020, mailed January 25, 2012, 4 pages	☐
	15	Fish & Richardson, P.C., Statement of the Substance of the Interview and Comments on Examiner's Interview Summary, in U.S. Serial No. 12,821,020, filed February 27, 2012, 7 pages	☐
	16	U.S. Examiner Ernst V. Arnold, Interview Summary in U.S. Serial No. 12/821,020, mailed April 17, 2012, 12 pages	☐
	17	Fish & Richardson P.C., Statement of Substance of Interview and Comments on Examiner's Interview Summary, in U.S. Serial No. 12/821,020 filed April 23, 2012, 8 pages	☐
	18	INO Therapeutics, "Comparison of Inhaled Nitric Oxide and Oxygen in Patient Reactivity during Acute Pulmonary Vasodilator Testing," downloaded from clinicaltrials.gov on April 23, 2012; first received on February 20, 2008; last updated on October 18, 2010.	☐

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	Attorney Docket Number	26047-0003005

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SIGNATURE

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Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-04-23
Name/Print	Janis K. Fraser	Registration Number	34819

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Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Lisa Gray
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
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Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

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EFS ID:	12607550
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Lisa Gray
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	23-APR-2012
Filing Date:	22-JUN-2010
Time Stamp:	20:48:39
Application Type:	Utility under 35 USC 111(a)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

94169 7590 05/03/2012

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

MAIL DATE	DELIVERY MODE
05/03/2012	PAPER

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The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

Examiner-Initiated Interview Summary	Application No. 12/821,041	Applicant(s) BALDASSARRE ET AL.	
	Examiner ERNST ARNOLD	Art Unit 1613	

All participants (applicant, applicant's representative, PTO personnel):

(1) ERNST ARNOLD. (3)_____.

(2) Janice Fraser. (4)_____.

Date of Interview: 23 April 2012.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The Examiner notified Applicant that the Office Action in this application will also be withdrawn for the same reasons discussed in 12/821020 and another Office Action will be forthcoming. Applicant alerted the Examiner that an IDS was filed in the copending '020 application and not to file anything before consideration of the IDS could be done.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL AMENDMENT AND REMARKS

This application has been granted special status under the prioritized examination (Track 1) program. An Office action was mailed February 10, 2012, setting a three-month deadline for response of May 10, 2012. As indicated in the Interview Summary mailed by the Office on May 3, 2012, the Examiner informed the undersigned in a telephone call on April 23, 2012, that the Office action would be replaced with a new Office action. In addition, the transaction history for this application on PAIR has an entry dated April 24, 2012, that says "Withdrawing/Vacating Office Action Letter," and a second entry dated May 3, 2012, that says "Mail Notice of Withdrawn Action." Applicants thus assume that there is no longer a pending deadline for response, and there will be no deadline for response until the new Office action is mailed and thereby resets a new deadline.

Applicants ask that the present amendment be entered, and the below remarks considered, prior to preparation of a new Office action in this case.

Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application.

Listing of Claims:

1-37. (Canceled)

38. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, said method comprising:

(a) performing echocardiography to identify~~identifying~~ a term or near-term neonate patient in need of inhaled nitric oxide treatment for pulmonary hypertension, wherein the patient is not ~~known to be~~ dependent on right-to-left shunting of blood;

(b) determining that the patient identified in (a) has ~~pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has ~~pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

39. (Currently amended) The method of claim 38, wherein step (b) comprises performing echocardiography~~the patient has pulmonary hypertension~~.

40. (Currently amended) The method of claim 38, wherein step (b) comprises measuring the patient's pulmonary capillary wedge pressure ~~the patient has a pulmonary capillary wedge pressure that is greater than or equal to 20 mm Hg~~.

41. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, said method comprising:

(a) ~~identifying a term or near-term neonate patient~~carrying out a diagnostic process comprising measuring blood oxygen level, to identify a term or near-term neonate patient as being in need of inhaled nitric oxide treatment for hypoxic respiratory failure, wherein the patient is not ~~known to be~~ dependent on right-to-left shunting of blood;

(b) performing echocardiography and/or measuring pulmonary capillary wedge pressure to determine that the patient has ~~determining by diagnostic screening that the patient identified in (a) has pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from treatment with inhaled nitric oxide based on the determination that the patient has ~~pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

42. (Currently amended) The method of claim 41, wherein the diagnostic process of step (a) further comprises performing~~screening comprises~~ echocardiography.

43. (Currently amended) The method of claim 41, wherein step (b) comprises performing echocardiography~~the patient has pulmonary hypertension~~.

44. (Currently amended) The method of claim 41, wherein in step (b), the patient's pulmonary capillary wedge pressure is measured and determined to be ~~the patient has a pulmonary capillary wedge pressure that is~~ greater than or equal to 20 mm Hg.

45. (Currently amended) A method of treatment ~~reducing the risk of occurrence of pulmonary edema associated with medical treatment comprising inhalation of nitric oxide gas, said method~~ comprising:

(a) performing echocardiography to identify~~identifying~~ a plurality of term or near-term neonate patients who are in need of inhaled nitric oxide treatment for pulmonary hypertension, wherein the patients are not ~~known to be~~ dependent on right-to-left shunting of blood;

(b) determining that a first patient of the plurality has ~~pre-existing~~ left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) determining that a second patient of the plurality does not have ~~pre-existing~~ left ventricular dysfunction;

(d) administering the inhaled nitric oxide treatment to the second patient; and

(e) excluding the first patient from treatment with inhaled nitric oxide, based on the determination that the first patient has ~~pre-existing~~ left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

46. (Currently amended) The method of claim 45, wherein step (a) further comprises measuring blood oxygen levels in the first and second patient and thereby determining that the first and second patient are hypoxic ~~have pulmonary hypertension~~.

47. (Previously presented) The method of claim 45, wherein the second patient has congenital heart disease.

48. (Currently amended) The method of claim 45, wherein step (b) comprises measuring the first patient's ~~the first patient has a~~ pulmonary capillary wedge pressure ~~greater than or equal to 20 mm Hg~~.

49. (Currently amended) The method of claim 45, wherein determining that the first patient of the plurality has pre-existing left ventricular dysfunction and the second patient of the plurality does not have pre-existing left ventricular dysfunction comprises performing echocardiography~~diagnostic screening~~.

50 - 52. (Canceled)

53. (New) A method of treatment comprising:

(a) identifying a plurality of term or near-term neonate patients who are in need of inhaled nitric oxide treatment, wherein the patients are not dependent on right-to-left shunting of blood;

(b) in a first patient of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the first patient of the plurality has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) in a second patient of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the second patient of the plurality does not have left ventricular dysfunction;

(d) administering inhaled nitric oxide treatment to the second patient; and

(e) excluding the first patient from treatment with inhaled nitric oxide, based on the determination that the first patient has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

54. (New) The method of claim 53, wherein step (a) comprises performing echocardiography to determine that the first and second patients have pulmonary hypertension.

55. (New) The method of claim 53, wherein step (a) comprises measuring blood oxygen levels in the first and second patients and thereby determining that the first and second patients are hypoxic.

56. (New) The method of claim 53, wherein the second patient has congenital heart disease.

57. (New) The method of claim 53, wherein step (b) comprises measuring the first patient's pulmonary capillary wedge pressure and determining that it is greater than or equal to 20 mm Hg.

58. (New) The method of claim 38, wherein the patient's left ventricular dysfunction is attributable to congenital heart disease.

59. (New) The method of claim 38, wherein the patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the patient is excluded from inhaled nitric oxide treatment based on the determination that the patient has left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide.

60. (New) The method of claim 41, wherein the left ventricular dysfunction is attributable to congenital heart disease.

61. (New) The method of claim 45, wherein the left ventricular dysfunction is attributable to congenital heart disease.

62. (New) The method of claim 46, wherein the left ventricular dysfunction is attributable to congenital heart disease.

63. (New) The method of claim 34, wherein the patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the patient is excluded from inhaled nitric oxide treatment based on the determination that the patient has left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

64. (New) The method of claim 63, wherein the left ventricular dysfunction is attributable to congenital heart disease.

65. (New) The method of claim 45, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

66. (New) The method of claim 45, wherein the first patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the first patient is excluded from inhaled nitric oxide treatment based on the determination that the first patient has left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

67. (New) The method of claim 66, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

68. (New) The method of claim 53, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

69. (New) The method of claim 53, wherein the first patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the first patient is excluded from inhaled nitric oxide treatment based on the determination that the first patient has pre-existing left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

70. (New) The method of claim 69, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

REMARKS

Upon entry of the above amendment, claims 38-49 and 53-70 will be pending, claims 50-52 having been newly canceled and new claims 53-70 added. Claims 1-37 were canceled in a prior amendment. Support for the amended and new claims can be found throughout the specification, e.g., in paragraphs [0004]-[0006], [0014], [0017], [0018], [0023], [0027]-[0029], [0033], [0039], [0040], and [0042]. No new matter has been added.

As there are only four independent claims and 30 total claims (and no multiply dependent claims) in the application following entry of the above amendment, this application continues to qualify for special status under the provisions for Prioritized Examination (Track 1).

Statement of the Substance of Multiple Telephonic Interviews

On April 23, 2012, Examiner Arnold telephoned the undersigned to confirm that the Office action mailed February 10, 2012 (the 2/10/12 Office action) was being withdrawn and would be replaced with a new Office action setting a new deadline for response.

On April 30, 2012, the undersigned spoke by telephone with SPE Brian Kwon, who noted that the Office actions in both the present case and a sister case (USSN 12/821,020) had been withdrawn and would be replaced with new Office actions.

Also on April 30, 2012, the undersigned spoke by telephone with SPE Marjorie Moran. SPE Moran confirmed that she had advised Examiner Arnold regarding how to apply the US Supreme Court's decisions concerning patent-eligible subject matter. SPE Moran provided some helpful, specific guidance for applicants as to what kinds of amendments might be useful in overcoming a potential rejection for lack of patent-eligible subject matter. Applicants are grateful for the guidance, and have closely followed SPE Moran's advice in drafting the present amendments.

Comments Regarding Some of the Present Amendments

The amendment deletes the term "pre-existing" from the phrase "pre-existing left ventricular dysfunction," wherever that phrase appears in the claims.

The amendment deletes the term "known to be" from the phrase "the patient is not known to be dependent on right-to-left shunting of blood," wherever that phrase appears in the claims.

The amendment adds at least one action step (e.g., "performing echocardiography") to each independent claim, as suggested by SPE Moran, in an effort to obviate any possible grounds for rejection for lack of patent-eligible subject matter under 35 USC § 101, and thereby expedite prosecution.

Request for Panel Decision

Applicants respectfully request that SPE Brian Kwon and QAS Julie Burke participate actively in the prosecution of this application as a panel with Examiner Arnold, as they are doing in a sister application, US application No. 12/821,020 (the '020 case). Applicants gratefully note that their perspective on the latter case has been very helpful to date in moving that case forward, and expect that it will similarly be helpful in the present case.

Discussion of technical points

The remarks below are intended to assist the Examiner in understanding some technical points that appear to applicants to be a source of confusion in this case and the '020 case. The topics covered are:

(1) the significance of the claim language "wherein the patient is not dependent on right-to-left shunting of blood";

(2) the description of the patient who is the subject of the claimed method;

(3) the disclosures of the various references cited in the obviousness rejection set forth in the 2/10/12 Office action; and

(4) the Examiner's conclusion that the art teaches that administering inhaled NO to babies with left ventricular dysfunction can cause pulmonary edema.

By resolving the apparent confusion regarding those four topics, applicants believe that these remarks should be very useful in moving the case forward efficiently.

(1) The significance of the claim language "*wherein the patient is not dependent on right-to-left shunting of blood.*"

The language "wherein the patient is not dependent on right-to-left shunting of blood" (or its equivalent "wherein the patients are not dependent on right-to-left shunting of blood") appears in step (a) of each of the pending independent claims, as amended above. It effectively narrows the scope of the claimed method by excluding outright some patients from the set of patients who are the subject of the method.

The term "dependent on right-to-left shunting of blood" is well understood in the medical art. See, for example, the use of this term in the 2007 INOmax® prescribing information[1] cited in the 2/10/12 Office action as the "INOmax insert" (page 2, left column, under "Contraindications"). The INOmax insert refers to a condition occasionally seen in neonates born with an absent or nonfunctional left ventricle -- the ventricle that normally pumps blood into the systemic circulation. Ordinarily, a neonate with an absent or nonfunctional left ventricle will die immediately from a lack of systemic circulation. Under certain circumstances, however, these neonates may survive: i.e., when two other independent conditions both happen to exist concurrently with the nonfunctional left ventricle: (i) an open (patent) ductus arteriosus, and (ii) an abnormally high level of pulmonary vascular resistance (routinely arising from pulmonary hypertension). When both of these conditions exist concurrently in a neonate who lacks a functional left ventricle, the neonate's right ventricle (which normally pumps blood only into the lungs) can take over the left ventricle's normal function of supplying blood flow to the systemic circulation. The right ventricle would have no outlet into the systemic circulation unless the infant's ductus arteriosus, a vascular connection between the pulmonary artery (which exits the right ventricle) and the aorta (which feeds the systemic circulation), remains open after birth. The ductus arteriosus normally closes at birth. If instead it remains open in a neonate who has

[1] Also commonly referred to as the "package insert" or "PI".

no functioning left ventricle, the ductus arteriosus will provide a conduit for some of the blood pumped by the right ventricle to shunt into the systemic circulation rather than taking its normal route into the lungs. This is termed a right-to-left shunt through a patent ductus arteriosus (PDA). If the neonate concurrently has pulmonary hypertension, this means relatively less blood goes from the right ventricle into the vasoconstricted lungs, thereby allowing more blood to shunt from the right ventricle through the PDA. In some cases, enough blood shunts through the PDA to sustain the systemic circulation. If the amount of blood flowing from the right ventricle through the PDA into the systemic circulation is sufficient to maintain life, and if the neonate's left ventricle is so severely dysfunctional that, absent this shunt through the PDA, the neonate would die from an inadequate systemic circulation, the neonate is said to be "***dependent*** on right-to-left shunting of blood." The reason this dependence on right-to-left shunting of blood has always been a contraindication on the INOmax® package insert since the product was first marketed is because it was known in the art that a patient who has pulmonary hypertension and is dependent on right-to-left shunting of blood, and who is treated with inhaled nitric oxide to open up the pulmonary blood vessels and thereby allow more blood to flow through the lungs, can suffer a catastrophic loss of the right-to-left blood flow through the PDA on which the patient depends for life.

There are many other situations in which a patient who is a candidate for treatment with inhaled nitric oxide (e.g., because the patient has pulmonary hypertension) exhibits a right-to-left shunt, a left-to-right shunt, or even a bi-directional shunt. Such a shunt can be through a PDA; through an open foramen ovale (a hole in the septum (wall) between the right and left atria); or through a hole in the septum between the left and right ventricles, termed a ventricular-septal defect. Except for the single situation described above with the particular combination of three conditions specified above (i.e., nonfunctional left ventricle, pulmonary hypertension, and a PDA through which blood shunts right-to-left in a volume that is sufficient to maintain the systemic circulation despite the nonfunctional left ventricle), the patient is not "dependent" on any of these shunts—i.e., his/her life does not depend on maintaining the shunt. In fact, it is more common that a shunt is harmful rather than helpful to the patient, because it diverts blood away from its normal path through the right side of the heart to the lungs (where it is oxygenated), then into the left side of the heart, and from there into the systemic circulation for delivery to all parts of the body. For example, a right-to-left shunt at the atrial level, i.e., through the foramen ovale, means some of the deoxygenated blood entering the right atrium is shunted into the left atrium instead of taking its normal path into the right ventricle and then into the lungs. In such a patient, the "shunted" deoxygenated blood then passes from the left atrium into the left ventricle and is pumped by the left ventricle into the systemic circulation, still in its deoxygenated state, leaving the infant chronically poorly oxygenated. Far from being "dependent" on this right-to-left-shunt through the foramen ovale, the patient would be much better off without it.

The articles cited by the Examiner in the obviousness rejection described in the 2/10/12 Office action discuss in various contexts right-to-left shunts and left-to-right shunts (sometimes referring to the shunt as "exclusively" right-to-left or "exclusively" left-to-right). These shunts may occur at an open foramen ovale, or at a PDA, or at a ventricular-septal defect. The sole situation in which the patient is "dependent" on a shunt is the one described above, where the patient has a combination of *pulmonary hypertension, a severely dysfunctional or absent left ventricle, and a right-to-left shunt through a PDA that permits the right ventricle's output to reach the systemic circulation through the shunt*. (As described on page 452, left column, of Atz & Wessel, Seminars in Perinatology 1997, 21(5): 441-455 (one of the references cited in the 2/10/12 Office action), such a patient may also have, in addition to that combination of conditions, a left-to-right shunt through an open foramen ovale; such a patient is still characterized as "dependent on a right-to-left shunt" because of the critical role played by the right-to-left shunt through the PDA.) Characterizing a shunt as "exclusively" right-to-left or "exclusively" left-to-right means that the blood flows only in the indicated direction through that shunt. It does not mean, and does not even imply, that the patient is "dependent" on the shunt. In fact, most patients who have a shunt that is exclusively in one direction are harmed by the shunt--far from being "dependent" on it.

Applicants hope that the above discussion helps to clarify the significance of the word "dependent" in the claim language "dependent on right-to-left shunting of blood."

(2) The description of the patient who is the subject of the claimed method.

During an in-person interview with Examiner Arnold, SPE Kwon, and QAS Burke in the '020 application on April 13, 2012 (hereinafter the "4/13/12 Interview"), QAS Burke mentioned that the negative limitations of claim 31 of that application made the claim somewhat difficult to parse. Claim 31 of that application is highly similar to claim 38 of the present application, differing only in that the former refers to "child" while the latter refers to "term or near-term neonate patient." Applicants have attempted to simplify the claims of both cases by omitting the words "known to be" in step (a) of each independent claim.

Claim 38 as presently amended is a drawn to a method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, where the method includes identifying a narrowly defined category of term or near-term neonate patients who are in need of nitric oxide treatment but who are at particular risk of pulmonary edema from that treatment, and excluding from the treatment any patient who falls into that defined category of at-risk patients. It is important to note that the prior art was unaware that any neonates were at particular risk of pulmonary edema when treated with inhaled nitric oxide. The prior art did know that some neonates (i.e., those who are dependent on right-to-left shunting of blood) were at risk of systemic hypotension when treated with inhaled nitric oxide, but this risk has nothing to do with a risk of pulmonary edema and does not predict a risk of pulmonary edema. *Thus, the claim would be novel and nonobvious regardless of how the category of neonate patients to be excluded from the treatment is defined in the claim.* Since the basis for the invention was the discovery that children (including neonates) who have left ventricular dysfunction are surprisingly at risk for pulmonary edema when they are treated with inhaled nitric oxide, the claims include a limitation that the neonate to be excluded from treatment due to this risk is determined to have left ventricular dysfunction. In addition to this limitation on the scope of the claim, applicants have chosen to narrow the scope even further by explicitly requiring that the category of neonates covered by the claim not include those who are dependent on right to left shunting of blood.

Applicants hope that this discussion of the claims will help the Examiner understand the nature of the claims and the effect of the various limitations on claim scope.

(3) The disclosures of the various references cited in the obviousness rejection set forth in the 2/10/12 Office action.

The comments below address the following six references that were cited by the Office in support of the obviousness rejection in the 2/10/12 Office action. The below comments focus on what applicants believe are misinterpretations of the references expressed in that Office action. Applicants realize that Office action has been withdrawn and so the prior obviousness rejection is presently moot, but are concerned that the same references may be cited in a new Office action. Thus, to facilitate efficient prosecution, applicants would like to clarify for the Examiner's benefit what those references actually say regarding the points raised in the Office action. The references considered below are:

Fraisse et al., Cardiol Young 2004; 14:277-283;
Atz & Wessel (mentioned above);
Kinsella et al., The Lancet 1999; 354:1061-1065;
Loh et al., Circulation 1994; 90:2780-2785;
Beghetti et al., J. Pediatrics 1997; page 844;
Henrichsen et al., Journal of Pediatrics 1996; 129(1):183; and
Ichinose et al., Circulation 2004; 109:3106-3111.

Fraisse et al.

Applicants first point out that the senior author on Fraisse et al. is David L. Wessel, M.D. Dr. Wessel is also the senior author of Atz & Wessel. His views about the nonobviousness of the present invention are set forth in the Declaration of David L. Wessel, M.D. under 37 CFR § 1.132 submitted with applicants' Reply filed January 6, 2012 (the 1/6/12 Reply), and are discussed in detail in the 1/6/12 Reply. In brief, Dr. Wessel, who was presumably fully aware of both of these articles that he co-authored, says that he did not expect that children who have pulmonary hypertension and LVD would be at increased risk of pulmonary edema upon inhalation of nitric oxide, until after the INOT22 clinical trial had proven, to his surprise, that this was indeed a real risk. That trial concluded long after Fraisse et al.'s 2004 publication date and Atz & Wessel's 1997 publication date. *This is a substantial clue that the Examiner's interpretation of these two articles as disclosing such a risk is incorrect.* That the Examiner's interpretation is indeed incorrect is borne out by a careful parsing of what Fraisse et al. and Atz & Wessel actually say. Applicants attempted to do that with respect to Atz & Wessel in the 1/6/12 Reply, and with respect to Fraisse et al. in the 4/13/12 Interview. Fraisse et al. is addressed in more detail here.

Fraisse et al. performed a retrospective analysis of echocardiographic features of newborns with persistent pulmonary hypertension who had been randomized to receive inhaled nitric oxide or other therapy in a previous clinical trial. The purpose of the Fraisse et al. analysis was to see whether these features could be used as a predictor of what the clinical trial had defined as a successful response to inhaled nitric oxide therapy. *See*, abstract. The clinical trial had defined a successful response to inhaled nitric oxide therapy as occurring when the patient survived without having to be placed on an alternative therapy (extracorporeal membrane oxygenation, "ECMO") to improve oxygenation. Fraisse et al. says nothing about pulmonary edema nor any other adverse events attributable to treatment with inhaled nitric oxide, except for noting that one patient whose systemic circulation was dependent on a right-to-left shunt through an open ductus arteriosus[2] experienced "haemodynamic deterioration" when inhaling nitric oxide

[2] The patient also reportedly had "an exclusively left-to-right shunt at the atrial level." In other words, the foramen ovale was open and allowed blood to flow in one direction, from the left atrium into the right atrium (i.e., left to right). In a patient who is dependent on a right-to-left shunt through a PDA, a left-to-right shunt through the foramen ovale has two effects: (1) it provides an outlet out of the left atrium for blood entering the left atrium from the lungs, thereby relieving pressure on the dysfunctional left ventricle; and (2) it allows oxygenated blood from the left atrium to mix with the deoxygenated blood being pumped from the right atrium into the right ventricle, which can pump it through the ductus arteriosus into the systemic circulation—i.e., it increases the oxygenation level of the blood entering the systemic circulation through the PDA.

(see page 281, upper left column). That haemodynamic deterioration was likely systemic hypotension,[3] i.e., not related to pulmonary edema.

> The 2/10/12 Office action at pages 4-5 characterizes Fraisse et al. in part as follows:
>
> Fraisse et al. teach that ***a left to right shunting of blood*** increases the risk of failing to respond to iNO including a patient with severe left ventricular dysfunction (Abstract and page 281 upper left column). (Emphasis in the original.)

The Fraisse et al. abstract and page 281, upper left column, does teach that left to right shunting of blood at the atrial level (i.e., through an open foramen ovale)[4] increased the risk of *failing to respond* to inhaled nitric oxide. Further, the cited part of Fraisse et al. at page 281, upper left column, does describe a patient with left to right shunting of blood at the atrial level who also had severe left ventricular dysfunction and who *failed to respond* to inhaled nitric oxide. However, the significance of those observations to the present claims is not clear, since the claims are not about identifying patients who will respond, or fail to respond, to inhaled nitric oxide. Rather, the claims are about reducing the risk of pulmonary edema. Pulmonary edema is a side effect that would be triggered by treatment with inhaled nitric oxide only when a patient's pulmonary hypertension responds well to the treatment—i.e., when the treatment is effective in relaxing the constricted pulmonary blood vessels, permitting an increased volume of blood to flow through the lungs and into the left side of the heart. It appears that the Examiner may have confused the concept of *failure to respond* to a given treatment with the concept of *adverse events* caused by the treatment. As noted by Dr. Greene during the 4/13/12 Interview, these are two entirely different concepts.

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[3] Elsewhere (page 280, top of right column) Fraisse et al. uses the term "haemodynamic instability" to mean "hypotension."

[4] A shunt at the "atrial level" is a shunt through the foramen ovale, a hole between the left atrium and right atrium (chambers of the heart). The word "atrial" should not be confused with the similar word "arterial", which refers to arteries and not chambers of the heart.

The Office action continues:

> Thus the patient is not known to be dependent on right to left shunting of blood and the patient had pre-existing left ventricular dysfunction before administration of iNO was performed.

The individual patient to which this sentence refers cannot be characterized, as the Office does, as "not known to be dependent on right to left shunting of blood." In fact, the description of that particular patient at page 281, upper left column, of Fraisse et al. says essentially the opposite:

> This last patient [who presented with persistent pulmonary hypertension], with an exclusively left-to-right shunt at the atrial level, also had a right-to-left ductal shunt. His left ventricular function was severely depressed, with echocardiographic evidence of a right ventricular dependent circulation. (Emphasis added)

A "right-to-left ductal shunt" is a right-to-left shunt through a patent ductus arteriosus (i.e., PDA). A "right ventricular dependent circulation" means, of course, that the right ventricle had taken over the job of supplying blood to the systemic circulation since the left ventricle's function was severely depressed. Fraisse et al. thus describes this neonatal patient as showing evidence of a combination of five conditions:

(i) persistent pulmonary hypertension;

(ii) an exclusively left-to-right shunt at the atrial level (i.e., through an open foramen ovale);

(iii) a right-to-left ductal shunt (i.e., through a PDA);

(iv) severely depressed left ventricular function (i.e., left ventricular dysfunction, or LVD); and

(v) evidence of a right ventricular dependent circulation (i.e., since his left ventricle was not functioning properly, the only way this patient survived was because his right ventricle had taken over the job of pumping blood into the systemic circulation, and that occurred only because the ductus arteriosus was open and permitted blood to flow from the pulmonary artery through the PDA into the aorta). This patient appears to fit the classic description of a neonatal LVD patient whose systemic circulation is dependent on right-to-left shunting of blood through a PDA, and who therefore should not be given inhaled nitric oxide because of the risk of systemic circulatory collapse, i.e., systemic hypotension. (See, e.g., the description of such newborns provided on page 452 of Atz & Wessel, as described in detail in applicants' 2/10/12 Reply at pages 12-15.)

Indeed, Fraisse et al. describes this particular patient as having "responded poorly to inhalation of nitric oxide, with persistence of hypoxaemia and haemodynamic deterioration." The "haemodynamic deterioration" was likely systemic hypotension induced by diversion of blood into the lungs and away from the PDA upon which the patient's systemic circulation depended, severely reducing the flow of blood into the systemic circulation. Applicants therefore submit that the Examiner is mistaken in asserting that this patient "is not known to be dependent on right to left shunting of blood." That plainly is not the case.

The 2/10/12 Office action continues by pointing to Table 2 of Fraisse et al. as giving clinical data and hemodynamic characteristics of 44 neonates who started treatment with inhaled nitric oxide. See, the 2/10/12 Office action at page 5. No explanation is provided as to what, if anything, in this table is considered to be relevant to the claims. Applicants note that, according to Table 2, three of the patients treated with inhaled nitric oxide reportedly had "moderately or severely depressed" left ventricular function. The table categorizes one of these as a "responder" (i.e., inhaled nitric oxide was effective) and two as "non-responders" (i.e., inhaled nitric oxide was not effective). Five other patients who were classified as having "mildly depressed" left ventricular function all were "responders." The table does not report any adverse events (pulmonary edema or otherwise) caused by the treatment in any patients. It therefore seems irrelevant to the claims, except as a possible *teaching-away*.

The 2/10/12 Office action then quotes extensively from pages 281 and 282 of Fraisse et al., without comment except to say on page 7: "The Examiner interprets 'reduced left ventricular compliance' to be a dysfunction of the left ventricle such that compliance is reduced." Absent the Examiner's views of why the lengthy quoted text is relevant to the claims, applicants are uncertain how to respond. *Below is a brief summary of the text that the 2/10/12 Office action quoted from pages 281 and 282 of Fraisse et al., with applicants' comments.*

The text from page 281 of Fraisse et al. is quoted on page 5 of the 2/10/12 Office action. It begins with a general description of how echocardiography is used in evaluating newborns with persistent pulmonary hypertension. It then discusses the authors' findings regarding left and right ventricular function in the patients included in the study, including an observation that some patients had significant depression of left ventricular function.

The text from page 282 appears on pages 6-7 of the Office action. It was extracted from a paragraph of Fraisse et al. that begins by noting that several studies have shown that inhaled nitric oxide is effective in improving oxygenation and reducing the need for ECMO in newborns with persistent pulmonary hypertension. The quoted paragraph then says that the results of the present study indicate that those newborns with an exclusively left-to-right shunt across the atrial septum (i.e., through an open foramen ovale) have an increased risk of failing to respond to nitric oxide. *(Note that the authors did not assess side effects of the treatment, but rather only response or failure to respond.)* Fraisse et al. discuss the phenomenon of left-to-right shunting across the atrial septum in the context of a predominantly left-to-right ductal shunt and <u>normal biventricular function</u>, saying that "[in] this subgroup of patients, systemic oxygenation is significantly less improved by inhalation of nitric oxide"—i.e., the treatment is not as effective as it is in other patients. (Note that this particular discussion in Fraisse et al. refers to patients with "normal biventricular function," meaning that their left and right ventricles both function normally, so there is plainly no LVD; furthermore, it is about effectiveness of the treatment, not adverse events caused by the treatment. It therefore appears to be irrelevant to the present claims.)

According to the authors, left to right shunting across the atrial septum may also occur in another context: a patient with decreased left ventricular compliance may have increased left atrial pressure, and this can produce "a resultant left-to-right shunt across the oval foramen." In other words, the increased pressure built up in the left atrium because the left ventricle has decreased compliance can cause blood to escape the left atrium through the open foramen ovale into the right atrium (i.e., left to right). In this situation, the open foramen ovale acts like a pressure relief valve for the left atrium. Note that there is <u>no</u> suggestion that, instead of escaping through the foramen ovale, the blood would back up into the pulmonary vessels and produce pulmonary edema; <u>rather, the only disclosed result of the increased left atrial pressure is a left to right shunt of blood from the left atrium into the right atrium</u>. This shunt would presumably serve to relieve at least some of the left atrial pressure, leaving one of skill in the art with no reason to expect that pulmonary edema would develop. Thus, this part of Fraisse et al. also appears to *teach away* from the presently claimed methods—and certainly does not support the rejection.

The reference goes on to explain what might cause decreased left ventricular compliance in patients with persistent pulmonary hypertension of the newborn. The causes listed by Fraisse et al. include adverse interaction between the ventricles (i.e., the adjacent left and right ventricles don't interact in a normal way, typically due to an enlarged right ventricle that is filled with blood at abnormally high pressure as it works hard to push blood into the constricted lung blood vessels); a leftward shift of the ventricular septum (i.e., the septum or wall shared by both ventricles is pushed "leftward" into the left ventricle's space by the enlarged right ventricle); decreased left ventricular diastolic filling (there is an inadequate volume of blood flowing from the vasoconstricted lungs into the left side of the heart, and less room in the left ventricle because of interference by the right ventricle, adding up to decreased filling of the left ventricle); and left ventricular systolic (emptying) dysfunction due to decreased preload (i.e., the "preload," or pressure exerted on the left ventricle by the blood present in the left atrium, is decreased due to the decreased flow of blood from the lungs into the left atrium and/or due to an open foramen ovale that permits blood to leak out of the left atrium into the right atrium; this decreased preload can make the left ventricle less efficient at contracting), hypoxaemia (low oxygenation), and acidosis (increased acidity of the blood). Fraisse et al. then describe what happens when left ventricular systolic (emptying) function is severely depressed in newborns with persistent pulmonary hypertension: the right ventricle takes over, providing blood flow to the systemic circulation by pumping blood through the patent (open) arterial duct (i.e., the PDA). In other words, this patient's systemic circulation is dependent on the right-to-left shunt through the PDA. As taught by Fraisse et al. on page 282, top of right column, treating such a patient with inhaled nitric oxide "may not give the desired clinical response, because the blood flowing across the duct is redistributed away from the systemic circulation towards the lungs, decreasing post-ductal systemic output, and increasing the left atrial pressure." Thus, Fraisse et al. points out that neonates whose systemic circulation is dependent on a right-to-left shunt through the open ductus are expected to suffer a loss of "post-ductal systemic output" (i.e., flow from the right side of the heart through the open ductus into the systemic circulation) if they are treated with inhaled nitric oxide—i.e., they may end up with life-threatening systemic hypotension. This is, of course, the well-known contraindication for inhaled nitric oxide in patients who are dependent on a right-to-left shunt, a set of patients explicitly <u>outside</u> the category of neonates defined in part (a) of each of the independent claims. This discussion by Fraisse et al. therefore has nothing to do with the category of patients to whom the claimed method applies. Furthermore, ***it has nothing to do with pulmonary edema.***

Applicants note for the record that Fraisse et al.'s reference to "increasing the left atrial pressure" as one of the effects of inhaled nitric oxide in these patients does not imply that pulmonary edema would result. For example: if, prior to the treatment, the left atrial pressure was below normal (as may occur when pulmonary hypertension has reduced the blood flow into the left atrium, and as confirmed by the reference in the quoted text to "decreased preload"[5]), the increase in left atrial pressure following the treatment may just bring the pressure up to a normal range. Thus, the observation about "increasing the left atrial pressure" does not in itself imply any pathology. Further, the cite provided by Fraisse et al. as support for the statement about "decreasing post-ductal systemic output, and increasing the left atrial pressure" is Henrichsen et al., *J. Pediatr.* 1996; 129:183, a case study of a single infant who was reported to be dependent on a right-to-left shunt and who suffered systemic hypotension (<u>not</u> pulmonary edema) after being treated with inhaled nitric oxide. Applicants submit that the sole relevance of this part of Fraisse et al. is as a description of patients who are dependent on a right-to-left shunt at the ductus arteriosus, a set of patients explicitly carved out of the category of neonates that is the subject of the claimed methods. ***Thus, Fraisse et al.'s teaching regarding what occurs in neonates dependent on right-to-left shunting of blood is entirely irrelevant to the claimed methods.***

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[5] Fifth line from the bottom of page 5 of the Office action. "Preload" in this context is the pressure exerted on the left ventricle by the volume of blood present in the left atrium. "Decreased preload" means the pressure is below normal.

The final passage that the 2/10/12 Office action quotes from Fraisse et al. is taken from the last paragraph on page 282. The sentence fragment "are at increased risk of death" that begins the quoted section is derived from a sentence that reads in full: "A pure right-to-left ductal shunt identified the patients who are at increased risk of death." This "risk of death" was not attributed to the treatment *per se*, but rather to the underlying condition. (*See*, e.g., page 281, right column, second full paragraph.) Further, Fraisse et al. does not suggest that the patients found to be at increased risk of death had LVD, nor that they suffered from pulmonary edema. That part of the quoted text is therefore, for several reasons, irrelevant to the present claims. The quoted section then says, "A pure left-to-right ductal shunt tends to be associated with greater need for extracorporeal membrane oxygenation, and should prompt cautious re-evaluation of the indication for further treatment aimed at increasing pulmonary vasodilation." Applicants cannot see how this statement is at all pertinent to the presently claimed methods. It does not suggest that the patients with the left-to-right ductal shunt had LVD, and it concerns the lack of efficacy of inhaled nitric oxide in patients with a left-to-right ductal shunt--not adverse events (pulmonary edema or anything else) attributable to this treatment. If the Examiner intends to cite Fraisse et al. (and these statements of Fraisse et al. in particular) in a new obviousness rejection, he is respectfully asked to clarify why he believes these statements of Fraisse et al. to be relevant. They appear to be as irrelevant as the other Fraisse et al. text discussed above.

In sum, Fraisse et al. is concerned with using echocardiography to identify neonates in whom inhaled nitric oxide is less likely to be efficacious—i.e., who died from their underlying condition despite the inhaled nitric oxide treatment, or who had to be put on ECMO in an effort to improve their oxygenation and keep them alive. Though some of the neonates in the trial analyzed by Fraisse et al. did have evidence of LVD, the authors do not link that observation to any identified risk—or even a reduction in efficacy--of the treatment, except for one patient in whom LVD was combined with dependence on a right-to-left shunt at the ductus arteriosus, so who is explicitly outside the population of patients defined as the subject of the present claims. In fact, the utter lack of any mention by Fraisse et al. of an actual or expected increased incidence of pulmonary edema in *any* subset of the neonates in the study following treatment with inhaled nitric oxide suggests that no such increased incidence was expected, much less found. Further, Fraisse et al. observed that increased left atrial pressure due to decreased left ventricular compliance was associated with an escape valve of sorts: a flow of blood from the left atrium to the right atrium through the open oval foramen.[6] ***Thus, Fraisse et al.'s only apparent relevance to the present claims is as a teaching away.***

If the Examiner disagrees with this assessment of the Fraisse et al. article, he is asked to explain why.

Atz & Wessel

The alleged teachings of Atz & Wessel are described on pages 7-8 of the 2/10/12 Office action:

> Atz et al. warn that sudden pulmonary vasodilation may produce **pulmonary edema** (page 452, left column). Atz et al. teach that: "Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension." (page 452, left column)…. Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively ***right to left shunting*** at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse outcomes*** in this circumstance." (page 452, left column) (Examiner added emphasis). Therefore it is known in the art that patients who had pre-existing LVD treated with NO for any duration may experience adverse outcomes…. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.
>
> To summarize, the methods disclosed by Atz et al. are interpreted to mean:
>
> - identifying a patient eligible for NO treatment;
> - diagnosing/identifying if the patient has left ventricular dysfunction;
> - excluding that patient with left ventricular dysfunction from treatment with NO but treating the patient with NO for other conditions discussed by Atz et al. with inhalation of NO thereby reducing the risk of adverse events associated with the medical treatment.

[6] Page 282, left column, last paragraph.

This characterization of Atz & Wessel is exactly the same as the one presented on pages 9-10 of the Office action dated June 27, 2011 (the "6/27/11 Office action"). Applicants' reply to the 6/27/11 Office action (the 1/6/12 Reply) included a detailed rebuttal of the Examiner's characterization of Atz & Wessel, pointing out that the Examiner's interpretation of the Atz & Wessel reference was far broader than what it really says. See pages 10-18 of the 1/6/12 Reply. Applicants' arguments were not simply opinion, but rather were supported by a careful parsing of the crucial paragraph on page 452 of the reference as well as by factual evidence submitted with the 1/6/12 Reply, and were intended to assist the Examiner in coming to a clearer understanding what the reference actually communicated to those of skill in the art. Unfortunately, rather than address applicants' arguments and evidence about what this reference says, either agreeing with them or pointing out any perceived errors or deficiencies in applicants' submission so that applicants can respond, the 2/10/12 Office action simply repeats, word for word, the prior overbroad characterization of the reference, dismissing applicants' entire submission regarding Atz & Wessel as "moot." Applicants fail to see how guidance as to how to interpret a reference's disclosure can possibly be "moot" if the reference is still being cited for exactly the same alleged disclosure. Forcing applicants to re-present the same arguments and evidence already of record, to address exactly the same points addressed by applicants' prior remarks, does not advance prosecution in an efficient way, wasting time, money and the Office's resources, and delaying a resolution in this case. Applicants request that the Examiner provide a substantive response, either accepting applicants' positions or explaining why, in the Examiner's view, the facts do not support these positions.

Rather than re-submit the entire nine pages of arguments (and related exhibits) about the Atz & Wessel reference submitted in the 1/6/12 Reply, applicants direct the Examiner's attention to pages 10-18 of the 1/6/12 Reply and to Exhibits A-C submitted with that reply. In those nine pages, supported by Exhibits A-C, applicants explained that the broad statement at the beginning of the pertinent paragraph of Atz & Wessel must be read in the context of the rest of the paragraph, which explains that the entire universe of LVD patients at risk from treatment with inhaled nitric oxide is limited to the two defined patient groups well known in the art to be at risk: adults with ischemic cardiomyopathy (who are at risk of pulmonary edema) and newborns who are dependent on a right-to-left shunting of blood (who are at risk of systemic circulatory collapse). Atz & Wessel did not suggest that inhaled nitric oxide treatment might pose a particular risk to any other patient group (whether with or without LVD), and certainly did not suggest that the treatment might trigger pulmonary edema in anyone but adults with LVD due to ischemic cardiomyopathy. The 2/10/12 Office action's purported summary of Atz & Wessel as implying that all patients (including all pediatric patients) with LVD should be excluded from treatment with inhaled nitric oxide is simply wrong. Further, the risk recited in the present claims is specified as being pulmonary edema, a risk that Atz & Wessel discussed solely in the context of adult patients—not the neonates specified in the claims. There was no recognition whatsoever in Atz & Wessel, or in any of the other cited art, that neonates or any other non-adult patients with LVD might be at risk of pulmonary edema upon treatment with inhaled nitric oxide. Dr. Wessel's declaration (Exhibit C submitted with the 1/6/12 Reply) establishes that in fact his Atz & Wessel article did not disclose that pediatric LVD patients--other than those dependent on a right-to-left shunt, who are known to be at risk of systemic hypotension, not pulmonary edema--were at any risk from the treatment, and that he was surprised when the new risk was discovered in the course of the INOT22 clinical trial that he helped design in 2006. As noted by Dr. Wessel, if he had expected children with LVD who are not dependent on a right-to-left shunt to be at risk from the treatment, he would not have allowed them to be included in the clinical trial. The Examiner is asked to give due consideration to the detailed explanation of Atz & Wessel provided on pages 10-18 of the 1/6/12 Reply, and to the factual evidence submitted in support thereof, and to acknowledge that the description of this reference provided in the last two Office actions does not accurate reflect what the reference discloses.

Kinsella et al.

As with the Atz & Wessel reference, the 2/10/12 Office action's characterization of Kinsella et al. at page 9 is word-for-word identical to the way Kinsella et al. was characterized in the 6/27/11 Office action. Also as with the Atz & Wessel reference, applicants' discussion of Kinsella et al. at pages 18-21 of the 1/6/12 Reply, though entirely relevant to how this reference is described and cited in the present rejection, was dismissed as "moot" by the 2/10/12 Office action, rather than being addressed on the merits. Applicants ask the Examiner to give due consideration to the detailed discussion of Kinsella et al. provided at pages 18-21 of the 1/6/12 Reply, including the factual evidence (Exhibits C and D) cited in support of that discussion. In brief, that discussion establishes that one of ordinary skill in the art would have viewed Kinsella et al. as irrelevant to the present claims. It is noted that the Examiner has not even attempted to rebut applicants' position.

Loh et al.

At risk of sounding repetitive, applicants point out that the 2/10/12 Office action's characterization of yet another reference--Loh et al.--is again word-for-word identical to the way this reference was characterized in the 6/27/11 Office action. See pages 9-10 of the 2/10/12 Office action. As with applicants' discussion of Atz & Wessel and Kinsella et al., applicants' discussion of Loh et al. at pages 21-22 of the 1/6/12 Reply, though entirely relevant to how this reference is described and cited in the present rejection, was inappropriately dismissed as "moot" by the 2/10/12 Office action rather than being addressed on the merits. Applicants ask the Examiner to give due consideration to the detailed discussion of Loh et al. provided at pages 21-22 of the 1/6/12 Reply, including the fact that Loh et al. is solely about adult patients who have an importantly different form of LVD than that typically found in neonates. That is, the adult form of LVD that concerns Loh et al. (diastolic LVD) renders the left ventricle stiff and unable to stretch readily to accept blood, while childhood LVD is generally characterized by a weak, flabby left ventricle that stretches easily but has weak contractions.[7] These assertions are supported by factual evidence submitted with the

[7] The 2/10/12 Office action at page 7 points to page 282 of Fraisse et al. as evidence that children can have "reduced left ventricular compliance." Dr. Greene addressed this phenomenon in the 4/13/12 Interview. According to Dr. Greene, the "reduced left ventricular compliance" to which Fraisse et al. referred is a temporary situation attributable to the fact that the patient has pulmonary hypertension. Pulmonary hypertension means that the right ventricle has to work extra hard to push blood into the vasoconstricted lungs. The increased pressure in the right

1/6/12 Reply, evidence that has not yet been considered on the record by the Examiner. Applicants have explained in detail in the 1/6/12 Reply why one of ordinary skill in the art would not have expected the results in adults (as reported by Loh et al.) to be duplicated in children, citing factual evidence to support this position. The Examiner is asked to address applicants' position and evidence on the record, rather than again dismissing it as "moot."

Beghetti et al. and Henrichsen et al.

Beghetti et al. is a newly cited brief Letter to the Editor in the Journal of Pediatrics, written in response to a prior Letter to the Editor in the same journal entitled "Inhaled nitric oxide can cause severe systemic hypotension" (Henrichsen et al., J. Pediatrics 129:183,1996; listed as "pertinent to applicant's disclosure" on page 19 of the 2/10/12 Office action). In order to put Beghetti et al.'s comments into context, it is necessary to review what Henrichsen et al. said.

Henrichsen et al. is a case study of a newborn baby who was given inhaled nitric oxide as a treatment for persistent pulmonary hypertension. The baby is said to have had severe left ventricular dysfunction and a PDA, and was diagnosed as being "dependent on the right-to-left shunt through the PDA." Because of that dependence on right to left shunting of blood, the baby described by Henrichsen et al. (and discussed after-the-fact by Beghetti et al.) is not within the population of patients that is the subject of each of the independent claims, all of which specify that the subject patient(s) "is/are not dependent on right-to-left shunting of blood." Treatment of Henrichsen et al.'s patient with inhaled nitric oxide "resulted in an immediate fall in the mean systemic arterial blood pressure from 48 to 35 mmHg, which reversed when NO therapy was discontinued," i.e., the baby experienced systemic hypotension upon inhalation of NO. According to Henrichsen et al., "This hypotensive episode was thought to have been caused by

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ventricle expands the size of the right ventricle, which pushes against the left ventricle and reduces its "compliance"—i.e., its ability to fill. When such a patient is treated with inhaled nitric oxide to open up the constricted pulmonary blood vessels, blood flows out of the right ventricle into the lungs, thereby reducing the pressure and size of the right ventricle so that it no longer interferes with the left ventricle. The left ventricle then recovers its normal level of compliance and is able to handle the increased flow from the lungs. Thus, there would be no expectation that pulmonary edema might develop upon treatment of such a patient with inhaled nitric oxide.

the NO's reversing the right-to-left shunt through the PDA on which the systemic circulation depended." In other words, the baby's systemic circulation was dependent on a right-to-left shunt through a PDA and was adversely affected, resulting in hypotension, when inhaled nitric oxide reduced the patient's pulmonary hypertension. This of course is exactly what is now well known to occur in neonates who are dependent on right-to-left shunting of blood, and is why the INOmax insert said that such neonates are contraindicated for treatment with inhaled nitric oxide. Henrichsen et al. says nothing about inhaled nitric oxide's having caused any problems other than systemic hypotension. ***In particular, there is no mention of pulmonary edema.*** As discussed by Dr. Greene during the 4/13/12 Interview, pulmonary edema and systemic hypotension are entirely different and conceptually inconsistent conditions, one being treated by decreasing fluids and the other being treated by increasing fluids.

Beghetti et al. read the case study published by Henrichsen et al. and offered their own interpretation of what may have been occurring in the infant. They dismissed Henrichsen et al.'s view that the baby was dependent on a right-to-left shunt and suggested that the systemic hypotension exhibited upon treatment with inhaled nitric oxide was instead due to further left ventricular failure caused by "overfilling"—i.e., the left ventricle was even less able to pump than it was before the treatment began, thereby reducing the blood flow out of the left ventricle and contributing to systemic hypotension. Though Beghetti et al. appeared perfectly willing to speculate about what might have been occurring, despite not having seen the baby or any data other than that provided in Henrichsen et al.'s letter, ***they do not even suggest that the proposed "overfilling" of the left ventricle might have precipitated pulmonary edema in the baby***. Beghetti et al. simply offered an alternative explanation for the observed fall in systemic blood pressure upon inhalation of nitric oxide. (Applicants again remind the Examiner that systemic hypotension is not pulmonary edema, and has nothing whatsoever to do with pulmonary edema.) By the time the INOmax® product was approved for marketing in December 1999, those of ordinary skill in the art at the priority date were aware that inhaled nitric oxide will precipitate systemic hypotension in newborns who, like Henrichsen et al.'s patient, are diagnosed as dependent on a right-to-left shunt, and understood this to happen by a mechanism essentially as postulated by Henrichsen et al., i.e., by interfering with the right-to-left shunt on which the systemic circulation depended. It could well be that the authors of Beghetti et al. were not aware of this fact when they wrote their letter in 1997 theorizing about another possible physiological mechanism to explain the observed systemic hypotension. At any rate, they do not propose that the patient in fact suffered an episode of pulmonary edema, rather than the reported systemic hypotension. One of ordinary skill in the art at the priority date would read the Henrichsen et al. case study as being a typical example of the systemic hypotension that happens when a neonate who is dependent on a right-to-left shunt is treated with inhaled nitric oxide, and would read the Beghetti et al. letter as mere second-hand speculation inconsistent not only with Henrichsen et al.'s first-hand report about the shunt-reliant nature of the baby's circulation, but also with what was learned in subsequent years about such patients. ***More to the point, even Beghetti et al. does not propose that the baby was ever at any risk of pulmonary edema due to the treatment.*** Rather, Beghetti et al. merely sought to "explain the observed hypotensive effect of iNO". Thus, Beghetti et al.'s caution regarding "LV overfilling" on which the 2/10/12 Office action focuses is based on unsubstantiated speculation about what was happening in the case report of Henrichsen et al. (speculation that is inconsistent with Henrichsen et al.'s first-hand diagnosis of dependence on a right-to-left shunt); and furthermore purports to relate to a risk of systemic hypotension, not its conceptual opposite, pulmonary edema. One of ordinary skill would not derive from the Beghetti et al. letter any information of relevance to the present claims. It is not clear why the Examiner places any reliance at all on Beghetti et al.'s unsubstantiated speculation about a patient the authors never saw, in preference to Henrichsen et al.'s first-hand observations that are more consistent with accepted wisdom in the art, and even less clear why the Examiner believes a discussion of a patient who suffered systemic hypotension has anything to do with predicting a risk of pulmonary edema.

Ichinose et al.

Ichinose et al. is briefly discussed on page 11 of the 2/10/12 Office action:

> Ichinose et al. teach inhalation of NO can increase left ventricle filling pressure in patients with severe left ventricle dysfunction and that it is important to be aware of the

> possibility that inhaled NO can produce pulmonary vasodilation and may overwhelm a failing left ventricle thereby producing **pulmonary edema** (page 3109 bottom left to top right columns). (Emphasis in the original)

Ichinose et al. is a review article entitled "Inhaled Nitric Oxide: A Selective Pulmonary Vasodilator: Current Uses and Therapeutic Potential." The 2/10/12 Office action focuses on one paragraph of the article, the paragraph spanning the left and right columns of page 3109. The paragraph begins with the sentence: "Inhaled NO has been demonstrated to be a selective pulmonary vasodilator in heart failure patients, although breathing NO was often accompanied by an elevation in LV filling pressure in patients with severe LV dysfunction," citing two publications, Semigram et al.[8] and Loh et al.[9] Both Semigram et al. and Loh et al. studied only adult patients suffering from severe heart failure. Thus, this quoted sentence from Ichinose et al. derives from observations made in adults with LVD associated with severe heart failure. Ichinose et al. goes on to say, "Investigators learned that the elevation in LV filling pressure that occurs with NO breathing is due to the augmentation of filling into a relatively noncompliant LV and is not caused by a negative inotropic effect," citing two more publications that again concern only adult conditions: Dickstein et al.[10] and Hare et al.[11] The statement of Ichinose et al. on which the 2/10/12 Office action relies ("Nonetheless, it is important to be aware of the possibility that inhaled NO can produce pulmonary vasodilation and may overwhelm a failing LV, thereby producing pulmonary edema") cites only the Beghetti et al. letter, a reference that (as discussed above) says nothing about pulmonary edema and in fact is about a (neonatal) patient who, when treated with inhaled nitric oxide, exhibited systemic hypotension, a condition that is nothing like pulmonary edema. Beghetti et al. hypothesized that inhaled NO induced "further LV failure," i.e., caused the dysfunctional left ventricle to lose even more of its pumping capacity, offering

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[8] Semigram et al., J Am Coll Cardiol 24:982-988, 1994 (cited in the 2/10/12 Office action on page 19 and in the Information Disclosure Statement filed June 22, 2010.

[9] This is the same Loh et al. as cited in the present rejection.

[10] Dickstein et al., J Heart Lung Transplant 15:715-721, 1996; cited in the Information Disclosure Statement filed April 23, 2012.

[11] Hare et al., Circulation 95:2250-2253, 1997; cited in the Information Disclosure Statement filed March 14, 2011.

this as an explanation for the drop in systemic blood pressure exhibited by the patient. It does not even begin to support an assertion that pulmonary edema could result in a neonate who is treated with inhaled nitric oxide. Thus, it appears doubtful that Ichinose et al. intended to imply, merely by citing Beghetti et al., that any patients other than adults might be at risk for pulmonary edema. This would have been a radical new assertion that would certainly have been discussed in detail with appropriate supporting evidence.

(4) The Examiner's conclusion that the art teaches that administering inhaled NO to babies with left ventricular dysfunction can cause pulmonary edema.

Pages 11-12 of the 2/10/12 Office action set out five rhetorical questions and the Examiner's view as to their answers. Since that Office action is being withdrawn and the points made by this part of the Office action may not be asserted in a new Office action, applicants will not belabor them here. However, applicants wish to note for the record that the Examiner's stated assumptions about what the "preponderance of art cited clearly indicates" and what the "art cautions and warns" are not supported by any of the references cited in the Office action. The only references to pulmonary edema in the cited art are in the context of adults, not neonates. All discussion in the art regarding risks to neonates in particular describe the risk as a risk of systemic hypotension, not pulmonary edema.

CONCLUSION

Applicants respectfully request that the above remarks, and the remarks and evidence (including objective evidence of nonobviousness) submitted in the 1/10/12 Reply, be taken into account by the Examiner when considering whether to assert an obviousness rejection (based on any of the above-discussed references or any others) in a new Office action. The 2/10/12 Office action reveals a misunderstanding of many physiological facts described in the cited references and a possible misunderstanding of the overall effect of the limitations of the claims on claim scope, leading to a rejection based on inappropriate grounds. Applicants would be happy to meet with the Examiner again (together with SPE Kwon and QAS Burke, if they are available) at the Office's convenience, if that would be helpful in clarifying the facts.

The excess claims fee of $300 is being paid concurrently herewith. If any other fees are due, please apply them to deposit account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: May 11, 2012

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Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Nancy Bechet
Attorney Docket Number:	26047-0003005

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	2202	10	30	300
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**300**

Electronic Acknowledgement Receipt

EFS ID:	12761489
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	11-MAY-2012
Filing Date:	22-JUN-2010
Time Stamp:	16:53:02
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$300
RAM confirmation Number	3510
Deposit Account	061050
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		supplementalamend003005.pdf	228064 9afd49973148bbcf67bc75fa4424f8763edfccb2	yes	32

Multipart Description/PDF files in .zip description

Document Description	Start	End
Supplemental Response or Supplemental Amendment	1	1
Claims	2	8
Applicant Arguments/Remarks Made in an Amendment	9	32

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
2	Fee Worksheet (SB06)	fee-info.pdf	30198 150049400b2d417dac3bb57876789ce3aafcaca7	no	2

Warnings:

Information:

Total Files Size (in bytes): 258262

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/821,041	Filing Date 06/22/2010	☐ To be Mailed

APPLICATION AS FILED – PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY ☒ RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 05/11/2012	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 30	Minus	** 20	= 10	X $30 =	300	OR	X $ =	
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $125 =	0	OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE	**300**	OR	TOTAL ADD'L FEE	

AMENDMENT	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/KIMBERLY WHITE/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	1	Fish & Richardson P.C., Supplemental Amendment in U.S. Serial No. 12/821,020, filed April 30, 2012, 10 pages	☐
	2	Fish & Richardson P.C., Supplemental Remarks in U.S. Serial No. 12/821,020, filed May 9, 2012, 22 pages	☐
	3	European Patent Office minutes of oral proceedings in EP 09 251 949.5, with allowable claims (7 pages), dated May 23, 2012	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		12821041
	Filing Date		2010-06-22
	First Named Inventor	Baldassarre	
	Art Unit		1613
	Examiner Name	Ernst V. Arnold	
	Attorney Docket Number		26047-0003005

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-05-25
Name/Print	Janis K. Fraser, Ph.D., J.D.	Registration Number	34819

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Lisa Gray
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
	Total in USD ($)			**180**

Electronic Acknowledgement Receipt

EFS ID:	12870179
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Lisa Gray
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	25-MAY-2012
Filing Date:	22-JUN-2010
Time Stamp:	17:53:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	4357
Deposit Account	061050
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Non Patent Literature	260470003004supplementalamendment.pdf	69946 84440328b63b24a52e49d769ee9680b100bac939	no	10
Warnings:					
Information:					
2	Non Patent Literature	260470003004supplementalremarks.pdf	204932 0721d68ac6e10e8fc3bf28c573050738bf97317f	no	22
Warnings:					
Information:					
3	Non Patent Literature	eporef.pdf	269381 0a706f61470c4522edb0c89352fa7d6d77e75063	no	7
Warnings:					
Information:					
4	Transmittal Letter	26047-0003005ids.pdf	62424 8d7e40e86aeebcf236c7ea4243ff9defb277753b	no	1
Warnings:					
Information:					
5	Information Disclosure Statement (IDS) Form (SB08)	SB0826047_0003005.pdf	612328 1c1e18dad0fbe2a1b2f2b075946884bbcc4e63dc	no	4
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
6	Fee Worksheet (SB06)	fee-info.pdf	30656 cc19a3c33f8e3561feed5edff8f9d58f2190a461	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1249667		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	James S. Baldassarre et al.	Art Unit	:	1613
Serial No.	:	12/821,041	Examiner	:	Ernst V. Arnold
Filed	:	June 22, 2010	Conf. No.	:	3219

Title : METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the documents listed on the enclosed PTO-1449 form and enclosed herewith.

The cited communication from the European Patent Office is the minutes of an oral hearing before the Examining Division in a European application that is in the same patent family as the present application. During the oral hearing, the Examining Division stated an intent to grant a European patent based on the revised claims submitted during the hearing. A copy of those revised claims is attached to the communication.

This statement is being filed after a first Office action on the merits, but before receipt of a final Office action or a Notice of Allowance. Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: May 25, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22851532.doc

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

94169 7590 06/19/2012
Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

EXAMINER	
ARNOLD, ERNST V	

ART UNIT	PAPER NUMBER
1613	

MAIL DATE	DELIVERY MODE
06/19/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 12/821,041	Applicant(s) BALDASSARRE ET AL.	
	Examiner ERNST ARNOLD	Art Unit 1613	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1/16/12 and 5/11/12*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *38-49 and 53-70* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *38-49 and 53-70* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/19/12, 2/7/12, 4/23/12, 5/25/12*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

In view of the interview of 4/13/12 (noted in copending 12/821020) and here (interview summary filed on 5/3/12), this is a supplemental Office Action addressing the issues discussed during the interview with the Quality Assurance Specialist and Supervisory Patent Examiner. The previous Office Action filed on 2/10/12 is hereby vacated and replaced by the instant Office Action by the Examiner.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 1/6/12 has been entered.

The amendment filed on 1/6/12 cancelled claims 1-37 and introduced new claims 38-52.

The amendment filed on 5/11/12 cancelled claims 50-52 and introduced new claims 53-70. Accordingly, claims 38-49 and 53-70 are pending and under examination.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 1/19/12, 2/7/12, 4/23/12 and 5/25/12 were filed after the mailing date of the Office Action on 6/27/11. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Withdrawn rejections:

Applicant's amendments and arguments filed 1/6/12 and 5/11/12 are acknowledged and have been fully considered. **The Examiner has re-weighed all the evidence of record.** Any rejection and/or objection not specifically addressed below is herein withdrawn.

The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set of rejections and/or objections presently being applied to the instant application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 38-49 and 53-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davidson et al. (Pediatrics 1998, 101 (3) pp 325-334) and The Neonatal Inhaled Nitric Oxide Study Group (The New England Journal of Medicine 1997, 336(9), pp597-604) and Macrae (Semin Neonatal 1997, 2, 49-58) and Miller et al. (Achives of Disease in Childhood 1994, 70, F47-F49) and Weinberger et al. (Toxicology Sciences 2001, 59, 5-16) and Hurford et al. (Nitric Oxide: Biology and Pathobiology 2000 Academic Press, Chapter 56, pages 931-945) and Kazerooni et al. (Cardiopulmonary Imaging 2004, Lippincott Williams & Wilkins, pp 234-235) and Wheeler et al. (Pediatric Critical Care Medicine 2007, Springer, page 278) and Moss et al. (Moss And Adams' Heart Disease in Infants, Children, and Adolescents, 2007, vol. 1, page 991 in part) and Bocchi et al. The American Journal of Cardiology 1994, 74, pp: 70-72. 4 pages) and Fraisse et al. (Cardiol Young 2004; 14: 277-283 IDS filed on 12/27/11) and Loh et al. (Circulation 1994, 90; 2780-2785; of record) and Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455; of record) and Ichinose et al. (Circulation 2004; 109:3106-3111: IDS filed on 1/10/12).

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicants claims, for example:

38. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas, said method comprising:

(a) performing echocardiography to identify~~identifying~~ a term or near-term neonate patient in need of inhaled nitric oxide treatment for pulmonary hypertension, wherein the patient is not ~~known to be~~ dependent on right-to-left shunting of blood;

(b) determining that the patient identified in (a) has ~~pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has ~~pre-existing~~ left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

Analysis:

What did Applicant actually do?

The instant specification teaches that children with pulonary hypertension where treated ***with 80 ppm NO inhalation gas*** therapy [0066] and two patients ***developed signs of pulmonary edema*** which has been ***previously reported*** in 1994 and 1996, over ten years before the present application was filed, with the use of iNO in patents with LVD due to decreasing PVRI and overfilling of the left atrium [0062].

The Examiner will now establish with an overwhelming preponderance of art a *prima facie* case of obviousness.

Determination of the scope and content of the prior art

(MPEP 2141.01)

1. Does the art teach administration of 80 ppm iNO to children and the exclusion of some patients from a plurality of patients?

Yes. Davidson et al. teaches providing iNO therapy to infants to improve oxygenation, (hence the patients are hypoxic) eligible within 72 hours of birth, thus neonates, with persistent **pulmonary hypertension** (PPHN) (determined by echocardiographic evidence) with iNO therapy up to **80 ppm NO** (5, 20 or 80 ppm NO) (Abstract; and page 326, left column). Davidson et al. teach that echocardiographic evidence of PPHN was either a right-to-left or bidirectional ductal shunt or if the ductus was closed, a right-to-left or bidirectional foramen ovale shunt (page 326, patient entry criteria) thus the subjects do not have to be dependent on right-to-left shunting of blood. Davidson et al. teach excluding neonates, hence a subgroup group from a plurality of children, with congenital heart disease as determined from **echocardiography** (diagnostic screening) from the therapy (page 326, patient entry criteria) but implicitly providing the treatment to those that do not have LVD. (*Please note that Applicant considers left ventricular dysfunction (LVD) as those with congenital heart disease [0014 and 0028]*). Consequently, Davidson et al. perform echocardiography and determine the types of heart conditions. Thus Davidson et al. clearly set forth excluding some patients from iNO therapy that have congenital heart disease while providing the therapy to a second group which does not have congenital heart disease, except as noted below, which intrinsically reduces the risk of pulmonary edema in those patients excluded from the therapy. Davidson et al. teach inclusion of patients with congenital heart disease such as ventricular septal defects that are insignificant but exclusion of others (page 326, patient entry criteria).

Davidson et al. also teach that nitrogen dioxide levels were higher in the 80 ppm group reaching levels of 2-3 ppm with 7 patients in the 80 ppm group reaching an NO_2

level of ≥ 3 ppm compared to lower NO doses which did not result in NO_2 levels above safety standards (page 332, right column).

Davidson et al. teach measuring the blood oxygen levels (page 326, right column; page 328, Table 2 and lower right column).

Davidson et al. teach using a NO delivery system with NO mixed with nitrogen in a pressurized tank (page 327, left column I-NO Delivery and page 329, Figure 2 and appropriate text).

Finding of Fact Summary: *The art already teaches and suggests administration of 80 pm iNO to neonates with the exclusion of neonatal child patients with pulmonary hypertension and congential heart disease from iNO therapy but to otherwise provide the therapy to patients in need of treatment.*

Yes. The Neonatal Inhaled Nitric Oxide Study Group teaches administration of 20, **80** and 100 ppm NO via inhalation to neonates less than 14 days old resulting in a mean NO_2 amount of 0.8 ± 1.2 ppm and one child had 5.1 ppm NO_2 (Abstract; page 602, Safety and Toxicity; and page 603, upper left column). Infants were excluded if they had congenital heart disease (page 598, study patients).

Finding of Fact Summary: *The art teaches and suggests administration of 80 ppm iNO to patients 2 weeks old, with exclusion of patients with congenital heart disease, resulting in a mean NO_2 amount of 0.8 ± 1.2 ppm.*

Yes. Fraisse et al. sought to identify the predictors of extracorporeal membrane oxygenation therapy, death and response to iNO by performing detailed diagnostic screening with Doppler echocardiographic screening of the patient, neonates, with suspected pulmonary hypertension (Abstract; page 278 Patients and methods). The non-invasive technique allows for measurement of ventricular function and estimates both the direction and degree of shunting including bi-directional shunting (page 277 right column; page 278, right column; and pages 279-280, Tables 1 and 2 and appropriate text). Fraisse et al. teach that right to left ductal shunting of blood was found to be an independent predictor of death (Abstract). Fraisse et al. teach that ***a left to right shunting of blood*** increases the risk of failing to respond to iNO including a patient with severe left ventricular dysfunction (Abstract and page 281 upper left column). Thus the patient is not known to be dependent on right to left shunting of blood and the patient had pre-existing left ventricular dysfunction before administration of iNO was performed. Furthermore, patients without LVD were provided the iNO therapy (see Tables 1 and 2).

Fraisse et al. teach that 44 neonates started iNO therapy at 40-**80 ppm** and the clinical data and hemodynamic characteristics are in Table 2 (page 280 right column).

Fraisse et al. teach on page 281:

A comprehensive echocardiographic examination is an integral element of the initial evaluation of newborns with persistent pulmonary hypertension, both in order to exclude structural congenital heart disease, and to assess cardiac function.[11] Echocardiography is also a valuable non-invasive method for evaluating the degree of pulmonary hypertension, the extrapulmonary shunt, and ventricular function.[3,5,8–12] In the present study, the majority of the patients had either normal, or only mildly depressed, left and right ventricular systolic function. Several factors can cause biventricular dysfunction in newborns with persistent pulmonary hypertension. These include pulmonary hypertension by itself, an alteration in the left ventricular geometry due to the pressure overloaded right ventricle, hypoxaemia causing generalised myocardial ischeamia, and metabolic acidemia.[13] As in our study, however, others have found significant depression of left ventricular function in less than one-fifth of patients with persistent pulmonary hypertension of the newborn.[8,11] Right ventricular dys-

And on page 282:

The results of our present study, however, indicate that an exclusively left-to-right shunt across the atrial septum increases the risk of failing to respond to nitric oxide, with an odds ratio of 7.46, and a p value equal to 0.028. Left-to-right shunting across the atrial septum is usual in newborns with a patent oval foramen and normally compliant ventricles. In persistent pulmonary hypertension of the newborn, a left-to-right atrial shunt associated with a predominantly left-to-right ductal shunt and a normal biventricular function may reflect intrapulmonary shunting. In this subgroup of patients, systemic oxygenation is significantly less improved by inhalation of nitric oxide.[10] Another potential pathophysiologic mechanism that underlies this finding may involve reduced left ventricular compliance, leading to increased left atrial pressure, with a resultant left-to-right shunt across the oval foramen. Decreased left ventricular compliance may occur in persistent pulmonary hypertension of the newborn due to adverse interaction between the ventricles, a leftward shift of the ventricular septum secondary to right ventricular hypertension, decreased left ventricular diastolic filling, and left ventricular systolic dysfunction due to decreased preload, hypoxaemia, and acidosis. Even when left ventricular systolic function is severely depressed in these patients, the right ventricle can maintain systemic output through the patent arterial duct. Selective pulmonary vasodilation with inhalation of nitric oxide in this circumstance may not give the desired clinical response, because the blood flowing across the duct is redistributed away from the systemic circulation towards the lungs, decreasing post-ductal systemic output, and increasing the left atrial pressure.[14]

And…

are at increased risk of death. A pure left-to-right ductal shunt tends to be associated with greater need for extracorporeal membrane oxygenation, and should prompt cautious re-evaluation of the indication for further treatment aimed at increasing pulmonary vasodilation. The direction of flow across the atrial

The Examiner interprets "reduced left ventricular compliance" to be a dysfunction of the left ventricle such that compliance is reduced.

Finding of Fact Summary: *The art teaches and suggests that **left to right** shunts exist in non-compliant left ventricle in PPHN of the newborn.*

Yes. Atz et al. teach methods using inhaled nitric oxide in a majority of children less than 1 year old (page 443, left column and page 444 Figure 1), which is a child, with cardiac disease, hence an **identified patient** in need of nitric oxide treatment, (title and Abstract) which intrinsically provides pharmaceutically acceptable NO gas for inhalation to a medical provider to provide to the patient. Figure 1 shows the age distribution:

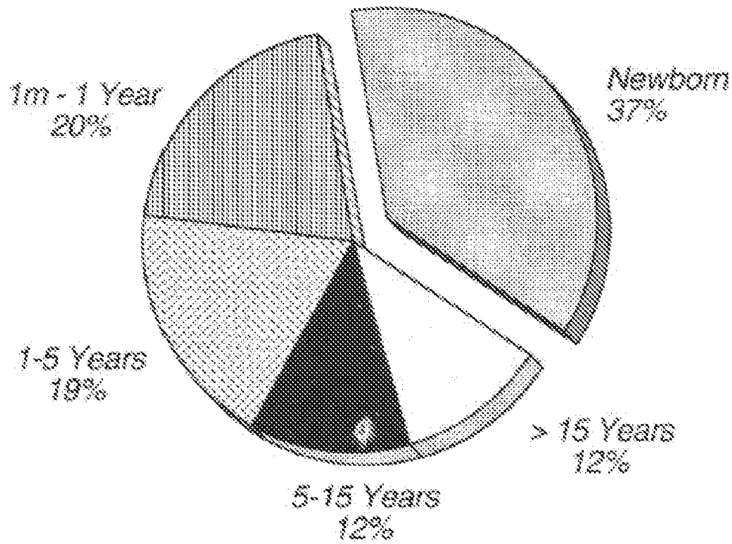

Figure 1. Age analysis of 405 consecutive patients who received inhaled NO at Children's Hospital, Boston.

Atz et al. administered **80 ppm NO** to the patients (Figures 2-5; and page 446, right column). Atz et al. warn that sudden pulmonary vasodilation may produce **pulmonary edema** (page 452, left column). Atz et al. teach that: “Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension.” (page 452, left column). Since the patients have pulmonary hypertension as claimed in instant claim 25 then they also intrinsically have hypoxic respiratory failure in the absence of evidence to the contrary. It is irrelevant how the hypoxic respiratory failure is associated with clinical or echocardiographic evidence of pulmonary hypertension because the hypoxia is intimately tied to the pulmonary hypertension regardless of how it is evidenced. Atz et al. continues with: “Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and exclusively ***right to left shunting*** at the ductus arteriosus, ***NO should be used with extreme caution, if at all***. We and others have reported ***adverse***

outcomes in this circumstance." (page 452, left column) (Examiner added emphasis). Therefore, it is known in the art that patients who had pre-existing LVD treated with NO for any duration may experience adverse outcomes. Artz et al. thus identify conditions in the patients which is screening of the patient. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.

Atz et al. teach neonates with pulmonary hypertension (Abstract and page 442, left column to right column) thus the hypertension is diagnosed in the patient population.

Finding of Fact Summary: *It is well known in the art that to administer iNO to children of all ages and iNO can cause adverse events in neonatal and adult patients with LVD.*

2. Does the art teach the consequences of inhaling 80 ppm NO?

Yes. Miller et al. teach that high doses of inhaled NO, such as **80 ppm NO**, and its oxidative product nitrogen dioxide, NO_2, can cause acute lung injury such as *pulmonary edema* (Abstract and page F47, right column).

Finding of Fact Summary: *The art already teaches and suggests that inhalation of 80 ppm can produce nitrogen dioxide resulting in pulmonary edema.*

Yes. Macrae teaches that inhalation of NO in PPHN can decrease pulmonary vascular resistance (PVR) and increase the return of blood to the pulmonary veins will worsen *pulmonary edema* in babies presenting with situations where pulmonary edema occurs due to pulmonary venous hypertension and that an *echocardiographic* *evaluation* ought to be obtained to *exclude* major cardiovascular anomalies in such infants (lower right page 55-56).

Finding of Fact Summary: *The art teaches and suggests the iNO in the treatment of PPHN in babies, hence a child, can worsen pulmonary edema and to exclude such patients with cardiovascular anomalies from such treatment.*

Yes. Weinberger et al. teach that the primary clinical indication for inhaled nitric oxide is hypoxic respiratory failure associated with PPHN in the newborn (page 5, Pulmonary Vasodilation). Weinberger et al. teach that most current dosing recommendations do not exceed 40 ppm and at this does there is little measureable short-term toxicity (page 5, Pulmonary Vasodilation). Weinberger et al. teach that at **80 ppm NO** formation of 5 ppm NO_2 is expected after 3 min of contact with air (page 6, Formation of Nitrogen Dioxide). At these higher doses of NO_2 the major toxicological effect of NO_2 is *pulmonary edema* (page 6, Formation of Nitrogen Dioxide). Weinberger et al. teach that studies have shown that lung NO_2 concentrations increased to over 18 ppm when the NO concentrations approached 80 ppm (page 7, lower left column).

Finding of Fact Summary: *The art teaches and suggests that inhalation of 80 ppm of NO results in toxic levels of nitrogen dioxide resulting in pulmonary edema.*

3. Does the art recognize that an increase in capillary wedge pressure is bad for a patient with left ventricular dysfunction?

Yes. Loh et al. teach inhalation of NO at 80 ppm caused a 23$\pm$7% increase in pulmonary artery wedge pressure (Abstract; and page 2782, left column) as measured by echocardiography (page 2782, right column) and may have adverse effects in patients with LV failure (Abstract; and page 2784, right column).

<u>Finding of Fact Summary:</u> *The art teaches that iNO causes an increase in the wedge pressure which can be measured by echocardiography.*

Yes. Hurford et al. teach on page 940, right column:

Adverse Hemodynamic Effects

Inhaled NO may also have adverse hemodynamic effects. Inhalation of NO may vasodilate the pulmonary circulation and increase blood flow entering the left ventricle. In patients with preexisting severe left ventricular dysfunction, an increased left ventricular end-diastolic pressure (Hayward *et al.*, 1996; Loh *et al.*, 1994; Semigran *et al.*, 1994) and pulmonary edema (Bocchi *et al.*, 1994) during NO breathing have been reported. This increase may be due to small increases in left ventricular volume associated with improved right ventricular function that, in turn, produced exaggerated increases in pulmonary capillary wedge pressure when the left ventricle is poorly compliant. Monitoring of left ventricular function may be indicated when inhaled NO is administered to patients with severe left ventricular dysfunction.

Hurford et al. teach that inhaled NO is useful for newborns and infants (page 932, Neonatal respiratory failure) but that carefully designed and conducted trials are needed to define additional groups of patients that *may benefit from or may be harmed* by inhaled NO (page 941, Summary).

<u>Finding of Fact Summary:</u> *The art teaches and suggests that when the left ventricle is poorly compliant as in patients with pre-existing left ventricular dysfunction, an increase in PCWP from iNO resulted in pulmonary edema.*

Yes. Kazerooni et al. teach that PCWP is used to reflect left-sided heart function and is a reflection of left ventricular function (page 234). Kazerooni et al. teach that normal PCWP is 6-12 mm Hg and as the PCWP rises to 18-25 mm Hg, it exceeds the normal colloid osmotic pressure of blood and a fluid transudate develops in the lung interstitium **edema** (page 236). (*Please note that Applicant teaches that normal PCWP in children is 10-12 mm Hg and in adults it is 15 mm Hg [0060]*).

Finding of Fact Summary: *The art teaches and suggests that as the PCWP rises to 18-25 mm Hg, edema in the lungs develops.*

Yes. Wheeler et al. teach that the Frank-Starling curve predicts that patients with diminished cardiac function and increasing PCWP results in decreased cardiac output and that increasing left ventricular end-diastolic volume results in overstretching of the fibers and worsening of the stroke volume (page 278).

Finding of Fact Summary: *The art teaches and suggests that a weakened left ventricle has decreased contractility and will have decreased cardiac output as the PCWP increases.*

Yes. Moss et al. teach that the immature myocardium is relatively ineffective in using compensatory mechanisms because as compared to the adult myocardium the neonatal left ventricular myocardium is poorly compliant and less able to preserve ventricular stroke volume via the Frank-Starling mechanism (page 991, right column).

Finding of Fact Summary: *The art teaches that the immature myocardium is different from adult myocardium and the neonatal left ventricle is poorly compliant and less able to handle increases in left ventricular end-diastolic volume to help maintain a normal stroke volume.*

Hurford, Kazerooni, Wheeler and Moss combined clearly teach that patients are at risk of pulmonary edema upon treatment with agents that increase PCWP especially neonates with a non-compliant/dysfunctional left ventricle.

Yes. Bocchi teach that patients that inhaled 40 ppm or 80 ppm NO caused an increase in pulmonary wedge pressure and resulted in pulmonary edema which was caused by the acute increment of blood return to the impaired left ventricle that caused the increase in wedge pressure and consequently pulmonary edema (pp: 70-72) (*Please note that this is the same reference cited by Applicant for teaching patients with elevated PCWP at baseline had a disproportionately high number of adverse events ([0062-0063])*).

Finding of Fact Summary: *The art teaches and suggests that inhaled 80 ppm NO increases pulmonary wedge pressure resulting in pulmonary edema.*

Yes. Ichinose et al. teach inhalation of NO can increase left ventricle filling pressure in patients with severe left ventricle dysfunction (LVD) and that it is important to be aware of the possibility that inhaled NO can produce pulmonary vasodilation and may overwhelm a failing left ventricle thereby producing **pulmonary edema** (page 3109 bottom left to top right columns).

Finding of Fact Summary: *The art teaches and suggests that iNO can increase the left ventricle filling pressure in patients with LVD resulting in pulmonary edema.*

Ascertainment of the difference between the prior art and the claims (MPEP 2141.02)

1. The difference between the instant application and Davidson et al. is that Davidson et al. do not expressly teach the neonate patient as one that is not dependent on right-to-left shunting of blood and that excluding the child from iNO treatment reduces the risk of pulmonary edema. This deficiency in Davidson et al. is cured by the

teachings of The Neonatal Inhaled Nitric Oxide Study Group and Macrae and Miller et al. and Weinberger et al. and Hurford et al. and Kazerooni et al. and Wheeler et al. and Moss et al., Bocchi et al. and Fraisse et al. and Loh et al. and Atz et al. and Ichinose et al.

2. The difference between the instant application and Davidson et al. is that Davidson et al. do not expressly teach measuring the neonate's pulmonary capillary wedge pressure and determining that it is greater than or equal to 20 mm Hg. This deficiency in Davidson et al. is cured by the teachings of The Neonatal Inhaled Nitric Oxide Study Group and Macrae and Miller et al. and Weinberger et al. and Hurford et al. and Kazerooni et al. and Wheeler et al. and Moss et al., Bocchi et al. and Fraisse et al. and Loh et al. and Atz et al. and Ichinose et al.

Finding of prima facie obviousness

Rational and Motivation (MPEP 2142-2143)

1. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Davidson et al. and exclude the neonatal child with LVD which child is not dependent on right-to-left shunting of blood from iNO treatment to reduce the risk of pulmonary edema and any other Serious Adverse Events, as suggested by The Neonatal Inhaled Nitric Oxide Study Group and Macrae and Miller et al. and Weinberger et al. and Hurford et al. and Kazerooni et al. and Wheeler et al. and Moss et al., Bocchi et al. and Fraisse et al. and Loh et al. and Atz et al. and Ichinose et al., and produce the instant invention.

One of ordinary skill in the art would have been motivated to do this because it is overwhelmingly well known in the art that inhaled nitric oxide, especially 80 ppm NO, can cause pulmonary edema especially in those with a dysfunctional left ventricle which would occur in any age child be they neonates "around 4 weeks" old or 18 years old because the principle of action remains the same. Administration of 80 ppm NO to the lungs results in toxic levels of nitrogen dioxide which is known to cause pulmonary edema. Consequently, the risk of pulmonary edema is greater with high dosages of iNO such as 80 ppm iNO. Not only that but the art also teaches that inhaled NO causes an increase in the PCWP that results in pulmonary edema and the Frank-Starling curve clearly predicts that a failing heart subjected to an increase in PCWP will fail. This is especially true in the case of neonates where Moss et al. teach that the immature myocardium is relatively ineffective in using compensatory mechanisms because as compared to the adult myocardium the neonatal left ventricular myocardium is poorly compliant and less able to preserve ventricular stroke volume via the Frank-Starling mechanism. In other words, the art is already aware that: 1) administration of 80 ppm NO results in the formation of nitrogen dioxide and consequent pulmonary edema; 2) administration of 80 ppm NO results in increased PCWP and subsequent pulmonary edema especially in those with impaired left ventricular function; and 3) patients with left ventricular dysfunction are at risk of pulmonary edema from iNO therapy and consequently it obvious to the ordinary artisan that this will occur regardless of the blood shunting direction. As stated in paragraph 22 of Dr. Greene's Declaration: "*On analyzing the data from the study, the inventors concluded that a correlation did, in fact, exist between the severe adverse events that had occurred during the study and the left* *ventricular dysfunction of the patients that had suffered them*." It is the Examiner's position that all Applicant has done is confirmed what was already known in the art and that the data is worthy of publication but is obvious to the ordinary artisan given the art as a whole. It is merely judicious selection of known methods such as echocardiography to determine the wedge pressure or presence of LVD and then exclude those subjects from therapy in view of the risk of pulmonary edema. Thus, it is obvious to exclude all patients, including children, with LVD, whether it is attributable to congenital heart disease or not, including those with or without right-to-left shunting of blood due to the risk of pulmonary edema from administration of the NO because the pulmonary edema can occur via two mechanisms: 1) toxic nitrogen dioxide in the lungs and 2) an increase in capillary wedge pressure on an impaired left ventricle heart due to the NO itself. This is all well known in the art. The expected and predictable outcome is a reduction in risk of occurrence of pulmonary edema and other Serious Adverse Events associated with iNO treatment in the excluded patient population because the iNO treatment is not performed. In other words, one cannot be at risk from pulmonary edema associated with iNO treatment if the iNO treatment is not performed.

2. It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Davidson et al. and measure the neonatal child's pulmonary capillary wedge pressure and determining that it is greater than or equal to 20 mm Hg and thus at risk of pulmonary edema.

One of ordinary skill in the art would have been motivated to do this because it is well known in the art that a wedge pressure over 20 mm Hg is already predisposed to pulmonary edema! See Kazerooni et al. above. Loh et al. teach inhalation of NO at 80 ppm caused a 23±7% increase in pulmonary artery wedge pressure as well. Thus, the artisan knowing full well that administration of 80 ppm of iNO can cause an increase in PCWP would measure the baseline pressure in the patient and exclude the patient that is in danger of pulmonary edema. iNO is known not only to increase pulmonary wedge pressure but also cause pulmonary edema via the formation of toxic nitrogen dioxide. Since the art teaches that a PCWP of 18-25 mg Hg is indicative of pulmonary edema then it is obvious to measure for at least 18 mm Hg and exclude the patient with any value higher than that because they already are or are about to suffer from pulmonary edema especially when it is known that administration of iNO will increase the PCWP value and predispose a patient with a higher than normal PCWP into the dangerous zone for pulmonary edema. The expected and predictable outcome is a reduction in risk of occurrence of pulmonary edema associated with iNO treatment in the excluded patient population because the iNO treatment is not performed. In other words, one cannot be at risk from pulmonary edema associated with iNO treatment if the iNO treatment is not performed.

In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a).

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Summary:

- In view of the cited art, it cannot be considered inventive to administer 80 ppm via inhalation to neonates or children or exclude those with congenital heart disease from the treatment.
- In view of the cited art, it cannot be considered inventive to perform echocardiography to identify a neonate in need of iNO treatment for pulmonary hypertension.
- In view of the cited art, it cannot be considered inventive to determine if the child has left ventricular dysfunction (LVD).
- In view of the cited art, it cannot be considered inventive to exclude a neonate with LVD from iNO treatment due to the risk of pulmonary edema and other Serious Adverse Events because the risk of pulmonary edema from iNO is more than likely when 80 ppm of NO is administered and a child with LVD is at a higher risk of pulmonary edema due to increased PCWP as per the Frank-Starling curve. In other words, pulmonary edema is an expected result from administration of 80 ppm of NO via inhalation especially in patients with LVD.
- The Examiner has carefully and thoroughly considered all Applicant's arguments and expert opinions and soundly rebutted all Applicant's arguments and expert opinions with factual evidence.

At the end of the day, in view of the overwhelming preponderance of evidence, the instantly claimed method to reduce the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of nitric oxide gas is obvious to the ordinary artisan and not patentable.

Response to Arguments:

Applicant's remarks are directed to a rejection that is no longer being applied. To the extent that the instant rejection is relevant to Applicant's remarks and Declarations, the Examiner will attempt to address any issues.

Applicant filed 150 pages of Remarks on 1/6/12 including 27 pages of argument and the remainder made of various Declarations of Dr. Greene, email correspondence, Declaration by Dr. Wessel, art, Declarations by Dr. Baldassarre, and the INOmax(R) insert. The Examiner has carefully read each and every Declaration and cited art above which soundly rebuts the expert opinions in the Declarations as discussed above.

Applicant asserts that the Examiner's interpretation of Atz and Wessel is far broader than what it really is and that Atz and Wessel are focused on a limited patient population of adults and newborns with a combination of conditions. That is not entirely true as noted above because children of all ages are disclosed as being treated with iNO thus the patient population is broad. That is what the Examiner has cited and clearly the disclosure of Atz and Wessel teach using caution when administering iNO with patients with pre-existing LVD whether they be adults or newborns. Furthermore, Applicant has admitted that the criticality is not the dependence on the direction of blood shunting but rather that children are at risk of pulmonary edema from iNO therapy. Applicant asserts that there was no reason to suspect that these or any other neonates might ever be at risk of some other serious adverse event, such as pulmonary edema, upon inhalation of NO (page 15 of 32). **Stop.** Let's review what Applicant actually did. It says in [0066]: "*Benefits and Risks Conclusions. The INOT22 study was designed to demonstrate the physiologic effect" of iNO in a well defined cohort of children (i.e., intended patient population) with pulmonary hypertension using a high concentration, 80 ppm, of iNO, i.e., one that would be expected to have the maximal pharmacodynamic effect.*" Applicant did not disclose using 0.1 ppm NO or 1 ppm NO. Applicant disclosed administration of a high concentration of 80 ppm NO. The Examiner has shown above what the consequences are from administration of 80 ppm NO via inhalation and the overwhelming concordance in the art is that the result from administration of 80 ppm NO is an increased risk of pulmonary edema; regardless of blood direction shunting and especially for those patients with LVD. Consequently, the art was well aware that administration of such high doses of NO via inhalation posed a risk. Indeed, Applicant comments on the observation that pulmonary edema has previously been reported with the use of iNO in patients with LVD [0062]. Applicant cites:

1) Bocchi 1994 whom teaches that pulmonary edema developed in adult patients with 40 and 80 ppm iNO via an increase in capillary wedge pressure (pages 70-71);
2) Hayward et al. 1996 cites Bocchi 1994 above for the development of pulmonary edema from 40 and 80 ppm iNO and suggest that iNO should not be used in the treatment of pulmonary hypertension associated with severe LV dysfunction (last two paragraphs). However, Hayward et al. note no adverse symptoms at lower doses of iNO (second to last paragraph) and one patient was 19 years old (Table 1)

which anatomically is no different from an 18 year old encompassed by the instant definition of "children" [0023].; and

3) Semigran 1994 teaches administration of 80 ppm iNO with noted an increase in capillary wedge pressure (Abstract; Figure 2 and Table 2).

Thus, Applicant was fully aware of an increase in capillary wedge pressure from the administration of 80 ppm iNO carried with it the risk of subsequent pulmonary edema. The Examiner reminds Applicant that the term “risk” is not an absolute and the plain and ordinary meaning is simply that there is only a possibility of loss or injury. Thus, the art fairly establishes such a risk from the administration of 80 ppm iNO.

Applicant arguments concerning adult stiff ventricles and overly elastic ventricles of the claimed patient population have been shown to be invalid with hard facts above.

Applicant cites Dr. Wessel as stating that “…*when I, the senior author of the Atz and et al., failed to anticipate or predict these unexpected outcomes at the time I participated in drafting the original INOT22 Study protocol. If so, I would have been acting negligently or intentionally to harm babies, and I most certainly was not*.” The Examiner can only let the facts speak for themselves and a determination of negligence is outside the examination of the instant application.

Atz & Wessel is not the primary reference and relied upon as discussed above in combination with the other references. The Examiner's position is unmoved by Applicant’s arguments. As show above, administration of 80 ppm NO will produce toxic levels of nitrogen dioxide resulting in pulmonary edema. *Was Applicant unaware of the basic chemistry of nitric oxide where NO reacts with oxygen to produce nitrogen dioxide?* As shown above, administration of 80 ppm NO will increase the PCWP resulting in pulmonary edema. *Was Applicant unaware that 80 ppm iNO will increase the PCWP and, in a case with a heart with a non-compliant left ventricle, that this would lead to a worsening situation as predicted by the Frank-Starling curve and an increased risk of pulmonary edema?* In other words, the ordinary artisan fully expects pulmonary edema to result from the administration of 80 ppm NO regardless of the patient's age because of either the production of toxic nitrogen dioxide or increase in PCWP overwhelming a non-compliant left ventricle where the heart muscle can only stretch so far before failing. The Examiner has cited the general teachings of Atz and Wessel because not only do they teach the instantly claimed patient population but also serve as a cautionary warning to be heeded by the ordinary artisan when working with iNO with LVD despite what Applicant may think.

Applicant argues that the reasons why iNO is not recommended in the Shunt Reliant Population are wholly different from the physiological reasons underlying the claimed invention and again launches into a discourse on the teachings of Atz and Wessel ultimately concluding that the newborn is dependent on right-to-left shunting of blood. That is correct. The Examiner is not citing Atz and Wessel for anything more than what is expressly stated above. In addition the Examiner points out that the instant claims do not contain any language describing the physiological reasons underlying the claimed invention whatsoever.

Applicant asserts that there is no suggestion that any other group of neonates/children might be at risk of systemic circulatory collapse when given iNO or suspect that these patients would be at risk of pulmonary edema. Applicant's conclusion is based upon an invalid premise and consequently the conclusion drawn is also invalid.

The Examiner has shown this to be invalid assertion as the art clearly teaches that administration of 80 ppm NO can produce pulmonary edema. Applicant in their INOT22 study administered 80 ppm NO [0066]. The expected and predictable result is pulmonary edema. This argument is not persuasive.

Applicant asserts that the instantly claimed population does not suffer from a stiff left ventricle but rather an elastic left ventricle then those of ordinary skill in the art had no reason to expect that increasing pulmonary blood flow due to iNO would produce a similar problem of harmfully augmenting preload resulting in pulmonary edema. The Examiner has shown this to be an invalid assertion. All normal hearts have some elasticity and can deal with preload but a non-compliant left ventricle will only stretch to a certain point after which there is a decrease in cardiac output as shown in the Frank-Starling curve of the Wheeler reference as well as discussed by Moss et al. The Examiner has shown with facts that this argument is invalid.

Applicant quotes Dr. Wessel as stating that it was unanticipated and surprising that children with LVD who are not dependent on right-to-left shunting of blood would be at increased risk of adverse events when administered iNO. While this might be true at low doses of NO, Applicant performed the instant method with 80 ppm iNO and the medical literature is clear that at 80 ppm of iNO therapy the expectation is pulmonary edema as discussed above. *Was Applicant unaware that the art has already taught treatment of children with 80 ppm iNO? Was Applicant unaware that the resulting consequence of such a high dose of iNO is the risk of pulmonary edema in the patient?*

Applicant's assertion that the *prima facie* case has been undermined is based on an invalid premise that Applicant's arguments are sound. The Examiner has shown with facts that a sound *prima facie* case of obviousness has been made and Applicant's arguments are unsound.

Applicant asserts that the Examiner offers no rationale as to why Atz & Wessel failed to note this supposedly "obvious" risk to the claimed patient population. The Examiner is not a mind reader and Atz & Wessel do caution the use of iNO with patients with LVD.

Kinsella is no longer a reference and therefore these arguments are moot.

With regard to Loh et al., Applicant asserts that the Examiner is reading it too broadly. The Examiner cannot agree as the teachings of Loh et al. mesh appropriately with all the other teachings cited above in that iNO increases the wedge pressure and in a non-compliant heart with left ventricular dysfunction this will create problems. Again, Applicant points to Dr. Greene's Declarations but the Examiner has shown with art that the Declaration opinions are not valid. Applicant states on page 21 of 31, that the LVD in children is typically a congenital form that leaves the left ventricles overly elastic. However, the ordinary artisan in the cardiac field is also aware of the Frank-Starling curve and knows that an increase in capillary wedge pressure with a heart that has a compromised left ventricle is going to result in pulmonary edema especially in neonates. This is a fact and not supposition.

Applicant then directs the Examiner's attention to the Declarations. Applicant notes on page 25 of 32:

> At no time did the study sponsor, any of the experts on the Steering Committee, any of the principal investigators, any of the IRBs, any of the IECs, any of the SAB members, any of the FDA experts, or any of the European Health Authority experts (altogether estimated to total at least 115 medical professionals) suggest that the exclusion criteria for the INOT22 Study protocol be amended to exclude patients who have LVD but were not dependent on a right-to-left shunt.[31] **In other words, of the estimated 115+ medical professionals tasked with the duty to consider potential safety issues for INOT22 Study patients, none—*not a single one*—suggested there was a chance that inhaled nitric oxide might increase the likelihood of pulmonary edema or other adverse events in the Claimed Patient Population.**[32]

Essentially what Applicant is asserting is that the estimated 115+ medical professionals were not aware of the basic chemistry of nitric oxide and consequent sequelae from administration of high doses of iNO. The Examiner's can only comment, based upon the preponderance of art in this crowded field, that the 115+ medical professionals, IRB/IEC members, FDA officials, four European Health Authorities and Dr. Wessel must have been unaware of the basic chemistry of NO and that administration of 80 ppm iNO results in not only the formation of nitrogen dioxide, which is responsible for pulmonary edema in any age patient, but also produces an increase in the PCWP further resulting in pulmonary edema, which is clearly taught in the art cited above, and well known in the art prior to the study. Thus administration of 80 ppm NO is not without risk especially for those with LVD where the PCWP will be increased resulting in pulmonary edema regardless of which direction the blood is shunting, especially in neonates with a non-compliant left ventricle. Applicant did not discover a new shunting of blood as at least the art of Fraisse et al. teaches that blood shunting can be bi-directional and left to right as well.

Applicant states that the original INOmax® lable included an express contraindication for the population taught by Atz and Wessel but was silent about the instant population. That is correct.

Applicant states that the FDA did require a label change upon notification of a discovered risk from the INOT22 study. That is correct.

Applicant states that, prior to Applicant's claimed subject matter, medical professionals working in the real world did not exclude the instantly claimed patient population from iNO therapy. This is incorrect. First, the Examiner has shown above that Davidson et al. did exclude patients with congenital heart disease which would include those with LVD. Secondly, the in the “real world” the art teaches administration of lower doses of iNO rather than simply the high dose of 80 ppm NO as studied by Applicant and the Examiner has very carefully explained the consequences of using 80 ppm NO for inhalation therapy. Applicant has not shown that inhalation of, for example, 0.1 or 1 or 10 ppm NO results in pulmonary edema in a child patient with pre-existing LVD but only at 80 ppm iNO, which the art already suggests would happen anyway contray to the opinions, arguments and Declarations presented by Applicant.

The multiple Declarations of Dr. Greene, Dr. Wessel and Dr. Baldassarre are not persuasive to overcome the rejection above because the Declarations do not adress this ground of rejection in this application and even if they did address the instant rejection in this application they are still insufficient because the Examiner has shown through sound facts and evidence why the instantly claimed subject matter is obvious, expected and predictable by the ordinary artisan.

In the 24 pages of Remarks filed on 5/11/12, Applicant first launches into a discourse on the prior references of record.

Fraisse et al., Cardiol Young 2004; 14:277-283;
Atz & Wessel (mentioned above);
Kinsella et al., The Lancet 1999; 354:1061-1065;
Loh et al., Circulation 1994; 90:2780-2785;
Beghetti et al., J. Pediatrics 1997; page 844;
Henrichsen et al., Journal of Pediatrics 1996; 129(1):183; and
Ichinose et al., Circulation 2004; 109:3106-3111.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

With regard to Fraisse et al., Applicant asserts that Dr. Wessel was surprised about the risk of pulmonary edema from inhalation of 80 ppm NO. The Examiner can only point to the art which already teaches and suggests this risk from inhalation of 80 ppm NO and the reference of Fraisse et al. is relied upon as discussed above. The Examiner has not confused any concepts of failure to respond with adverse events because the art is clear that inhalation of 80 ppm NO results in the risk of pulmonary edema. Pages 281 and 282 which speak for themselves as to what is known in the art especially with respect to decreasd left ventricular compliance and iNO. Table 2 of Fraisse et al. clearly shows different shunting of blood in the patients that recieved iNO including a patient with depressed left ventricular function. The Examiner does not agree with Applicant's interpretation of the Examiner's art rejection as patients that live because they have a different direction of blood shunting are clearly dependent on that blood shunting to live. Thus the reference supports that the Examiner's position that not only is echocardiography well known in the art for medical use but also patients can be dependent on more than one direction of blood shunting. The Examiner reminds Applicant that this is a combination of references making the rejection.

Applicant claims that they cannot see how the citation is pertinent to the presently claimed methods (page 22 of 32). The citation indicates that subjects with left-to-right shunts also require cautious re-evaluation before further treating with vasodilators which includes iNO. It is a further cautionary note for the population not dependent on right-to-left shunts and therefore directly relevant to the instant claims.

The reference of Atz & Wessel has been discussed *ad nauseum* above and the Examiner's position is the same and any discussion concerning Atz & Wessel is hereby incorporated by reference.

The reference of Loh et al. is discussed in detail above and relied upon as discussed above. The Examiner’s position has not changed.

Beghetti et al. and Henrichsen et al. are no longer references in the rejection.

Ichinose et al. is discussed in detail above and relied upon as discussed above. The Examiner’s position has not changed.

Applicant lastly notes that the Examiner’s stated assumptions are not supported by any of the referneces. The Examiner cannot agree for the reasons presented above.

In anticipation of an argument based on an excessive number of references cited by the Examiner, the Examiner reminds Applicant that reliance on a large number of references in a rejection does not, without more, weigh against the obviousness of the claimed invention. See *In re Gorman*, 933 F.2d 982, 18 USPQ2d 1885 (Fed. Cir. 1991).

In summary, the Examiner has shown through facts and evidence that the preponderance of art as a whole teaches and suggests that administration of 80 ppm iNO results in pulmonary edema especially in those with left ventricular dysfunction due to an increase in PCWP regardless of patient age or the formation of toxic nitrogen dioxide. Indeed, the Examiner has shown with multiple references that these concepts are well known in the art. Consequently, the ordinary artisan has more than a reasonable expectation of success to exclude those patients with LVD, regardless of the direction of blood shunting, from 80 ppm iNO therapy to reduce the risk of pulmonary edema and other Serious Adverse Events that can occur from the iNO therapy.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422

F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

1. Claims 38-49 and 53-70 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 29-42 of copending Application No. 12/820980. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy because iNO therapy may cause pulmonary edema.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a <u>provisional</u> obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

2. Claims 38-49 and 53-70 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 31-42 and 46-63 of copending Application No. 12/821041. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy because iNO therapy may cause pulmonary edema.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

3. Claims 31-42 and 46-63 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 28-42 of copending Application No. 12/820866. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant subject matter embraces or is embraced by the subject matter of the copending subject matter. Both applications are drawn to methods of reducing one or more adverse events in a patient population by excluding from treatment anyone with pre-existing left ventricular dysfunction from iNO therapy.

Therefore one of ordinary skill in the art would have recognized the obvious variation of the instant application over the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Response to Arguments:

Applicant did not appear to traverse these rejections.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ERNST ARNOLD whose telephone number is (571)272-8509. The examiner can normally be reached on M-F 7:15-4:45.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Kwon can be reached on 571-272-0581. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

Notice of References Cited	Application/Control No. 12/821,041	Applicant(s)/Patent Under Reexamination BALDASSARRE ET AL.	
	Examiner ERNST ARNOLD	Art Unit 1613	Page 1 of 3

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
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	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Davidson et al. (Pediatrics 1998, 101 (3) pp 325-334)
	V	The Neonatal Inhaled Nitric Oxide Study Group (The New England Journal of Medicine 1997, 336(9), pp597-604)
	W	Macrae (Semin Neonatal 1997, 2, 49-58)
	X	Miller et al. (Achives of Disease in Childhood 1994, 70, F47-F49)

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
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*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Weinberger et al. (Toxicology Sciences 2001, 59, 5-16)
	V	Hurford et al. (Nitric Oxide: Biology and Pathobiology 2000 Academic Press, Chapter 56, pages 931-945)
	W	Kazerooni et al. (Cardiopulmonary Imaging 2004, Lippincott Williams & Wilkins, pp 234-235)
	X	Wheeler et al. (Pediatric Critical Care Medicine 2007, Springer, page 278)

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	Examiner ERNST ARNOLD	Art Unit 1613	Page 3 of 3

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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Moss et al. (Moss And Adams' Heart Disease in Infants, Children, and Adolescents, 2007, vol. 1, page 991 in part)
	V	Bocchi et al. The American Journal of Cardiology 1994, 74, pp: 70-72. 4 pages)
	W	
	X	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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	1	Azeka, et al., "Effects of Low Doses Of Inhaled Nitric Oxide Combined with Oxygen for the Evaluation Of Pulmonary Vascular Reactivity in Patients with Pulmonary Hypertension," Pediatric Cardiol, Vol. 23, pages 20-26 (2002)	☐
	2	Barst et al., "Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension," Pediatr. Cardiol., Vol. 31, pages 598-606 (2010)	☐
	3	Beghetti et al., "Inhaled nitric oxide and congenital cardiac disease," Cardiol. Young, Vol. 11, pages 142-152 (2001).	☐
	4	Bichel et al., "Successful weaning from cardiopulmonary bypass after cardiac surgery using inhaled nitric oxide," Pediatric Anaesthesia, Vol. 7, pages 335-339 (1997)	☐
	5	Bin-Nun et al., "Role of iNO in the modulation of pulmonary vascular resistance," Journal of Perinatology, Vol. 28, pages S84-S92 (2008)	☐
	6	Dickstein et al., "A theoretic analysis of the effect of pulmonary vasodilation on pulmonary venous pressure: Implications for inhaled nitric oxide therapy," J Heart Lung Transplant, Vol. 15, pages 715-21 (1996)	☐
	7	Haddad et al., "Use of inhaled nitric oxide perioperatively and in intensive care patients," Anesthesiology, Vol. 92, pages 1821-1825 (2000)	☐
	8	Hayward et al., "Inhaled Nitric Oxide in Cardiac Failure: Vascular Versus Ventricular Effects," Journal of Cardiovascular Pharmacology," Vol. 27, pages 80-85 (1996)	☐
	9	Kieler-Jensen et al., "Inhaled nitric oxide in the evaluation of heart transplant candidates with elevated pulmonary vascular resistance," J Heart Lung Transplant, Vol. 13, pages 366-375 (1994)	☐
	10	Kulik, "Inhaled nitric oxide in the management of congenital heart disease," Current Opinion in Cardiology, Vol. 11, pages 75-80 (1996)	☐
	11	Madriago M.D. et al., "Heart Failure in Infants and Children," Pediatrics in Review, Fol. 31, pages 4-12 (2010)	☐

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	Attorney Docket Number	26047-0003005

	12	Steudel et al., "Inhaled nitric oxide," Anesthesiology, Vol. 91, pages 1090-1121 (1999)	☐
	13	Wessel et al., "Managing low cardiac output syndrome after congenital heart surgery," Crit. Care Med., Vol. 29(10) pages S220-S230 (2001)	☐
	14	U.S. Examiner Ernst V. Arnold, Interview Summary in U.S. Serial No. 12/821,020, mailed January 25, 2012, 4 pages	☐
	15	Fish & Richardson, P.C., Statement of the Substance of the Interview and Comments on Examiner's Interview Summary, in U.S. Serial No. 12,821,020, filed February 27, 2012, 7 pages	☐
	16	U.S. Examiner Ernst V. Arnold, Interview Summary in U.S. Serial No. 12/821,020, mailed April 17, 2012, 12 pages	☐
	17	Fish & Richardson P.C., Statement of Substance of Interview and Comments on Examiner's Interview Summary, in U.S. Serial No. 12/821,020 filed April 23, 2012, 8 pages	☐
	18	INO Therapeutics, "Comparison of Inhaled Nitric Oxide and Oxygen in Patient Reactivity during Acute Pulmonary Vasodilator Testing," downloaded from clinicaltrials.gov on April 23, 2012; first received on February 20, 2008; last updated on October 18, 2010.	☐

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EXAMINER SIGNATURE

Examiner Signature	/Ernst Arnold/	Date Considered	06/15/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-04-23
Name/Print	Janis K. Fraser	Registration Number	34819

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	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

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	1	Barst et al., "Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension," Received: 14 September 2009 / Accepted: 19 January 2010 Springer Science + Business Media, LLC, 2010, 9 pages	☐
	2	Beggs et al., "Cardiac Failure in Children," 17th Expert Committee on the Selection and Use of Essential Medicines, Geneva, March 2009, 31 pages	☐
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	4	UTMB Respiratory Care Services, "Delivery of Inhaled Nitric Oxide Therapy through an Adult or Pediatric Nasal Cannula," (4 pages) July 2003	☐
	5	Douwes et al., "The Maze of Vasodilator Response Criteria," Published online: 26 November 2010, Pediatr Cardiol, (2011) 32: pp. 245-246	☐
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	16	Lee & Hayes, Amendment in Reply to Office Action in US Serial No. 12/820,866, mailed June 8, 2011, filed July 8, 2011, 105 pages	☐
	17	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/820,866, mailed August 24, 2011, 27 pages	☐
	18	Fish & Richardson P.C., Brief on Appeal in US Serial No. 12/820,866, filed October 4, 2011, 211 pages	☐
	19	U.S. Examiner Ernst V. Arnold, Examiner Answer in US Serial No. 12/820,866, mailed November 1, 2011, 27 pages	☐
	20	Fish & Richardson P.C., Reply Brief in US Serial No. 12/820,866, filed December 16, 2011, 21 pages	☐
	21	U.S. Examiner Ernst V. Arnold, Non-Final Office Action for US Patent Application 12/820,980, mailed June 10, 2011, 30 pages	☐
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	34	U.S. Examiner Ernst V. Arnold, Final Office Action in US Serial No. 12/821,020, mailed June 27, 2011, 29 pages	☐
	35	Fish & Richardson P.C., Supplement to the Reply Brief, US Serial No. 12/820,866, filed January 3, 2012, 3 pages	☐
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Name/Print	Janis K. Fraser	Registration Number	34819

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/E.A./	2	Krohn, "Effect of inhaled nitric oxide on left ventricular and pulmonary vascular function," The Journal of Thoracic and Cardiovascular Surgery, Vol. 117(1), pages 196-196 (1999).	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	Baldassarre
	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

	1	Fish & Richardson P.C., Supplemental Amendment in U.S. Serial No. 12/821,020, filed April 30, 2012, 10 pages	☐
	2	Fish & Richardson P.C., Supplemental Remarks in U.S. Serial No. 12/821,020, filed May 9, 2012, 22 pages	☐
	3	European Patent Office minutes of oral proceedings in EP 09 251 949.5, with allowable claims (7 pages), dated May 23, 2012	☐

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EXAMINER SIGNATURE

Examiner Signature	/Ernst Arnold/	Date Considered	06/15/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

 ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
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	Art Unit	1613
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	26047-0003005

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Janis K. Fraser/	Date (YYYY-MM-DD)	2012-05-25
Name/Print	Janis K. Fraser, Ph.D., J.D.	Registration Number	34819

 ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12821041	BALDASSARRE ET AL.
	Examiner	**Art Unit**
	ERNST V ARNOLD	1616

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
inventor name EAST/PALM	8/11/10	eva
EAST 424/718 text limted all databases	8/11/10	eva
google	8/10/10	eva
various disucssions with QAS Bennett Celsa and Jean Vollano concering proper incorporation by reference as well as patentabilityn	6/18/11	eva
updated IDS	2/9/12	eva
consultation QAS Jean Vollano	2/2/12	eva
updated IDS	6/14/12	eva
Consultation 103 rejection SPE Brian Kwon	6/6/12	eva

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN REPLY TO ACTION OF JUNE 19, 2012

This application has been granted special status under the prioritized examination (Track 1) program.

Please amend the application as follows:

CERTIFICATE OF (A) MAILING BY FIRST CLASS MAIL OR (B) TRANSMISSION
I hereby certify under 37 CFR §1.8(a) that this correspondence is either (A) addressed as set out in 37 CFR §1.1(a) and being deposited with the United States Postal Service as first class mail with sufficient postage, or (B) being transmitted by facsimile in accordance with 37 CFR § 1.6(d) or via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4), on the date indicated below.

August 15, 2012
Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application.

Listing of Claims:

1-37. (Canceled)

38. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) performing echocardiography to identify a term or near-term neonate patient in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the patient is not dependent on right-to-left shunting of blood;

(b) determining that the patient identified in (a) has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, ~~and~~ so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from inhaled nitric oxide treatment based on the determination that the patient has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

39. (Previously presented) The method of claim 38, wherein step (b) comprises performing echocardiography.

40. (Previously presented) The method of claim 38, wherein step (b) comprises measuring the patient's pulmonary capillary wedge pressure.

41. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) carrying out a diagnostic process comprising measuring blood oxygen level, to identify a term or near-term neonate patient as being in need of 20 ppm inhaled nitric oxide treatment for hypoxic respiratory failure, wherein the patient is not dependent on right-to-left shunting of blood;

(b) ~~performing echocardiography and/or measuring pulmonary capillary wedge pressure to determine~~ determining that the patient has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, ~~and~~ so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the patient from treatment with inhaled nitric oxide based on the determination that the patient has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

42. (Previously presented) The method of claim 41, wherein the diagnostic process of step (a) further comprises performing echocardiography.

43. (Previously presented) The method of claim 41, wherein step (b) comprises performing echocardiography.

44. (Currently amended) The method of claim 41, wherein ~~in~~ step (b)[[,]] comprises measuring the patient's pulmonary capillary wedge pressure ~~is measured and determined to be greater than or equal to 20 mm Hg~~.

45. (Currently amended) A method of treatment comprising:

(a) performing echocardiography to identify a plurality of term or near-term neonate patients who are in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the patients are not dependent on right-to-left shunting of blood;

(b) determining that a first patient of the plurality has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) determining that a second patient of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second patient; and

(e) excluding the first patient from treatment with inhaled nitric oxide, based on the determination that the first patient has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

46. (Currently amended) The method of claim 45, wherein step (a) further comprises measuring blood oxygen levels in the first and second patients and thereby determining that the first and second patients are hypoxic.

47. (Previously presented) The method of claim 45, wherein the second patient has congenital heart disease.

48. (Previously presented) The method of claim 45, wherein step (b) comprises measuring the first patient's pulmonary capillary wedge pressure.

49. (Currently amended) The method of claim 45, wherein determining that the first patient of the plurality has pre-existing left ventricular dysfunction and the second patient of the plurality does not have pre-existing left ventricular dysfunction comprises performing echocardiography on the first and second patients.

50 - 52. (Canceled)

53. (Currently amended) A method of treatment comprising:

(a) identifying a plurality of term or near-term neonate patients who are in need of 20 ppm inhaled nitric oxide treatment, wherein the patients are not dependent on right-to-left shunting of blood;

(b) in a first patient of the plurality, measuring ~~performing echocardiography and/or measurement of~~ pulmonary capillary wedge pressure to determine that the first patient of the plurality has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) in a second patient of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the second patient of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second patient; and

(e) excluding the first patient from treatment with inhaled nitric oxide, based on the determination that the first patient has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

54. (Previously presented) The method of claim 53, wherein step (a) comprises performing echocardiography to determine that the first and second patients have pulmonary hypertension.

55. (Previously presented) The method of claim 53, wherein step (a) comprises measuring blood oxygen levels in the first and second patients and thereby determining that the first and second patients are hypoxic.

56. (Previously presented) The method of claim 53, wherein the second patient has congenital heart disease.

57. (Currently amended) The method of claim 53, wherein step (b) comprises measuring the first patient's pulmonary capillary wedge pressure ~~and determining that it is greater than or equal to 20 mm Hg~~.

58. (Previously presented) The method of claim 38, wherein the patient's left ventricular dysfunction is attributable to congenital heart disease.

59. (Previously presented) The method of claim 38, wherein the patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the patient is excluded from inhaled nitric oxide treatment based on the determination that the patient has left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide.

60. (Previously presented) The method of claim 41, wherein the left ventricular dysfunction is attributable to congenital heart disease.

61. (Previously presented) The method of claim 45, wherein the left ventricular dysfunction is attributable to congenital heart disease.

62. (Previously presented) The method of claim 46, wherein the left ventricular dysfunction is attributable to congenital heart disease.

63. (Currently amended) The method of claim [[34]]41, wherein the patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the patient is excluded from inhaled nitric oxide treatment based on the determination that the patient has left ventricular

dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

64. (Previously presented) The method of claim 63, wherein the left ventricular dysfunction is attributable to congenital heart disease.

65. (Previously presented) The method of claim 45, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

66. (Previously presented) The method of claim 45, wherein the first patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the first patient is excluded from inhaled nitric oxide treatment based on the determination that the first patient has left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

67. (Previously presented) The method of claim 66, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

68. (Previously presented) The method of claim 53, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

69. (Previously presented) The method of claim 53, wherein the first patient is determined to be at particular risk not only of pulmonary edema, but also of other Serious Adverse Events, upon treatment with inhaled nitric oxide, and the first patient is excluded from inhaled nitric oxide treatment based on the determination that the first patient has pre-existing left ventricular dysfunction and so is at particular risk not only of pulmonary edema, but also other Serious Adverse Events, upon treatment with inhaled nitric oxide.

70. (Previously presented) The method of claim 69, wherein the left ventricular dysfunction of the first patient is attributable to congenital heart disease.

REMARKS

Claims 38-49 and 53-70 remain pending in the case. Claims 1-37 and 50-52 were previously canceled. The amendments to claims 38, 41, 44, 45, 53, 57, and 63 are supported by the specification, e.g., at paragraphs [0012], [0039] and [0042]. Claims 46 and 49 are amended to increase clarity. No new matter has been added.

Statement of the Substance of the Interview

In a related case with similar claims (US application no. 12/821,020), Applicants faxed a draft proposed amendment to the Examiner on August 1, 2012, in which Applicants proposed adding a "20 ppm" limitation to each of the four independent claims in the '020 application. A copy of that proposed amendment is attached. The Examiner agreed to consider the amendment. On August 14, 2012, the Examiner left a voicemail message for the undersigned concerning the proposed amendment. Applicants then drafted a second proposed amendment for the '020 application and faxed it to the Examiner on August 14. (A copy of that second proposed amendment is also attached.) The Examiner telephoned the undersigned later the same day suggesting that Applicants submit it as an official amendment in the '020 application and do a similar amendment in the present application as well. Also discussed was the need for appropriate Terminal Disclaimers.

The above amendment amends the independent claims of the present application consistent with the amendments shown in the second proposed amendment for the '020 application reviewed by the Examiner on August 14, 2012, except that the wording of part (b) of claim 53 has been revised compared to part (b) of the corresponding claim (claim 46) in the second proposed amendment, to specify "measuring" rather than "determining" pulmonary capillary wedge pressure. Dependent claims 44 and 57 are amended to ensure they remain consistent with, and further limit, the claims from which they depend. As noted above, claims 46 and 49 are amended to increase clarity.

Rejection under 35 USC § 103(a)

The Office action dated June 19, 2012, rejected all of the pending claims as obvious over a combination of fourteen references. Applicants do not agree that the claims as they stood prior to the present amendment were obvious. Nonetheless, in the interest of advancing prosecution, Applicants have amended the claims in a manner consistent with the above-described communications with the Examiner. Withdrawal of the rejection is therefore respectfully requested.

Rejection for obviousness-type double patenting

All of the claims were provisionally rejected for obviousness-type double patenting over claims 29-42 of Application No. 12/820,980; claims 31-42 and 46-63 of Application No. 12/821,041; and claims 28-42 of Application No. 12/820,866. Applicants first note that Application No. 12/820,980 is abandoned, as Applicants did not file a response to the last Office action in that case (the Office action dated September 9, 2011). Thus, the provisional rejection over the claims of that application is moot. The second application cited (Application No. 12/821,041) appears to have been cited in error, as that is the serial number of the present application. Based on the claim numbers cited for that application (claims 31-42 and 46-63), Applicants believe the Examiner meant to cite related co-pending and co-owned Application No. 12/821,020. Thus, the obviousness-type double patenting rejection is presumed to be over the claims of Application Nos. 12/821,020 and 12/820,866.

In order to address the obviousness-type double patenting rejection over the claims of Application Nos. 12/821,041 and 12/820,866, Applicants submit herewith an appropriate Terminal Disclaimer with its associated fee.

It is believed that all rejections have been addressed and overcome. If any issues remain, the Examiner is asked to telephone the undersigned to discuss.

Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: August 15, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
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Facsimile: (877) 769-7945

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Applicant : James S. Baldassarre et al. Art Unit : 1613
Serial No. : 12/821,020 Examiner : Ernst V. Arnold
Filed : June 22, 2010
Title : Methods of Reducing the Risk of Occurrence of Pulmonary Edema in Children in Need of Treatment with Inhaled Nitric Oxide

PROPOSED AMENDMENT

FOR DISCUSSION PURPOSES ONLY

We propose to amend independent claims 31, 34, 38, and 46, as shown below. The "20 ppm" limitation added to these claims is supported at paragraphs [0039] and [0042] of the specification as filed. It is also supported by material incorporated into the application by reference at [0020]: see page 2, col. 2, "Dosage and administration," of the 2007 INOmax® prescribing information attached hereto, which says that "The recommended dose of INOmax is 20 ppm." The language added to step (b) of each claim below is derived from claim 37.

31. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) performing echocardiography to identify a child in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the child is not dependent on right-to-left shunting of blood;

(b) determining that the child identified in (a) has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the child from inhaled nitric oxide treatment based on the determination that the child has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

34. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) carrying out a diagnostic process comprising measuring blood oxygen level, to identify a child as being in need of 20 ppm inhaled nitric oxide treatment for hypoxic respiratory failure, wherein the child is not dependent on right-to-left shunting of blood;

(b) ~~performing echocardiography and/or measuring pulmonary capillary wedge pressure to determine~~ determining that the child has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction~~, and~~ so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the child from treatment with inhaled nitric oxide based on the determination that the child has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

38. (Currently amended) A method of treatment comprising:

(a) performing echocardiography to identify a plurality of children who are in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the children are not dependent on right-to-left shunting of blood;

(b) determining that a first child of the plurality has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) determining that a second child of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second child; and

(e) excluding the first child from treatment with inhaled nitric oxide, based on the determination that the first child has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

46. (Currently amended) A method of treatment comprising:

(a) identifying a plurality of children who are in need of 20 ppm inhaled nitric oxide treatment, wherein the children are not dependent on right-to-left shunting of blood;

(b) in the first child of the plurality, ~~performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine~~ determining that the first child of the plurality has a pulmonary capillary wedge pressure greater than or equal to 20 mm Hg and thus has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) in the second child of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the second child of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second child; and

(e) excluding the first child from treatment with inhaled nitric oxide, based on the determination that the first child has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

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Applicant : James S. Baldassarre et al. Art Unit : 1613
Serial No. : 12/821,020 Examiner : Ernst V. Arnold
Filed : June 22, 2010
Title : Methods of Reducing the Risk of Occurrence of Pulmonary Edema in Children in Need of Treatment with Inhaled Nitric Oxide

PROPOSED AMENDMENT

FOR DISCUSSION PURPOSES ONLY

We propose to amend independent claims 31, 34, 38, and 46, as shown below. The "20 ppm" limitation added to these claims is supported at paragraphs [0039] and [0042] of the specification as filed. It is also supported by material incorporated into the application by reference at [0020]: see page 2, col. 2, "Dosage and administration," of the 2007 INOmax® prescribing information attached hereto, which says that "The recommended dose of INOmax is 20 ppm."

31. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) performing echocardiography to identify a child in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the child is not dependent on right-to-left shunting of blood;

(b) determining that the child identified in (a) has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the child from inhaled nitric oxide treatment based on the determination that the child has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

34. (Currently amended) A method of reducing the risk of occurrence of pulmonary edema associated with a medical treatment comprising inhalation of 20 ppm nitric oxide gas, said method comprising:

(a) carrying out a diagnostic process comprising measuring blood oxygen level, to identify a child as being in need of 20 ppm inhaled nitric oxide treatment for hypoxic respiratory failure, wherein the child is not dependent on right-to-left shunting of blood;

(b) performing echocardiography and/or measuring pulmonary capillary wedge pressure to determine that the child has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide; and

(c) excluding the child from treatment with inhaled nitric oxide based on the determination that the child has left ventricular dysfunction and so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

38. (Currently amended) A method of treatment comprising:

(a) performing echocardiography to identify a plurality of children who are in need of 20 ppm inhaled nitric oxide treatment for pulmonary hypertension, wherein the children are not dependent on right-to-left shunting of blood;

(b) determining that a first child of the plurality has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) determining that a second child of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second child; and

(e) excluding the first child from treatment with inhaled nitric oxide, based on the determination that the first child has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

46. (Currently amended) A method of treatment comprising:

(a) identifying a plurality of children who are in need of 20 ppm inhaled nitric oxide treatment, wherein the children are not dependent on right-to-left shunting of blood;

(b) in the first child of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the first child of the plurality has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide;

(c) in the second child of the plurality, performing echocardiography and/or measurement of pulmonary capillary wedge pressure to determine that the second child of the plurality does not have left ventricular dysfunction;

(d) administering the 20 ppm inhaled nitric oxide treatment to the second child; and

(e) excluding the first child from treatment with inhaled nitric oxide, based on the determination that the first child has left ventricular dysfunction, so is at particular risk of pulmonary edema upon treatment with inhaled nitric oxide.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TERMINAL DISCLAIMER UNDER 37 C.F.R. §§ 3.73(b) AND 1.321(c)

Pursuant to 37 C.F.R. § 3.73(b), IKARIA HOLDINGS, INC., a corporation, certifies that it is the assignee of the entire right, title, and interest in the present patent application (a 100% ownership interest) by virtue of an assignment from the inventors of the present patent application. The assignment was recorded in the Patent and Trademark Office at Reel 026606, Frame 0245, on July 18, 2011.

To the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned is empowered to act on behalf of the assignee.

Pursuant to 37 C.F.R. § 1.321(c), and to obviate a double patenting rejection, the assignee identified above hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application that would extend beyond the expiration date of the full statutory term of any patent granted on application serial no. 12/820,866. The assignee hereby agrees that any patent granted on the instant application shall be enforceable only for and during such period that it is commonly owned with any patent granted on application serial no. 12/820,866.

The assignee identified above does not disclaim any terminal part of any patent granted on the present application that would extend to the expiration date of the full statutory term of

CERTIFICATE OF (A) MAILING BY FIRST CLASS MAIL OR (B) TRANSMISSION
I hereby certify under 37 CFR §1.8(a) that this correspondence is either (A) addressed as set out in 37 CFR §1.1(a) and being deposited with the United States Postal Service as first class mail with sufficient postage, or (B) being transmitted by facsimile in accordance with 37 CFR § 1.6(d) or via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4), on the date indicated below.

August 15, 2012
Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

any patent granted on application serial no. 12/820,866 in the event that such patent granted on application serial no. 12/820,866 later expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321, has all claims cancelled by a reexamination certificate, is reissued, or is otherwise terminated prior to expiration of its full statutory term. The full statutory term of any patent includes any term adjustment as defined in 35 U.S.C. § 154 and § 173. Assignee herein does not disclaim or otherwise affect any part of any patent granted on application serial no. 12/820,866.

Pursuant to 37 C.F.R. § 1.321(c), and to obviate a double patenting rejection, the assignee identified above hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application that would extend beyond the expiration date of the full statutory term of any patent granted on application serial no. 12/821,020. The assignee hereby agrees that any patent granted on the instant application shall be enforceable only for and during such period that it is commonly owned with any patent granted on application serial no. 12/821,020.

The assignee identified above does not disclaim any terminal part of any patent granted on the present application that would extend to the expiration date of the full statutory term of any patent granted on application serial no. 12/821,020 in the event that such patent granted on application serial no. 12/821,020 later expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321, has all claims cancelled by a reexamination certificate, is reissued, or is otherwise terminated prior to expiration of its full statutory term. The full statutory term of any patent includes any term adjustment as defined in 35 U.S.C. § 154 and § 173. Assignee herein does not disclaim or otherwise affect any part of any patent granted on application serial no. 12/821,020.

This disclaimer runs with any patent granted on the present application and is binding upon the grantee, its successors or assigns.

The fee of $160 required under 37 C.F.R. § 1.20(d) is being paid concurrently. Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: August 15, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Fish & Richardson P.C.
Customer No. 94169
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22896264.doc

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Nancy Bechet
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Statutory or terminal disclaimer	1814	1	160	160
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**160**

Electronic Acknowledgement Receipt

EFS ID:	13506697
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	15-AUG-2012
Filing Date:	22-JUN-2010
Time Stamp:	17:22:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$160
RAM confirmation Number	4328
Deposit Account	061050
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		response260470003005.pdf	454898 fd708f13f3f6128c1ffed5028253a2e347f486f0	yes	17

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	8
Applicant Arguments/Remarks Made in an Amendment	9	17

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
2	Terminal Disclaimer Filed	terminaldisclaimer0003005.pdf	74277 277c0b363c5e0abb46035563d3af6228f77b4a85	no	3

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
3	Fee Worksheet (SB06)	fee-info.pdf	30356 1f9d8f046af1a85d3555bb9805d533cee2aa497a	no	2

Warnings:

Information:

Total Files Size (in bytes):	559531

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/821,041	Filing Date 06/22/2010	☐ To be Mailed

APPLICATION AS FILED – PART I

SMALL ENTITY ☒ OR OTHER THAN SMALL ENTITY

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)		OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

SMALL ENTITY OR OTHER THAN SMALL ENTITY

AMENDMENT	08/15/2012	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0	X $30 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $125 =	0	OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE	**0**	OR	TOTAL ADD'L FEE	

AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/BRENDA HARRISON/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219

Title : METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL LETTER

In an interview that took place on April 13, 2012, with Examiner Arnold, SPE Kwon, and QAS Burke, QAS Burke informed applicants' representative that some of applicants' previously-submitted declarations in the above-captioned application were formally inadequate in that they are copies of declarations originally filed in a different, related application, and some of the comments in the declarations referenced an Office action in that different application rather than an Office action in the present application. QAS Burke said that the declarations should eventually be resubmitted in revised form in order to correct the record, but meanwhile would be substantively fully considered by the Office. (See the Statement of Substance of Interview and Comments on Examiner's Interview Summary submitted by applicants on April 23, 2012.) Applicants are grateful for QAS Burke's guidance on this. To correct the record as suggested by QAS Burke, applicants submit herewith four slightly revised declarations, two by Douglas A. Greene, M.D., and two by James S. Baldassarre, M.D., to replace the four corresponding, previously-submitted declarations signed by Dr. Greene and Dr. Baldassarre. The present declarations do not refer to the Office action of any application other than the present application.

CERTIFICATE OF (A) MAILING BY FIRST CLASS MAIL OR (B) TRANSMISSION
I hereby certify under 37 CFR §1.8(a) that this correspondence is either (A) addressed as set out in 37 CFR §1.1(a) and being deposited with the United States Postal Service as first class mail with sufficient postage, or (B) being transmitted by facsimile in accordance with 37 CFR § 1.6(d) or via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4), on the date indicated below.

August 17, 2012
Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

It is believed that there is no need to submit a revised version of the previously-submitted Declaration of David L. Wessel, M.D., since Dr. Wessel's declaration did not refer to an Office action of any application. If this understanding is incorrect, applicants request further guidance on this point.

Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: August 17, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22897907.doc

Attorney Docket No.: 26047-0003005 / 3000-US-0008CON4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

FOURTH DECLARATION OF JAMES S. BALDASSARRE, M.D.
UNDER 37 C.F.R. § 1.132

I, James S. Baldassarre, declare the following:

1. I am a co-inventor on U.S. Patent Application No. 12/821,041.

2. I currently hold the position of Vice President of Clinical Research at Ikaria, Inc. ("Ikaria"), the assignee of U.S. Patent Application No. 12/821,041. My *curriculum vitae* is attached as Exhibit 1.

3. I have over 20 years of experience as a physician, and over fifteen years of experience directing clinical research in the pharmaceutical industry.

4. Ikaria markets pharmaceutical grade nitric oxide (NO) gas under the brand name INOMAX® (nitric oxide) for inhalation. INOMAX® was approved by the U.S. Food and Drug Administration ("FDA") in December 1999, for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure (HRF) associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (ECMO).

5. In May 2004, INO Therapeutics LLC[1] initiated a clinical trial entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing," designated the "INOT22" trial, to compare the utility and side effects of oxygen (O_2), inhaled nitric oxide (iNO), and a combination of iNO and O_2 for determining pulmonary reactivity.

6. The INOT22 study was to be an open, prospective, randomized, multi-center trial, with an expected total enrollment of a minimum of 150 patients in approximately 18 study sites over approximately 2 years.

7. The expected patient population for enrollment into the INOT22 trial was subjects between the ages of four (4) weeks and eighteen (18) years undergoing diagnostic right heart catheterization scheduled to include acute pulmonary vasodilation testing to assess pulmonary vasoreactivity. The expected population was subjects with idiopathic pulmonary arterial hypertension, congenital heart disease (with or without intravascular shunt) with pulmonary hypertension and cardiomyopathies.

8. The INOT22 study was established and designed by the study sponsor, INO Therapeutics LLC (INO), and a Steering Committee comprising internationally recognized experts in the field of pediatric heart and lung disease, whose members would assist INO to develop the INOT22 protocol, monitor the progress of the trial, and provide recommendations to INO on changes in the procedures and conduct of the trial.

[1] INO Therapeutics LLC is a wholly owned subsidiary of Ikaria, Inc., and holder of the NDA for INOMAX.

9. The Steering Committee consisted of:

a. David L. Wessel, MD,[2] presently Division Chief, Pediatric Critical Care of Medicine at Children's National Medical Center, Washington, DC);

b. Robyn J. Barst, MD, presently Professor Emeritus of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York; and

c. Duncan J. Macrae, MD,[3] presently Director, Pediatric Intensive Care, Royal Brompton Hospital, London, U.K.

10. The original INOT22 protocol designed by INO and the Steering Committee contained the following inclusion and exclusion criteria:

Inclusion Criteria

The patient must meet the following criteria:

1. *Have any one of the three disease categories:*
   a. *Idiopathic Pulmonary Arterial Hypertension*
      i. *PAPm >25mmHg at rest, PCWP≤ 15mmHg, and PVRI>3 u·m^2 or diagnosed clinically with no previous catheterization.*

---

[2] Dr. Wessel co-authored two scientific publications that were cited in the Office action dated June 19, 2012: Azt & Wessel, "Inhaled Nitric Oxide in the Neonate with Cardiac Disease," Seminars in Perinatol. 21:441-455, 1997; and Fraisse et al., "Doppler echocardiographic predictors of outcome in newborns with persistent pulmonary hypertension," Cardiol Young 14:277-283, 2004.

[3] Dr. Macrae is the author or co-author of two scientific publications that were cited in the Office action dated June 19, 2012: Macrae, "Drug therapy in persistent pulmonary hypertension of the newborn," Semin. Neonatol. 2:49-58, 1997; and Miller et al., "Guidelines for the safe administration of inhaled nitric oxide," Archives of Disease in Childhood 70:F47-F49, 1994.

b. CHD with pulmonary hypertension repaired and unrepaired,

i. PAPm > 25mmHg at rest, and PVRI >3 u· m^2 or diagnosed clinically with no previous catheterization.

c. Cardiomyopathy

i. PAPm>25mmHg at rest, and PVRI>3u·m^2 or diagnosed clinically with no previous catheterization.

2. *Scheduled to undergo right heart catheterization to assess pulmonary vasoreactivity by acute pulmonary vasodilation testing.*

3. *Males or females, ages 4 weeks to 18 years, inclusive.*

4. *Signed IRB/IEC approved informed consent (and assent if applicable).*

Exclusion Criteria

The patent will be excluded from enrollment if any of the following are true:

1. *Focal pulmonary infiltrates on chest radiograph.*
2. *Diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.*
3. *Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.*
4. *Pregnant (urine HCG +).*

11. The INOT22 investigational plan and study protocol was further reviewed and approved by the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the participating study institutions, including review by the principal investigator within each study institution.

12. At no time did any member of the Steering Committee, nor any member of an IRB, IEC, or individual principal investigator, appreciate, recognize or otherwise suggest that the exclusion criteria be amended to exclude study subjects with pre-existing left ventricular

dysfunction (LVD), due to an anticipated or predicted risk of adverse events or serious adverse events arising from the use of iNO in patients with pre-existing LVD and/or elevated pulmonary capillary wedge pressure. Nor was it, in my expert opinion, common sense to any expert in this field of medicine to exclude neonates, near-term neonates or children diagnosed with pre-existing LVD from having iNO administered for diagnostic or treatment purposes, unless, of course, the subject was also known to be dependent on right-to-left shunting of blood (a contraindication on the prescribing information for INOMAX®).

13. After initiation and enrollment of the first 24 subjects in INOT22, there were 5 serious adverse events (SAEs) – a rate much higher than expected by INO and the Steering Committee based on prior clinical experience. These were all cardiovascular events, and included pulmonary edema, cardiac arrest and hypotension (low blood pressure).

14. Thereafter, in February 2005, INO and the Steering Committee convened to review the unexpected SAEs described above, and upon review and discussion, expressed concern that the unexpected SAEs may be due to the administration of iNO in subjects having pre-existing LVD. Accordingly, based upon a review of the cases, the exclusion criteria of the INOT22 protocol were amended to thereafter exclude subjects with pre-existing LVD. For purposes of the study, the exclusion criteria were amended to exclude subjects from enrollment if the subjects demonstrated an elevated pulmonary capillary wedge pressure (PCWP), defined within the study as subjects having a PCWP greater than 20 mmHg. All study sites were notified immediately. The amended exclusion criteria (see point 5.) were as follows:

Exclusion Criteria

The patient will be excluded from enrollment if any of the following are true:

1. *Focal pulmonary infiltrates on chest radiograph.*
2. *Diagnosed with severe obstructive or restrictive pulmonary disease that is significantly contributing to the patient's pulmonary hypertension.*

3. *Received treatment with nitric oxide for inhalation within 30 days prior to study initiation, are on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin.*

4. *Pregnant (urine HCG +).*

5. *Baseline PCWP > 20 mmHg*

15. Upon conclusion of the INOT22 study and completion of the final study report, INO noted that, subsequent to excluding patients with pre-existing LVD, the rate of SAEs (including SAEs associated with heart failure) was significantly reduced. There were 5 SAEs among the first 24 subjects prior to the additional exclusion criterion, but only 2 SAEs among the last 80 subjects in the study after the additional exclusion. Furthermore, there were 2 SAEs among the 4 subjects with evidence of pre-existing left ventricular dysfunction, but only 5 SAEs amongst the 120 subjects without evidence of left ventricular dysfunction.

16. Based upon this unexpected finding, on February 25, 2009, INO submitted a labeling supplement to the FDA seeking to amend the prescribing information for INOMAX to include a warning statement for physicians indicating that the use of iNO in patients with pre-existing LVD could cause SAEs, such as pulmonary edema.

17. On August 28, 2009, the FDA approved the INOMAX labeling supplement to include (i) a statement in the Warnings and Precautions section of the INOMAX prescribing information that states, **"Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema;"** and (ii) new section 5.4 of the INOMAX prescribing information that states, **"Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema)."**

18. Based upon my review of the Office Action dated June 19, 2012, and the references cited therein, none of the cited prior art suggests, appreciates or otherwise recognizes that exclusion of neonates, near-term neonates or children with LV dysfunction from administration of iNO for diagnostic or treatment purposes would reduce the risk of adverse events and/or SAEs such as pulmonary edema, as such terminology is generally understood in the medical arts.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from this patent application.

Dated: August 17, 2012

James S. Baldassarre, M.D.

22896364.doc

EXHIBIT 1

CURRICULUM VITAE

James S. Baldassarre, MD

HOME:	145 Pebble Woods Dr Doylestown, Pa 18901
PHONE: **EMAIL:**	215-348-2835 jbaldassarre@comcast.net
BUSINESS :	**Ikaria/ INO Therapeutics** Perryville III Corporate Park 53 Frontage Rd, Third Floor PO Box 9001 Hampton, NJ 08827
PHONE: **CELL:**	908-238-6363 908-500-8111

EDUCATION:	S.U.N.Y. Downstate Medical Center Brooklyn, NY 1986 - M.D.
	S.U.N.Y., Binghamton, NY 1982 - Biology, B.S.

EMPLOYMENT:

2007- present **Ikaria (INO Therapeutics)**
Vice President, Clinical Research

- Project Team Leader/Medical Leader IK 5001, injectable device for prevention of congestive heart failure
- Medical Leader IK 3001 (INOmax) in prevention of BPD in premature infants
- Participation on R&D Leadership Team, Business Development Team, and management of clinical research, as well as selection of investigator-sponsored trials.
- Supervised Director of Drug Safety and two Research Directors, as well as the IK 5001 project team.
- Business Development activities included preliminary and detailed diligence on numerous compounds, with 3 compounds in-licensed over the last year. Also a member of the Research Management Committee (RMC) with monthly review and critique of all research projects (drug and device) including all development projects: IK 5001 for prevention of left ventricular remodelling after acute MI, carbon monoxide for organ transplant (especially delayed graft function in kidney transplant), sulfide for ischemia -reperfusion injury and small peptides for hepato-renal syndrome and capillary leak syndrome.
- Successfully sNDA for INOmax which also ensured pediatric exclusivity

2008-2010 Project Team Leader: INOmax®

- Lead cross-functional team to manage life cycle for the company's flagship product.
- Design and execution of phase 2 and 3 trials for additional indications, selection an oversight of investigator-initiated trials, pharmacovigilance and safety review, numerous interactions with FDA including successful sNDA, and numerous Type B and Type C meetings.
- Contributed medical input to clinical study protocols, statistical analysis plans, clinical study reports and manuscripts.
- Wrote and revise numerous additional documents including IND annual updates, PSURs and investigational drug brochures.
- Named inventor on an additional 'method of use' patent (now under review).
- Reviewed and approval promotional materials, educational materials and press releases.
- Provided medical input to device development and design specifications.
- Therapeutics areas studied under INOmax include neonatology (persistent pulmonary hypertension, respiratory distress syndrome and bronchpulmonary dysplasia), acute myocardial infarction, cardiovascular surgery and congenital heart disease, pulmonary hypertension and sickle cell disease.

2003- 2007 **INO Therapeutics**
Senior Director, Clinical Research

- Drove clinical research strategy and operations in several areas of interest for INOmax.
- Selected KOLs for steering committees, wrote and executed 6 clinical trials, leading to 3 manuscripts and 1 sNDA .
- Also interacted with numerous independent clinical researchers to coordinate research strategy for INOmax; this involved negotiating access to NIH-sponsored study data for use in FDA submissions, reanalysis of this data and preparation of reports suitable for FDA submission.
- Developed relationships with highest-level academic experts to maximize credibility of trial data and analysis.
- Oversaw all of clinical operations staff of 20, including clinical operations, data management, biostatistics and pharmacovigilance.

2003 **Johnson & Johnson Pharmaceutical Research and Development LLC**
Compound Development Team Leader/Clinical Leader-REGRANEX®

- Wound healing product based on recombinant platelet-derived growth factor. Regranex was a marketed product with post-approval clinical commitments.
- Led project team to successful resolution of commitments with EMEA.

2001-2003 **Johnson & Johnson Pharmacentical Research and Development LLC**
Senior Director, Operations Team Management

- Project management leadership for several project teams, with primary emphasis in oral hypoglycaemic and anti-obesity drugs. Applied methodologies to improve project planning, and risk and cost management. Assisted with implementation of pilot eDC project.

1999-2001 **Janssen Research Foundation**
Director of Clinical Research Italy/Greece/Spain et alia

- Member of European R&D leadership team, reporting to EVP in Belgium. Managed Clinical Research staff in several countries, implementing new organization structure and processes, including country specific enrolment metrics.
- Created skills mapping tool for staff development and succession planning.

1997 -1999 **Janssen-Cilag Limited, UK**
Head of Clinical Research and Senior Medical Advisor

- Head of Clinical Research: managed a group of 5 clinical research managers in all therapeutic areas of interest to Janssen Research Foundation, including epilepsy (Topamax), schizophrenia (Risperdal), pain (Ultram) and gastric dysmotility.
- Oversaw execution of Phase 1-4 clinical trials
- Senior Medical Advisor also reviewed and approved promotional materials, training materials, educational materials etc. Participated on the Johnson & Johnson Signature of Quality internal assessment as lead from Clinical Research.

1993 - 1997 **R.W. Johnson Pharmaceutical Research Institute**
Spring House, PA
1995-1997 Associate Director, Clinical Research
1993-1995 Assistant Director, Clinical Research

1992 - 1993 **Presbyterian Medical Center**
Philadelphia, PA
Attending Physician, Division of Infectious Diseases

1986 - 1993 **Medical College of Pennsylvania**
Philadelphia, PA
1990-1993 Fellow, Division of Infectious Diseases
1989-1990 Medical Director (half time)
1986-1989 Internship/Residency Internal Medicine

1989 - 1990 **Philadelphia Department of Health**
Philadelphia, PA
Medical Director, Sexually Transmitted Diseases Clinic (half time)

ACADEMIC APPOINTMENT :

John Radcliffe Hospital, Oxford, UK

1999-2000 Honorary SHO, Dept of Clinical Pharmacology

Medical College of Pennsylvania, Philadelphia, USA

1994 -	Clinical Assistant Professor, Department of Medicine
1991 - 1993	Instructor in Medicine

CERTIFICATION:

Diplomat, A.B.I.M.
Internal Medicine, 1989
Infectious Diseases, 1992
Limited GMC registration, 1999

EMPLOYMENT-RELATED ACTIVITIES/COMMITTEES:

RWJ-PRI Continuous Process Improvement Committee	1995-1996
Johnson & Johnson Signature of Quality submission	1997 and 1999
JJ PRD New Product Development Committee Implementation Team	2002-2003
Ikaria Opportunity Review Team	2007-present

PUBLICATIONS:

1. Levison M E and Baldassarre J S: Intra-Abdominal Infections. *Current Practice of Medicine* 1993.
2. Baldassarre J S and Abrutyn E: Antibiotic-Resistant Streptococcus pneumoniae. Infectious Disease Practice 1993; 17 (9).
3. Baldassarre J S and Abrutyn E: Genital Ulcer Disease. *Infectious Disease Practice* 1992; 16 (9); 1-7.
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5. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Keys to Making the Diagnosis. *Modern Med* 1992; 60: 11 42-58.
6. Baldassarre J S, Ingerman M J, Nansteel J, and Santoro J: Development of Listeria Meningitis during Vancomycin Therapy: A Case Report. *J Infect Dis* 1991; 164: 221-222.
7. Baldassarre J S, Update on the Management of Sexually Transmitted Diseases. *Phila Med* 1991; 87-5 230-233.
8. Baldassarre J S and Kaye D: Special Problems in Urinary Tract Infection in the Elderly. *Med Clin North Am* 1991; 75:2 375-390.
9. Baldassarre J S, Johnson CC and Levison M E: Peritonitis: Update on Pathophysiology, Clinical Manifestations and Management. *Clinical Infectious Diseases* 1997; 24(6); 1035-47.
10. Baldassarre JS and Levison ME: Intra-abdominal Infections *Current Practice of Medicine* 1999, vol 2 (4)591-605
11. Baldassarre JS and Pledger GW Clinical Trial Design for New Antiepileptic Drugs: Determination of Dose and Titration Schedules *Rev Contemp Pharmacother* 1999; 10

12. Mercier JC, Hummler H, Durrmeyer X, Sanchez-Luna M, Carnielli V, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, Baldassarre J; EUNO Study Group. Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial. *Lancet.* 2010 Jul 31;376(9738):346-54.

13. Barst RJ, Agnoletti G, Fraisse A, Baldassarre J, Wessel DL; NO Diagnostic Study Group. Vasodilator testing with nitric oxide and/or oxygen in pediatric pulmonary hypertension *Pediatr Cardiol.* 2010 Jul; 31(5):598-606.

14. Mark T. Gladwin; Gregory J. Kato; Debra Weiner; Onyinye C. Onyekwere; Carlton Dampier; Lewis Hsu; R. Ward Hagar; Thomas Howard; Rachelle Nuss; Maureen M. Okam; Carole K. Tremonti; Brian Berman; Anthony Villella; Lakshmanan Krishnamurti; Sophie Lanzkron; Oswaldo Castro; Victor R. Gordeuk; Wynona A. Coles; Marlene Peters-Lawrence; James Nichols; Mary K. Hall; Mariana Hildesheim; William C. Blackwelder; James Baldassarre; James F. Casella; for the DeNOVO Investigators Nitric Oxide for Inhalation in the Acute Treatment of Sickle Cell Pain Crisis: A Randomized Controlled Trial *JAMA.* 2011;305:893-902

15. E. Potapov, D. Meyer, M. Swaminathan, M. Ramsay, A. El Banayosy, C. Diehl et al. Inhaled Nitric Oxide After Left Ventricular Assist Device Placement: A Prospective, Randomized, Double-Blind, Multicenter, Placebo-Controlled Trial *J Heart Lung Transplant* 28 Apr 2011 (epub: 28 4 2011), ISSN: 1557–3117.

16. B. Goldstein, J. Baldassarre, J. Young Effects Of Inhaled Nitric Oxide on Hemostasis in Healthy Adults Treated with Heparin: A Randomized, Controlled, Blinded Crossover Study *Thrombosis Journal* 2012; 10:1

Book Chapters

Baldassarre J S and Kaye D: Principles and Overview of Antibiotic Use in Infective Endocarditis. In: Kaye D (ed) *Infective Endocarditis* 2nd ed. New York: Raven Press, 1992; 169-190.

Abstracts

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6. Glauser TA, Olberding L, Clark P, Reife R, Baldassarre J, Conover D. Topiramate monotherapy substitution in children with partial epilepsy. Epilepsia 1996; 37(suppl 4):98.

7. JC Mercier, H. Hummler, X Durrmeyer, M. Sanchez-Luna, V Carnielli, D Field, A. Greenough, B. Van Overmeire, B Jonsson, M Hallman, J Baldassarre, for the EUNO Study Group. The effects of inhaled nitric oxide on the development of bronchopulmonary dysplasia (BPD) in preterm infants: the 'EUNO' multicentre randomised clinical trial. European Academy of Pediatrics; Nice, France October 2008

8. RJ Barst, G Agnoletti, A Fraisse, J Baldassarre, DL Wessel. Nitric Oxide in Combination with Oxygen Versus Either Oxygen Alone or Nitric Oxide Alone for Acute Vasodilator Testing in Children with Pulmonary Hypertension: A Multicenter, Randomized Study. Pediatric Academic Societies Scientific Meeting, Baltimore Md; May 2009 [3861.195]

9. EV Potapov; D Meyer; M Swaminathan; M Ramsay; A El Banayosy; C Diehl; B Veynovich; ID Gregoric; J Baldassarre; M J Zucker; R Hetzer Use of Inhaled Nitric Oxide After Left Ventricular Assist Device Placement: Results of a Prospective, Randomized, Double-Blind, Multicenter, Placebo-Controlled Trial. American Heart Association Scientific Sessions Orlando, Fl; Nov 2009 [3663]

10. X. Durrmeyer, H. Hummler, M. Sanchez-Luna, V. Carnielli, D. Field, A. Greenough, B. Van Overmeire, B. Jonsson, M. Hallman, J. Baldassarre, N. Marlow, J.-C. Mercier Neurodevelopmental and Respiratory Outcomes at 2 Years Oaf Age an Preterm Infants Treated With Inhaled Nitric Oxide: The EUNO Trial Follow Up *Eur Society for Ped Res* Oct 2011 Newcastle, UK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD DECLARATION OF JAMES S. BALDASSARRE, M.D.
UNDER 37 C.F.R. § 1.132

I, James S. Baldassarre, do hereby declare the following:

1. I am a co-inventor on U.S. Application No. 12/821,041.

2. I currently hold the position of Vice President of Clinical Research at INO Therapeutics LLC ("INO"), which is a wholly-owned subsidiary of Ikaria, Inc. A copy of my *curriculum vitae* is attached as **Exhibit 1**.

3. I have over 20 years of experience as a physician and over fifteen years of experience directing clinical research in the pharmaceutical industry.

4. In 2004, I was the Medical Monitor responsible for the design and execution of the INOT22 study.

5. The INOT22 study, entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilatory Testing," was a randomized, multi-center study having an expected enrollment of 150 patients, aged four weeks to 18 years, in approximately 18 study sites over approximately 2 years.

6. The INOT22 study was established and designed by the study sponsor, INO Therapeutics LLC ("INO"), and a Steering Committee comprising internationally recognized experts in the field of pediatric heart and lung disease, whose members would assist INO to develop the INOT22 protocol, monitor the progress of the trial, and provide recommendations to INO on changes in the procedures and conduct of the trial.

7. The Steering Committee consisted of:

a. David L. Wessel, MD, presently Senior Vice President, The Center for Hospital Based Specialties, and Division Chief, Pediatric Critical Care Medicine, at Children's National Medical Center, Washington, DC;
b. Robyn J. Barst, MD, presently Professor Emeritus of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York; and
c. Duncan J. Macrae, MD, presently Director, Pediatric Intensive Care, Royal Brompton Hospital, London, UK.

8. The original INOT22 study protocol designed by INO and the Steering Committee did not exclude study patients with pre-existing left ventricular dysfunction who were not dependent on right-to-left shunting of blood.

9. After the INOT22 study protocol design, but prior to study initiation and enrollment, the original INOT22 study protocol was reviewed by an Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the 18 participating study institutions, including review by the principal investigator within each study institution. In addition, prior to study initiation and enrollment, the original INOT22 study protocol was reviewed by the US Food and Drug Administration (FDA) and separately reviewed by each national Health Authority (European equivalent to FDA) within the four European countries participating in the INOT22 trial (United Kingdom, France, Netherlands and Spain). In addition,

INO regularly requested input and scientific guidance on the clinical trial from its own Scientific Advisory Board. At no time did any member of the Steering Committee, INO, an IRB or IEC, an individual principal investigator, an Advisory Board member, FDA or European Health Authority appreciate, recognize or otherwise suggest that subjects with pre-existing left ventricular dysfunction who are not dependent on right-to-left shunt should be excluded from the INOT22 study or that such subjects would be anticipated or predicted to have an increased risk of adverse events or serious adverse events arising from the administration to them of inhaled nitric oxide.

10. Under FDA regulations, an IRB is an appropriately constituted group that has been formally designated to review and monitor biomedical research involving human subjects. In accordance with FDA regulations, an IRB has the authority to approve, require modifications in (to secure approval), or disapprove research. This group review serves an important role in the protection of the rights and welfare of human research subjects. The purpose of IRB review is to assure, both in advance and by periodic review, that appropriate steps are taken to protect the rights and welfare of humans participating as subjects in the research. To accomplish this purpose, IRBs use a group process to review research protocols to ensure protection of the rights and welfare of human subjects of research. An IRB must have at least five members and each member must have enough experience, expertise and diversity to make an informed decision on whether the research is ethical, informed consent is sufficient and the appropriate safeguards have been put in place (see 21 CFR Part 56).

11. In Europe, an Ethics Committee is an independent body in an EC Member State consisting of healthcare professionals and non-medical members whose responsibility is to protect the rights, safety and well-being of human subjects involved in a clinical trial and to provide public assurance of that protection by expressing an opinion on a proposed clinical trial protocol, the suitability of the investigators, and the adequacy of facilities involved in a trial (see Directive 2001/20/EC).

12. In total, at least 115 individuals experienced in, and responsible for, the review of clinical trial protocols for patient safety, in addition to the FDA and four European Health Authorities, reviewed the original INOT22 protocol prior to initiating the INOT22 study. Again, not a single individual or authority suggested, predicted or raised a concern about an increased risk associated with the use of inhaled nitric oxide in study subjects with pre-existing left ventricular dysfunction who were not dependent on right-to-left shunt.

13. On the contrary, it was only after unexpected serious adverse events (including at least one death) occurred during the course of the INOT22 study that the study protocol was amended to exclude study subjects with pre-existing left ventricular dysfunction who were not dependent on right-to-left shunt. In particular, the exclusion criteria of the INOT22 study were amended to exclude subjects having an elevated pulmonary capillary wedge pressure greater than 20 mm Hg.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent that may issue on the present application.

Dated: August 17, 2012

James S. Baldassarre, M.D.

22787574.doc

EXHIBIT 1

CURRICULUM VITAE

James S. Baldassarre, MD

HOME: 145 Pebble Woods Dr
Doylestown, Pa
18901

PHONE: 215-348-2835
EMAIL: jbaldassarre@comcast.net

BUSINESS : **Ikaria/ INO Therapeutics**
Perryville III Corporate Park
53 Frontage Rd, Third Floor
PO Box 9001
Hampton, NJ 08827

PHONE: 908-238-6363
CELL: 908-500-8111

EDUCATION: S.U.N.Y. Downstate Medical Center
Brooklyn, NY
1986 - M.D.

S.U.N.Y., Binghamton, NY
1982 - Biology, B.S.

EMPLOYMENT:

2007- present **Ikaria (INO Therapeutics)**
Vice President, Clinical Research

- Project Team Leader/Medical Leader IK 5001, injectable device for prevention of congestive heart failure
- Medical Leader IK 3001 (INOmax) in prevention of BPD in premature infants
- Participation on R&D Leadership Team, Business Development Team, and management of clinical research, as well as selection of investigator-sponsored trials.
- Supervised Director of Drug Safety and two Research Directors, as well as the IK 5001 project team.
- Business Development activities included preliminary and detailed diligence on numerous compounds, with 3 compounds in-licensed over the last year. Also a member of the Research Management Committee (RMC) with monthly review and critique of all research projects (drug and device) including all development projects: IK 5001 for prevention of left ventricular remodelling after acute MI, carbon monoxide for organ transplant (especially delayed graft function in kidney transplant), sulfide for ischemia -reperfusion injury and small peptides for hepato-renal syndrome and capillary leak syndrome.
- Successfully sNDA for INOmax which also ensured pediatric exclusivity

2008-2010 Project Team Leader: INOmax®

- Lead cross-functional team to manage life cycle for the company's flagship product.
- Design and execution of phase 2 and 3 trials for additional indications, selection an oversight of investigator-initiated trials, pharmacovigilance and safety review, numerous interactions with FDA including successful sNDA, and numerous Type B and Type C meetings.
- Contributed medical input to clinical study protocols, statistical analysis plans, clinical study reports and manuscripts.
- Wrote and revise numerous additional documents including IND annual updates, PSURs and investigational drug brochures.
- Named inventor on an additional 'method of use' patent (now under review).
- Reviewed and approval promotional materials, educational materials and press releases.
- Provided medical input to device development and design specifications.
- Therapeutics areas studied under INOmax include neonatology (persistent pulmonary hypertension, respiratory distress syndrome and bronchpulmonary dysplasia), acute myocardial infarction, cardiovascular surgery and congenital heart disease, pulmonary hypertension and sickle cell disease.

2003- 2007 **INO Therapeutics**
Senior Director, Clinical Research

- Drove clinical research strategy and operations in several areas of interest for INOmax.
- Selected KOLs for steering committees, wrote and executed 6 clinical trials, leading to 3 manuscripts and 1 sNDA .
- Also interacted with numerous independent clinical researchers to coordinate research strategy for INOmax; this involved negotiating access to NIH-sponsored study data for use in FDA submissions, reanalysis of this data and preparation of reports suitable for FDA submission.
- Developed relationships with highest-level academic experts to maximize credibility of trial data and analysis.
- Oversaw all of clinical operations staff of 20, including clinical operations, data management, biostatistics and pharmacovigilance.

2003 **Johnson & Johnson Pharmaceutical Research and Development LLC**
Compound Development Team Leader/Clinical Leader-REGRANEX®

- Wound healing product based on recombinant platelet-derived growth factor. Regranex was a marketed product with post-approval clinical commitments.
- Led project team to successful resolution of commitments with EMEA.

2001-2003 **Johnson & Johnson Pharmaceutical Research and Development LLC**
Senior Director, Operations Team Management

- Project management leadership for several project teams, with primary emphasis in oral hypoglycaemic and anti-obesity drugs. Applied methodologies to improve project planning, and risk and cost management. Assisted with implementation of pilot eDC project.

1999-2001 **Janssen Research Foundation**
Director of Clinical Research Italy/Greece/Spain et alia

- Member of European R&D leadership team, reporting to EVP in Belgium. Managed Clinical Research staff in several countries, implementing new organization structure and processes, including country specific enrolment metrics.
- Created skills mapping tool for staff development and succession planning.

1997 -1999 **Janssen-Cilag Limited, UK**
Head of Clinical Research and Senior Medical Advisor

- Head of Clinical Research: managed a group of 5 clinical research managers in all therapeutic areas of interest to Janssen Research Foundation, including epilepsy (Topamax), schizophrenia (Risperdal), pain (Ultram) and gastric dysmotility.
- Oversaw execution of Phase 1-4 clinical trials
- Senior Medical Advisor also reviewed and approved promotional materials, training materials, educational materials etc. Participated on the Johnson & Johnson Signature of Quality internal assessment as lead from Clinical Research.

1993 - 1997 **R.W. Johnson Pharmaceutical Research Institute**
Spring House, PA
1995-1997 Associate Director, Clinical Research
1993-1995 Assistant Director, Clinical Research

1992 - 1993 **Presbyterian Medical Center**
Philadelphia, PA
Attending Physician, Division of Infectious Diseases

1986 - 1993 **Medical College of Pennsylvania**
Philadelphia, PA
1990-1993 Fellow, Division of Infectious Diseases
1989-1990 Medical Director (half time)
1986-1989 Internship/Residency Internal Medicine

1989 - 1990 **Philadelphia Department of Health**
Philadelphia, PA
Medical Director, Sexually Transmitted Diseases Clinic (half time)

ACADEMIC APPOINTMENT :

John Radcliffe Hospital, Oxford, UK

1999-2000 Honorary SHO, Dept of Clinical Pharmacology

Medical College of Pennsylvania, Philadelphia, USA

1994 - Clinical Assistant Professor, Department of Medicine

1991 - 1993 Instructor in Medicine

CERTIFICATION:

Diplomat, A.B.I.M.
Internal Medicine, 1989
Infectious Diseases, 1992
Limited GMC registration, 1999

EMPLOYMENT-RELATED ACTIVITIES/COMMITTEES:

RWJ-PRI Continuous Process Improvement Committee	1995-1996
Johnson & Johnson Signature of Quality submission	1997 and 1999
JJ PRD New Product Development Committee Implementation Team	2002-2003
Ikaria Opportunity Review Team	2007-present

PUBLICATIONS:

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3. Baldassarre J S and Abrutyn E: Genital Ulcer Disease. *Infectious Disease Practice* 1992; 16 (9); 1-7.

4. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Time to Reassess Treatment Strategies. *Modern Med* 1992; 60:12 86-91.

5. Levison M E and Baldassarre J S: Community Acquired Pneumonia: Keys to Making the Diagnosis. *Modern Med* 1992; 60: 11 42-58.

6. Baldassarre J S, Ingerman M J, Nansteel J, and Santoro J: Development of Listeria Meningitis during Vancomycin Therapy: A Case Report. *J Infect Dis* 1991; 164: 221-222.

7. Baldassarre J S, Update on the Management of Sexually Transmitted Diseases. *Phila Med* 1991; 87-5 230-233.

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6. Glauser TA, Olberding L, Clark P, Reife R, Baldassarre J, Conover D. Topiramate monotherapy substitution in children with partial epilepsy. Epilepsia 1996; 37(suppl 4):98.

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10. X. Durrmeyer, H. Hummler, M. Sanchez-Luna, V. Carnielli, D. Field, A. Greenough, B. Van Overmeire, B. Jonsson, M. Hallman, J. Baldassarre, N. Marlow, J.-C. Mercier Neurodevelopmental and Respiratory Outcomes at 2 Years Oaf Age an Preterm Infants Treated With Inhaled Nitric Oxide: The EUNO Trial Follow Up *Eur Society for Ped Res* Oct 2011 Newcastle, UK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
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Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

FOURTH DECLARATION OF DOUGLAS A. GREENE, M.D.
UNDER 37 C.F.R. § 1.132

I, Douglas A. Greene, do hereby declare the following:

1. I currently hold the position of Executive Vice President and Head of Research and Development at INO Therapeutics LLC ("INO"), which is a wholly-owned subsidiary of Ikaria, Inc. A copy of my *curriculum vitae* is attached as **Exhibit 1.**

2. I received an undergraduate degree in biology (*cum laude*) from Princeton University in 1966 and a doctoral degree in medicine (M.D.) from Johns Hopkins School of Medicine in 1970.

3. I spent the next thirty years of my medical career (1970-2000) practicing and teaching medicine at some of America's foremost academic medical centers, including Johns Hopkins, the University of Pennsylvania, the University of Pittsburgh, and the University of Michigan. At Michigan, I was a full professor of internal medicine, director of the Michigan Diabetes Research and Training Center, and chief of the Division of Endocrinology and Metabolism.

4. In 2000, I left Michigan to join Merck as Executive Vice President in charge of clinical sciences and product development. In this role, I supervised and directly managed all clinical research at Merck Research Laboratories, among other duties.

5. In 2003, I left Merck for Sanofi-Aventis, where I became a Senior Vice President and Chief Medical Officer. My duties at Sanofi-Aventis included overseeing all aspects of pre-clinical and clinical regulatory development of the company's products and overseeing all medical aspects of the company's US business.

6. In 2010, I joined INO, where – as noted above – I am presently Executive Vice President and Head of Research and Development.

7. I have been shown a Final Office Action issued by the United States Patent and Trademark Office (USPTO) on June 27, 2011 (the "6/27/11 Office Action") in the present patent application. This Office Action rejected the then-pending claims as "obvious" based on clinical interpretations presented by the USPTO regarding the teaching and disclosure of Atz & Wessel (Seminars in Perinatology 1997, 21(5), 441-455), Kinsella et al. (Lancet 1999, 354 1061-1065) and Loh et al. (Circulation 1994, 90, 2780-2785). Below is my professional opinion and interpretation of the arguments and clinical interpretations presented by the USPTO within the 6/27/11 Office Action.

8. On page 9 of the 6/27/11 Office Action, the Examiner states:

> *"Atz et al. teach that: 'Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension.' (page 452, left column)."*

A more complete excerpt from Atz & Wessel, p. 452, left column, is as follows:

> Caution should be exercised when administering NO to patients with severe left ventricular dysfunction and pulmonary hypertension. In **adults with ischemic cardiomyopathy**, sudden pulmonary vasodilation may occasionally unload the right ventricle sufficiently to increase pulmonary blood flow and

> harmfully augment preload in a compromised left ventricle. The attendant increase in left atrial pressure may produce pulmonary edema. ... A different but related phenomenon may be operative in the newborn .... (emphasis added)

Thus, although Atz & Wessel warns that "[c]aution should be exercised when administering nitric oxide (NO) to patients with severe left ventricular dysfunction and pulmonary hypertension[,]" this caution is specifically limited to two populations of patients. In the first population, the statement in Atz & Wessel p. 452, left column, is directed to adult patients with ischemic cardiomyopathy who also exhibit severe left ventricular dysfunction and pulmonary hypertension. This patient population is clearly different from the neonatal population that is the object of the teaching of the present claims.

9. Further in the same paragraph, Atz & Wessel specifically refers to a <u>second patient population, which is also distinct from that of the present patent application</u>, to whom inhaled NO should not be administered, namely, neonates depending on right-to-left shunting of blood:

> A different but related phenomenon may be operative in the newborn with severe left ventricular dysfunction and pulmonary hypertension. In these patients, the **systemic circulation may depend in part on the ability of the right ventricle to sustain cardiac output through a right-to-left shunt across the patent ductus arteriosus.** Selective pulmonary vasodilation may redirect the right ventricular output to the lungs and away from the systemic circulation. (emphasis added)

For this second patient population, Atz & Wessel state that these patients exhibit a "different but related phenomenon" from that observed in adults with ischemic cardiomyopathy. This second population of patients consists of newborn patients with congenital heart disease and left ventricular dysfunction who are **dependent on a right-to-left shunt through a ductus arteriosus** in order to maintain the peripheral circulation necessary to survive. In these patients, a patent ductus provides the only alternate pathway for blood being pumped by the right ventricle to **bypass the dysfunctional left ventricle** and thereby substitute for the dysfunctional left ventricle in providing life-sustaining blood flow to the peripheral circulation. Blood emerging from the right ventricle has only two possible pathways, either through the pulmonary circulation and then back to the dysfunctional left ventricle, or through the patent ductus arteriosus in a right-to-left shunt to reach the systemic circulation. Inhaled NO dilates the pulmonary circulation, and therefore would divert blood to the lungs at the expense of the patent ductus arteriosus and systemic circulation, causing systemic vascular collapse and death. Again, this second patient population described by Atz & Wessel is also completely different from the patient population addressed in the present claims, which is term or near term neonates with left ventricular dysfunction who are **NOT dependent upon right-to-left shunting.**

10. The risk of circulatory collapse in the subset of newborns with congenital heart disease and severe left ventricular dysfunction **who are dependent upon a right-to-left shunt** through a patent ductus arteriosus was well known in this field long before the Atz & Wessel publication, as evidenced by the contraindication stated in the US Food and Drug Administration (FDA) prescribing information for INOMAX® (nitric oxide) for inhalation from the time of its initial approval by the FDA in 1999: "**CONTRAINDICATIONS:** Neonates known to be dependent on right-to-left shunting of blood".

11. As a result of the INOT22 study, it was recognized that a second population of neonates existed, distinct from the population described in Atz & Wessel, that had an increased risk of adverse events when inhaled NO was administered, namely: pediatric patients with left ventricular dysfunction **who are not dependent upon right-to-left shunting of blood**. In view of this newly identified risk, the FDA imposed the addition of a distinct and separate precaution to the prescribing information for INOMAX specifically cautioning about an additional risk of pulmonary edema for patients with left ventricular dysfunction (see paragraph 15). It is important to note that patients covered in the pre-existing contraindication (specifically neonates known to be dependent on right-to-left shunting of blood) were completely excluded from INOT22 by virtue of the labeled contraindication. The newly discovered risk of adverse events in neonates and children with left ventricular dysfunction **who are not dependent on right-to-**

left shunting was not addressed, suggested or otherwise inferred from the teachings of Atz & Wessel, because when Atz and Wessel recommend that inhaled NO should be used with caution "if at all", that warning relates to neonates **who are dependent upon right-to-left shunting of blood** – a completely different population of patients than the population that is addressed in the present claims.

12. On page 9 of the 6/27/11 Office Action, the Examiner further states:

> *Since the patients have pulmonary hypertension as claimed in instant claim 25, then they also intrinsically have hypoxic respiratory failure in the absence of evidence to the contrary.*

This statement is not medically accurate. Pulmonary hypertension occurs in many conditions other than hypoxic respiratory failure, such as congenital heart disease, maternal use of serotonin reuptake inhibitors, idiopathic pulmonary hypertension, etc.

13. On page 10 of the 6/27/11 Office Action, the Examiner states:

> *Atz et al. continues with: "Therefore, in newborns with severe left ventricular dysfunction, predominantly left to right shunting at the foramen ovale and **exclusively right to left shunting at the ductus arteriosus**, NO should be used with extreme caution, if at all. We and others have reported **adverse outcomes** in this circumstance." (p. 452, left column) (emphasis differing from original).*

This statement merely reiterates the "caution" delivered by Atz & Wessel for the second population of patients identified in that publication, namely neonates **dependent upon a right-to-left shunt** at the ductus arteriosus. In this statement, Atz & Wessel simply teach that patients with severe left ventricular dysfunction dependent upon an exclusively right-to-left shunt at the ductus arteriosus often have coexistent predominantly left-to-right shunt at the foramen ovale. This additional left-to-right shunt at the foramen ovale, upstream from the dysfunctional left ventricle, permits blood to bypass the dysfunctional left ventricle and enter the right side of the heart, thereby enhancing the ability of the right ventricle to pump sufficient blood through the ductus arteriosus to maintain the systemic circulation. The population of patients dependent upon right-to-left shunting of blood (with or without shunting at the foramen ovale) was already excluded by the pre-existing FDA-mandated contraindication for inhaled NO, and is distinct from the patient population addressed in the present claims.

14. On page 10 of the 6/27/11 Office Action, the Examiner states:

> *Atz et al. thus identify conditions in the patients which is screening of the patient. Thus, Atz et al. fairly teaches excluding patients which include pediatric patients with left ventricular dysfunction from inhaled NO treatment because the Examiner interprets "if at all" to mean no treatment and hence exclusion from treatment. The left ventricular dysfunction is intrinsically pre-existing.*

This statement misinterprets the teaching of Atz & Wessel. Specifically, "if at all" refers to the second patient population, wherein no treatment is allowed in the population of newborn "patients dependent upon right-to-left shunting of blood" who are at risk for circulatory collapse. Because these patients were already contraindicated in the drug labeling for inhaled NO prior to INOT22 (see paragraph 10 above), they were excluded from INOT22 and, more importantly, are distinct from the patients identified in the new inhaled NO safety warnings mandated by the FDA in view of the risk that was newly identified as a result of the INOT22 study.

15. On February 25, 2009, INO Therapeutics LLC (owner of NDA 20845) submitted a label supplement to the FDA seeking to amend the prescribing information (i.e., the "label") for INOMAX® (nitric oxide) for inhalation, to include a new warning statement based on the unexpected outcome of the INOT22 study. On August 28, 2009, the FDA approved the INOMAX® label supplement to include the following new information:

> *WARNINGS AND PRECAUTIONS*
>
> *Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).*

> *5 WARNINGS AND PRECAUTIONS*
>
> *5.4 Heart Failure: Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).*

Thereafter, similar warnings were added to the INOMAX label by Health Authorities in Japan, Europe, Canada and Australia. The FDA (and its counterparts in foreign nations) would not add new warnings and precautions to the label of an approved drug that merely restate a known contraindication already existing on the approved drug label. Indeed, the new FDA-approved warnings for the use of nitric oxide are clinically distinct from the existing, original INOMAX contraindication disclosed by Atz & Wessel, with respect to neonates dependent on right-to-left shunt.

16. On page 11 of the 6/27/11 Office Action, the Examiner states:

> *Kinsella et al. teach excluding patients (premature neonates) from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease (Abstract and page 1062, Methods). Since left ventricular dysfunction is a congenital heart disease, as acknowledged by Applicant, (see specification [0028]), and it would be pre-existing, then the methods of Kinsella et al. intrinsically exclude this patient population from the method. ... The intended patient population is intrinsically at risk of one or more adverse events. Patients are intrinsically identified for nitric oxide inhalation treatment, diagnosed for congenital heart disease which intrinsically includes left ventricular dysfunction, and if the patient meets the criteria then treatment with NO is performed thereby reducing the risk of adverse events associated with the treatment.*

Based on these statements, it is clear that the Examiner fails to understand several critical aspects of the study of Kinsella et al.

17. First and foremost, the patients included in the Kinsella et al. trial were premature neonates who have severe respiratory failure due to immature lungs and surfactant deficiency, rather than term and near-term neonates suffering from pulmonary hypertension. In addition, none of the premature neonates enrolled in Kinsella et al. suffered from pulmonary hypertension.

Thus, the patients included in Kinsella et al. were clinically differentiated, by age, etiology and pathophysiology, from the term and near-term neonates addressed in the present claims.

18. Secondly, exclusion of patients from a particular study may occur for a variety of reasons. For example, clinical trial inclusion and exclusion criteria are often chosen to define or restrict the study population in order to maximize homogeneity, thereby minimizing the presence of potentially confounding factors. This exclusion greatly facilitates the interpretation of the study results, and increases the soundness of the conclusions reached in the study. Accordingly, patients with background disease sufficiently severe to overwhelm or confound an expected treatment effect are systematically identified and excluded quite independently from considerations of anticipated safety or efficacy of the test article in this particular patient group.

19. For example, patients with malignancy are often excluded from non-oncologic clinical trials, not because the test agents are unsafe, pose any specific risk in this population, or will not work, but rather because the clinical results will be confounded by the wholly unrelated effects of the underlying malignancy, thereby reducing the power of the clinical trial to answer a specific hypothesis regarding the test treatment. As a specific example, exclusion of patients with malignancy or advanced heart failure from cholesterol lowering trials does not imply that statins are unsafe or ineffective in these patients, but rather that their inclusion would confound the potential effects of statins on overall mortality or cardiovascular events.

20. In the specific case of Kinsella et al., it is clear that one of ordinary skill in the art would understand that the patients having fatal congenital anomalies or congenital heart disease were excluded not because of a suspected safety risk of treating these patients with inhaled NO (e.g., a risk of pulmonary edema), but rather solely because the inclusion of such patients would have made it much more difficult – if not impossible - for Kinsella et al. to interpret the target outcomes of the study (i.e., would have "confounded" the results).

21. On pages 11-12 of the 6/27/11 Office Action, the Examiner states:

> *Loh et al. teach that inhaled nitric oxide in patients with left ventricular dysfunction may have adverse effects in patients with LV failure (Title and Abstract). Loh et al. clearly teaches that patients with pulmonary artery wedge pressure, which is synonymous with the instantly claimed pulmonary capillary wedge pressure, of greater than or equal to 18mm Hg had a greater effect of inhaled NO due to the greater degree of reactive pulmonary hypertension present in such patients (p. 2784, left column). Loh et al. state: "Since the degree of reactive pulmonary hypertension is generally related to the severity of hemodynamic compromise in patients with LV failure, it might be anticipated that patients with more severe heart failure will have a more marked hemodynamic response to inhaled NO." Loh et al. examined this prediction further and verified it (p. 2784, left column).* Original emphasis omitted.

The Examiner apparently neglects to consider that the acute hemodynamic effect of inhaled NO was studied by Loh et al. only in **adult** patients with New York Heart Association Class III or IV congestive failure due to coronary artery disease or dilated cardiomyopathy, not in neonates or children. Thus, their observations do not teach, or even suggest, the risk of inhaled NO in neonates or children with pulmonary hypertension and left ventricular dysfunction who are not dependent on right-to-left shunting of blood, the population that is addressed in the present claims.

22. The underlying etiologies and hemodynamic characteristics of both the primary heart disease and the increased pulmonary vascular resistance are drastically different in adults, as compared to non-adults, such that one cannot readily assume or anticipate clinical results observed in adults to translate to neonates or children. In particular, left ventricular dysfunction in neonates with congenital heart disease is primarily due to developmental structural disease of the heart, inborn errors of metabolism that impair energy generation in the heart muscle, or viral infection. Class III or class IV congestive heart failure in adults (in contrast to congenital heart disease in neonates or children) is due to ischemic or dilated cardiomyopathy, mostly secondary to coronary artery disease and/or chronic systemic hypertension. Pulmonary hypertension

associated with neonatal congenital heart disease is secondary to chronic hypoxemia, developmental abnormalities of the pulmonary blood vessels and/or pulmonary vascular damage from abnormally high blood flow and/or pressure through the pulmonary vasculature, resulting in evident disease of the lung vasculature. In contrast, increased pulmonary vascular resistance in adult Class III or IV congestive heart failure is due to reactive pulmonary vasoconstriction secondary to increased sympathetic tone or circulating vasoactive molecules (Loh et al., p. 2780, left column) in otherwise structurally normal blood vessels. Therefore, the hemodynamic responses to pulmonary vasodilation by inhaled NO in children or neonates without right-to-left shunting of blood but with significant pulmonary hypertension and left ventricular dysfunction cannot be reasonably predicted from the hemodynamic responses to pulmonary vasodilation by inhaled NO of adults with advanced atherosclerotic congestive heart failure and reactive neuro-humoral pulmonary vascular constriction (with or without pulmonary hypertension) as described by Loh et al.

23. On pages 12-13 of the 6/27/11 Office Action, the Examiner states:

> *It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform the method of Atz et al. and identify patients with a LVD characterized by the conditions of instant claim 24 and informing the medical provider that separately and independent from patients dependent on right to left shunt that patients with pre-existing LVD who are not dependent on right to left shunting of blood iNO may increase PWCP leading to pulmonary edema or using a pressurized cylinder of NO and recommending a dose of 20 ppm NO, as suggested by Loh et al., and Kinsella et al., and produce the instant invention.*

24. Atz & Wessel do not recommend exercising "caution" when treating term or near-term neonates who are not dependent upon right-to-left shunting, but rather refer to two **other** patient populations, namely (i) neonatal patients whose systemic circulation is dependent upon right-to-left shunting of blood and who therefore might suffer from systemic circulatory collapse if given inhaled NO (a well-known contraindication for inhaled NO) and (ii) adult patients with

New York Heart Association Class III-IV heart failure due to ischemic or dilated cardiomyopathy and increased neuro-humorally-mediated pulmonary vascular resistance who might be hemodynamically at risk for pulmonary edema if given inhaled NO (the same population discussed by Loh et al.).

25. On page 13 of the 6/27/11 Office Action, the Examiner states:

> *One of ordinary skill in the art would have been motivated to do this because: 1) if the pediatric patient is not healthy and has left ventricular dysfunction (LVD), which would intrinsically be characterized by any of the instantly claimed conditions of instant claim 24, then Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with LVD which would also render obvious all conditions/risk factors associated with LVD; 2) the art of Kinsella et al. establishes excluding patients from inhaled nitric oxide treatment if they have fatal congenital anomalies or congenital heart disease which are structural heart diseases as claimed in instant claim 24;....*

The conclusions presented by the Examiner are not clinically accurate, nor do they accurately reflect the expectations or motivations of a clinician of ordinary skill in the art at the time of the invention. Their expectation would have been quite the opposite. It is by no means true that "*if the pediatric patient is not healthy and has left ventricular dysfunction (LVD)... then Atz et al. clearly teach using extreme caution or not using NO at all in the treatment of patients with LVD.*" Atz & Wessel teach "using extreme caution or not using NO at all" only in neonates dependent upon right-to-left shunting of blood in order to avoid systemic circulatory collapse, and makes no statement regarding neonates with left ventricular dysfunction **who are not dependent upon right-to-left shunting**. Kinsella et al. do not teach about the safe or unsafe use of inhaled NO in neonates or children, let alone term or near-term neonates not dependent upon right-to-left shunting, but merely noted that they had excluded premature babies with fatal malformations or congenital heart disease from a clinical trial of inhaled NO in premature babies suffering from the respiratory distress of prematurity. Loh et al. doesn't make up for the deficiencies of Atz et al. and Kinsella et al., because Loh et al. merely teach about the effect of inhaled NO on hemodynamic measurements in adults with advanced heart failure and secondary neuro-humorally-mediated increased pulmonary vascular resistance, and speculate that these adults may be at increased risk for pulmonary edema, but do not teach anything about the use of inhaled NO in neonates or children not dependent upon right-to-left shunting.

26. On pages 14-15 of the 6/27/11 Office Action, the Examiner states:

> *Furthermore, it is already known through the teachings of Loh et al. that a pulmonary capillary wedge pressure (PCWP) of greater than 18 mg Hg serves as a guidepost for alerting the artisan to adverse events from inhaled NO. Thus, it is not inventive to exclude patients with a PCWP of greater than 20 mm Hg when the art already suggests the risk of trouble of treating patients with a PCWP of 18 mm Hg because inhaled NO increases the wedge pressure as taught by Loh et al. (see entire document).*
>
> *In summary, it remains the position of the Examiner, which is in alignment with the written opinion of the international search authority, that it is simply not inventive to 'inform' a medical provider that a neonate with LVD is at risk of adverse/serious adverse events from iNO therapy when the art already has established that fact and the ordinary artisan is alerted to this fact. If the patient has LVD then they are at risk of adverse and/or serious adverse events from iNO therapy and it is not inventive to further identify other secondary conditions associated with LVD and provide further warnings for secondary conditions that are separate and independent from the first condition but nevertheless associated with LVD to the medical provider. Screening for conditions that predispose the patient to adverse/serious adverse effects from medical treatment of iNO is obvious given the teachings above.* (emphasis in original)

It is inaccurate to represent Loh et al as "*serving as a guidepost for alerting the artisan to adverse events from inhaled NO*," as Loh et al. reported no adverse events during administration of inhaled NO for 10 minutes to 19 stable patients with advanced heart failure. Rather, Loh et al. speculated that a finding of an elevation in PCWP in a subgroup of such patients could pose an increased risk of pulmonary edema in adults with congestive heart failure due to ischemic or dilated cardiomyopathy. As discussed above, extrapolation of that theoretical risk to neonates and children with different forms of heart disease, different cardiovascular hemodynamics, and different pulmonary vasculature physiology, pathophysiology and pathology was not obvious, as evidenced by the fact that the members of the INOT22 Screening Committee (including Dr. Wessel) who designed the INOT22 study protocol and the approximately 18 Institutional Review Boards and/or Independent Ethics Committees and 5 National Health Authorities (FDA and the national Health Authority for each of United Kingdom, France, Netherlands and Spain) who reviewed and approved the INOT22 study protocol prior to its initiation all failed to predict that any untoward effects would be caused by the administration of inhaled NO within a pediatric patient population having left ventricular dysfunction who are not dependent on right-to-left shunting of blood. Only after being informed of the present invention did the FDA mandate a change to the drug labeling for inhaled NO to include a new warning (separate and distinct from the pre-existing contraindication pertaining to neonates dependent on right-to-left shunting of blood) concerning the use of inhaled NO in patients with pre-existing left ventricular dysfunction.

27. On page 15 of the 6/27/11 Office Action the Examiner states:

> *Respectfully, the instantly claimed method steps are in the realm of common sense and not in the realm of invention because it is already known in the art that patients with pre-existing LVD are at risk of adverse effects from iNO. It is obvious to the ordinary artisan that if the child/neonate has LVD with or without any number of conditions then, in order to avoid the risk of adverse or serious adverse events associated with iNO, to then exclude the neonate from iNO therapy. In other words, given the art as a whole, determination of further conditions associated with LVD that would exclude the neonate from iNO therapy is obvious given the teachings in the art as discussed above which direct the artisan to screen neonates about to undergo treatment with NO by inhalation and to exclude those with LVD from such treatment.*
>
> *In light of the forgoing discussion, the Examiner concludes that the subject matter defined by the instant claims would have been obvious within the meaning of 35 USC 103(a).*
>
> *From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the*

> *claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.* Emphasis omitted.

The arguments by which this conclusion is supported are both medically and scientifically unsound. To summarize, the teaching of Atz & Wessel is inaccurately portrayed by the Examiner due to his confusion of the known risk of systemic vascular collapse if inhaled NO is administered to neonates dependent upon right-to-left shunting of blood, and the opposite case of adults where inhaled NO may be less effective than in children. The Examiner misconstrues Kinsella et al.'s clinical trial inclusion/exclusion criteria as a teaching of risk associated with inhaled NO administration, rather than as a routine practical measure in the design of clinical trials to minimize confounding factors and heterogeneity in the study population. Lastly, the Examiner grossly over-interprets the hemodynamic findings of Loh et al. in adults with ischemic or dilated cardiomyopathy and congestive heart failure (a disease process differing in etiology, physiology, pathophysiology and pathology from childhood congenital heart disease) as "*a guidepost to the artisan*" regarding the use of inhaled NO in neonates with pulmonary hypertension and left ventricular dysfunction, but not dependent on right-to-left shunting of blood. These inaccurate and erroneous interpretations of all three supporting publications cited by the Examiner lead the Examiner to draw incorrect conclusions regarding what is or is not taught or suggested by the prior art.

28. On June 28, 2011, I met with Dr. David L. Wessel, the chair of the INOT22 Steering Committee and the senior author of *Atz & Wessel, Seminars in Perinatology 1997, 21(5), pp 441-455.* During our discussion, I informed Dr. Wessel of two patent applications (application nos. 12/820,866 and 12/820,980) related to the present application and the fact that, in both applications, the Examiner was citing Atz & Wessel to allege that it would have been obvious to predict the adverse events and outcomes of the INOT22 study that led to the inventions claimed in those two applications.

29. Dr. Wessel disagreed with the Examiner's allegation and found it ironic that his own publication would be cited to suggest the obviousness of the unexpected outcomes of the INOT22 study, when Dr. Wessel himself, the senior author of Atz & Wessel, failed to predict that neonatal and child patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at increased risk of adverse events when administered inhaled NO. A copy of a June 29, 2011 letter from Dr. Wessel to me stating this opinion is attached hereto as Exhibit 2.

30. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on the present application.

Dated: August 17, 2012

Douglas A. Greene, M.D.

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EXHIBIT 1

CURRICULUM VITAE

PERSONAL DATA

Name: Douglas Alan Greene, M.D.

EDUCATION

High School — Columbia High School, South Orange, NJ, 1962

Undergraduate — Princeton University, Princeton, NJ, BA Biology(cum laude), 1962-1966

Graduate/Professional — Johns Hopkins School of Medicine, Baltimore, MD, M.D., 1966-1970

POSTDOCTORAL TRAINING

Medical Internship: Department of Medicine, Johns Hopkins, Baltimore, MD, 1970-1971

Medical Residency: Department of Medicine, Johns Hopkins, Baltimore, MD, 1971-1972

Fellowship: Medical Fellowship, Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, 1970-1972

Post-doctoral Research Fellow, Diabetes, George S. Cox Medical Research Institute; Hospital of the University of Pennsylvania, Philadelphia, PA (Dr. Albert I. Winegrad, preceptor), 1972-1975

Medical Fellowship, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA, 1972-1975

NON-ACADEMIC EMPLOYMENT

2000-2003 — Executive Vice President, Clinical Sciences and Product Development (CSPD), Merck Research Laboratories, Rahway, New Jersey, and Corporate Officer, Merck, Inc. Supervised and directly managed all clinical research, regulatory affairs, clinical and non-clinical quality assurance and pharmaco-vigilance at Merck Research Laboratories.

2003-2006 Vice President, Head Corporate Regulatory Development, Sanofi-Aventis, Bridgewater, NJ. Overseeing all aspects of corporate regulatory development of all pre-clinical and clinical development projects/life-cycle products in Research & Development.

2006-2009 Senior Vice Prescident, Chief Medical Officer, Sanofi-Aventis, Bridgewater, NJ. Overseeing medical, regulatory, pharmocovigilance, risk management, education and medical communications for US region, Member US Executive Committee, Member Committee Operational de Development, International Clinical Development.

2009-present Senior Vice President, Senior Scientific Advisor, Sanofi-Aventis, Bridgewater, New Jersey. Member Corporate Portfolio Valuation Process and Drug Development Committees. The position at the interface between the Research and Development and Pharmaceutical Operations is responsible for providing key scientific and medical guidance for sanofi-aventis' scientific strategy within U.S. and global contexts to enhance the quality and effectiveness of the company's research and product portfolio, including assessment and guidance of internal R&D product pipeline and franchise portfolio and external commercial and academic innovation opportunities.

ACADEMIC APPOINTMENTS

1975-1980	Assistant Professor of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania
1980-1986	Associate Professor of Medicine, Director, General Clinical Research Center and Diabetes Research Laboratories, University of Pittsburgh, School of Medicine
1986-2000	Professor of Internal Medicine, Director, Michigan Diabetes Research and Training Center, University of Michigan School of Medicine
1991-2000	Chief, Division of Endocrinology & Metabolism, University of Michigan School of Medicine
2000-Present	Adjunct Professor, Internal Medicine, Division of Endocrinology & Metabolism, University of Michigan, School of Medicine

SELECTED SCIENTIFIC ACTIVITIES

1988-1994	Chairman, Endocrinologic and Metabolic Drug Advisory Board, Food and Drug Administration, Washington D.C (Chair, 1990-1994)
1994-2000	Chairman, Merck Scientific Board of Advisors

SELECTED SCIENTIFIC PRIZES AND AWARDS

1986	First Annual Raymond A. and Robert L. Kroc Lecturer, Eisenhower Medical Center, Palm Springs, California
1987	Moore Award, The American Association of Neuropathologists, Seattle, Washington
1987	Carol Sinicki Manuscript Award (The Diabetes Educator), American Association of Diabetes Educators, Chicago, Illinois
1988	Kellion Lecture, International Diabetes Federation, Sydney, Australia
1989	Banting and Best Lecture, Toronto General Hospital, Toronto, Canada
1994	Charles H. Best Lecturer, Toronto Diabetes Association, Toronto, Canada
1996	Invited Speaker, Seventy-fifth Anniversary Celebrating the Discovery of Insulin, Toronto, Canada
1996	First Alan Robinson Lecturer, University of Pittsburgh
1998	Outstanding Foreign Investigator Award, Japan Society of Diabetic Complications

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EXHIBIT 2

Children's National
Medical Center

David L. Wessel, MD
Senior Vice President
Center for Hospital-Based Specialties
Ikaria Distinguished Professor of
Critical Care Medicine

June 29, 2011

Douglas Greene, M.D.,
Executive Vice President and Head of Research & Development
Ikaria, Inc.
Perryville III Corporate Park
53 Frontage Road, 3rd Floor
PO Box 9001
Hampton, NJ 08827-9001

RE: **USSN 12/820,866 and 12/820,980**
Atz et al., Seminars in Perinatology 1997,21(5), pp 441-455

Dear Doug:

In 2005, I chaired the Steering Committee of the Sponsor, INO Therapeutics LLC (INOT), to establish, design and oversee the INOT22 Study. Presently, I am Chief, Division of Critical Care Medicine and Senior Vice President, Children's National Medical Center, Washington, D.C.[1]

In addition to being the Chair of the INOT22 Steering Committee, I also am the senior author of *Atz et al., Seminars in Perinatology 1997,21(5), pp 441-455* (Atz et al.).

At the time of the design of the INOT22 Study protocol, neither myself, the other Steering Committee members, nor the study Sponsor appreciated or anticipated that a child with left ventricular dysfunction who is not dependent on right-to-left shunting of blood would be at additional risk when treated with inhaled nitric oxide (INO). This is the reason such children were not originally excluded from the INOT22 Study entry criteria.

Neither the Atz et al. article that I co-authored, nor the medical literature or medical experience of which I was aware at the time, predict this risk. Instead, Atz et al describes two distinct, independent precautions with respect to the use of INO. First, with respect to adults, Atz et al. stated that INO may be more effective in newborns than in older patients, and noted that it

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[1] In the interest of full disclosure, I formerly served as a consult for INO Therapeutics LLC. I currently serve without remuneration as a member of the Ikaria Scientific Board of Advisors. In 2010 I was appointed by my institution as the Ikaria Distinguished Professor of Critical Care Medicine.

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Children's National Medical Center

should be used with caution in adults with ischemic cardiomyopathy in whom a risk of pulmonary edema is a consideration (see page 462, left column). Second, with respect to neonates, we stated the well-known contraindication (currently found in the INOMAX® prescribing information) that INO should not be used in newborns dependent upon right-to-left shunting of blood across a patent ductus arterious to avoid circulatory collapse. What we did not disclose or predict was that neonatal patients with left ventricular dysfunction who are not dependent on right-to-left shunting of blood would be at greater risk of adverse events.

It is ironic that my own publication would be cited to suggest that it would have been obvious to predict the adverse events and outcomes of the INOT22 Study when I, the senior author of Atz et al., failed to anticipate or predict these unexpected outcomes at the time I participated in drafting the original INOT22 Study protocol. If so, I would have been acting either negligently or intentionally to harm babies, and I most certainly was not. Furthermore, to my knowledge, none of the other members of the INOT22 Steering Committee who assisted me in designing the study, nor the approximately 18 Institutional Review Boards and 2 National Health Authorities who reviewed and approved the study prior to its initiation, predicted the adverse events in children with left ventricular dysfunction who are not dependent on right-to-left shunting of blood.

In summary, although it was known that neonates whose systemic circulation was dependent on right-to-left shunt should not receive INO; and it had been reported that adults with pre-existing left ventricular dysfunction (from coronary artery disease) may be at risk when provided INO, it was unanticipated and surprising that children with left ventricular dysfunction who are not dependent on right-to-left shunting would be at increased risk of adverse events when administered INO.

Sincerely,

David L. Wessel, M.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit	: 1613
Serial No.	: 12/821,041	Examiner	: Ernst V. Arnold
Filed	: June 22, 2010	Conf. No.	: 3219
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM AND NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD DECLARATION OF DOUGLAS A. GREENE, M.D.
UNDER 37 C.F.R. § 1.132

I, Douglas A. Greene, do hereby declare the following:

1. I currently hold the position of Executive Vice President and Head, Research and Development at INO Therapeutics LLC ("INO"). A copy of my *curriculum vitae* is attached as **Exhibit 1**.

2. I received an undergraduate degree in biology (*cum laude*) from Princeton University in 1966 and a doctoral degree in medicine (M.D.) from Johns Hopkins School of Medicine in 1970.

3. I spent the next thirty years of my medical career (1970-2000) practicing and teaching medicine at some of America's foremost academic medical centers, including Johns Hopkins, the University of Pennsylvania, the University of Pittsburgh, and the University of Michigan. At Michigan, I was a full professor of internal medicine, director of the Michigan Diabetes Research and Training Center, and chief of the Division of Endocrinology and Metabolism.

4. In 2000, I left Michigan to join Merck as Executive Vice President in charge of clinical sciences and product development. In this role, I supervised and directly managed all clinical research at Merck Research Laboratories, among other duties.

5. In 2003, I left Merck for Sanofi-Aventis, where I became a Senior Vice President and Chief Medical Officer. My duties at Sanofi-Aventis included overseeing all aspects of pre-clinical and clinical regulatory development of the company's products and overseeing all medical aspects of the company's US business.

6. In 2010, I joined INO, where – as noted above – I am presently Executive Vice President and Head of Research and Development.

7. INO markets pharmaceutical grade nitric oxide (NO) gas under the brand name INOmax®. INOmax® is administered to patients using INO's proprietary INOvent® and INOmax® DS devices.

8. INOmax® was approved for sale in the United States by the U.S. Food and Drug Administration ("FDA") in 1999 for the treatment of term and near-term (≥ 34 weeks gestational age) neonates with hypoxic respiratory failure ("HRF") associated with clinical or echocardiographic evidence of pulmonary hypertension, a condition also known as persistent pulmonary hypertension in the newborn ("PPHN"). From 2000 to the present, INO has been selling INOmax® throughout the United States, Canada and certain other overseas markets.

9. In addition to the approved indication, physicians employ INOmax® to treat or prevent pulmonary hypertension and improve blood oxygen levels in a variety of other clinical settings, including in both pediatric and adult patients suffering from acute respiratory distress syndrome ("ARDS"), in pediatric and adult patients undergoing cardiac or transplant surgeries, in pediatric and adult patients for testing to diagnose reversible pulmonary hypertension, and in pediatric patients with congenital diaphragmatic hernia. In most, if not all, of these applications,

INOmax® acts by preventing or treating reversible pulmonary vasoconstriction, and improves pulmonary gas exchange.

10. The mechanism of action of INOmax® - the selective relaxation of pulmonary blood vessels - is particularly relevant to the transition of the newborn from the fetal to the neonatal environment. During *in utero* development, the fetal lungs are not filled with air. Accordingly, the fetus obtains oxygen from the mother across the placenta into the systemic circulation, whereas the circulation through the lungs is largely shut down because the pulmonary vessels are tightly constricted. Instead of the blood's being pumped from the right side of the heart through the fetal lungs and then returning to the left side of the heart to be pumped to the rest of the body, as it is normally after birth, blood from the right side of the fetal heart bypasses the fetal lungs through a patent ductus arteriosis, a blood vessel connecting the outflow of the right heart directly to the systemic circulation.

11. In addition to the patent ductus arteriosis, the fetal heart contains a second anatomical distinction from the neonatal heart - the foramen ovale - as a means for fetal blood to circumvent the nonfunctional fetal lungs while the fetus obtains its oxygen from the placenta. The foramen ovale is a "hole" located in the wall that separates the right and left atria of the heart. The foramen ovale is usually covered by a flap of tissue known as the septum primum, which is located on the inner wall of the left atrium. The septum primum and the foramen ovale together act as a one-way valve that permits blood to be shunted from the right atrium, where blood pressure is usually high due to the high vascular resistance present in the non-functional fetal lungs, into the left atrium for distribution to the body via the left ventricle. As discussed below, nonclosure of a patent foramen ovale after birth, as well as other forms of congenital heart disease, are often associated with a large persistently patent ductus arteriosis.

12. After birth, the pressure in the pulmonary circulatory system drops, reducing the right atrial pressure below that of the left atrium. This shift in pressure causes the septum primum to close off the foramen ovale, and this flap of tissue eventually becomes incorporated into the intra-atrial wall. In certain instances, however, the foramen ovale may remain open or

"patent" after birth. In one such case, elevation of pressure in the pulmonary circulatory system (i.e.: pulmonary hypertension due to various causes) can prevent the pressure shift that leads to the closure of the foramen ovale. This condition is known as patent foramen ovale, and the use of inhaled nitric oxide to decrease pulmonary hypertension is known to be a successful treatment for right-to-left shunting through a patent foramen ovale.[1]

13. At birth, the ductus arteriosis closes and pulmonary vessels relax, thereby redirecting the outflow of the right heart to the now oxygenated lungs, with oxygenated blood then returning to the left side of the heart to be pumped to the rest of the body from the left ventricle. However, in some instances, neonates are born with severe congenital heart disease involving the left ventricle, wherein the left side of the heart lacks the ability to pump blood to the rest of the body. In these instances, a ductus arteriosis that remains open or "patent" is actually beneficial, and in fact is life-saving when combined with pulmonary hypertension, because the reverse pressure created by the pulmonary hypertension creates a right-to-left shunt through the patent ductus arteriosis, thereby permitting the right ventricle to pump oxygenated blood directly to the systemic circulation to maintain organ function; simply put, the patent ductus arteriosis permits the right ventricle to subsume the role of a nonfunctional left ventricle in circulating blood to the body. In these circumstances, stealing blood circulation away from the ductus arteriosis would be potentially fatal, and significantly, pulmonary vasoconstriction is also absolutely essential for survival in order to divert sufficient blood from the right heart through the patent ductus arteriosis to the systemic circulation, thus bypassing the non-functional left side of the heart to maintain life. The terminology to describe this situation is "neonates dependent upon right-to-left shunting of blood" for survival.

14. Administration of inhaled nitric oxide (iNO) in the context of such right-to-left shunting would be catastrophic, because reducing or eliminating the pulmonary vasoconstriction

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1 *See* Fessler MB et al., *Right-to-left shunting through a patent foramen ovale in right ventricular infarction: improvement of hypoxemic and hemodynamics with inhaled nitric oxide*. J. Clin. Anesth. 15: 371-4, 1993, at 371.

would permit blood to be diverted to the lungs and away from the patent ductus arteriosis.[2] Accordingly, an absolute contraindication for the use of iNO in babies dependent upon right-to-left shunting of blood has been contained in the INOmax® prescribing information since the original approval of INOmax® by the FDA in December, 1999.[3]

15. Pulmonary engorgement may occur in adults with serious left-sided heart disease due to coronary artery disease ("ischemic cardiomyopathy"), hypertensive heart disease ("hypertensive cardiomyopathy") or obstructive valvular disease or other conditions that similarly restrict the inflow of blood to the left side of the heart such that engorgement of the pulmonary blood vessels ensues. It is important to note that restriction of left-sided inflow is particularly prominent in the above cardiomyopathies, and is described as diastolic dysfunction.[4] Diastolic dysfunction is extremely common in adult heart disease, especially in the elderly, but is

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2 *See, e.g.,* Atz AM, Wessel DL. *Inhaled nitric oxide in the neonate with cardiac disease.* Sem. Perinatol. 21:441-455, 1997, at 452.

3 *See*, Exhibit 2, section 4, Prescribing Information, INOMAX.

4 *See* "Diastolic Dysfunction" American Heart Association "Learn and Live" website visited April 13, 2011: "The heart contracts and relaxes with each heartbeat. The contraction part of this cycle is called **systole** (SIS'-to-le). The relaxation portion is called **diastole** (di-AS'-to-le). In some people with heart failure, the contraction function is normal but there's impaired relaxation of the heart. This affects the heart's lower, pumping chambers (the ventricles) specifically. If the relaxation part of the cycle is abnormal, it's called diastolic (di"as-TOL'-ik) dysfunction. Because the ventricle doesn't relax normally, the pressure in it increases and exceeds what's normal as blood for the next heartbeat. (It's harder for all of the blood to go into the ventricle.) This can cause increased pressure and fluid in the blood vessels of the lungs. (This is called pulmonary congestion.) It can also cause increased pressure and fluid in the blood vessels coming back to the heart. (This is called systemic congestion.) People with certain types of cardiomyopathy (kar"-de-o-my-OP'-ah-the) may also have diastolic dysfunction."

extremely rare in childhood heart disease, which is generally caused by either congenital malformations or viral infections.[5]

16. To summarize, in adults, left-sided ventricular dysfunction is generally ischemic or hypertensive in origin, and is associated with a stiff, non-compliant left ventricle that cannot fill properly ("diastolic dysfunction"). In contrast, in children, left-sided ventricular dysfunction is generally not ischemic or hypertensive in origin and is not associated with impaired filling, but rather is associated with a soft, overly elastic heart that cannot push blood out, resulting in impaired emptying ("systolic dysfunction"). Thus, adult left ventricular diastolic dysfunction, but not childhood left ventricular systolic dysfunction, would lead to pulmonary vascular engorgement, requiring caution in the use of iNO.

17. Since the approval of iNO in December 1999, INO has from time-to-time sponsored, supported or otherwise facilitated - under its own FDA Investigational New Drug (IND) application or IND applications filed by other investigators - clinical research exploring the efficacy and safety of iNO in clinical contexts outside the approved indication for PPHN. The results of these investigations are submitted to the FDA and are often published in the medical literature. In May 2004, following detailed consultations with an expert steering committee composed of leading world authorities in pediatric heart and lung disease,[6] INO initiated a multinational randomized controlled 150-patient study entitled "Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation Plus Oxygen in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing" ("INOT22"). Prior to its initiation, the INOT22 study was reviewed and approved by the

5 Diastolic dysfunction in children has been described in rare genetic diseases such as Marfan's syndrome [that directly affects the elasticity of connective tissue of the heart and elsewhere], Kawasaki's disease [that creates cardiac ischemia similar to that in adult ischemic cardiomyopathy] or sickle cell disease [that produces fibrotic scars in the myocardium].

6 The steering committee included Dr. David Wessel of the Department of Cardiology, Children's Hospital and the Department of Pediatrics, Harvard Medical School.

Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC) at each of the 18 participating study institutions, and by the U.S. FDA and the European Medicines Agency (EMEA). At no time did any of the members of these boards, committees or agencies counsel against giving inhaled nitric oxide to the proposed patient population because of the risk of severe adverse events in pediatric patients (i.e., children) with left ventricular dysfunction.

18. INOT22 was designed and purposed to compare the diagnostic utility of short-term (10 minute) inhalation of iNO alone, iNO plus oxygen ("O_2") or O_2 alone to children between the ages of 4 weeks and eighteen years with either idiopathic pulmonary arterial hypertension, congenital heart disease with pulmonary arterial hypertension, or childhood forms of cardiomyopathy undergoing diagnostic right heart catheterization and acute pulmonary vasodilatation testing to assess pulmonary vasoreactivity. The rationale for INOT22 was: (1) in patients with right ventricular failure and lung disorders, the prognosis and course of treatment are determined by acute pulmonary vasodilatation testing (APVT); (2) a reduction in the mean pulmonary artery pressure and pulmonary vascular resistance with acute vasodilator treatment may be used to predict therapeutic efficacy of long-term vasodilator medication; and (3) APVT is also used to evaluate patients being considered for heart or heart/lung transplantation; elevated pulmonary artery pressures and pulmonary vascular resistance place a strain on the right ventricle leading to an increased risk of perioperative morbidity and mortality due to right heart failure post heart transplant. Accordingly, the primary objective of INOT22 was to compare the number of patients who exhibited reversible pulmonary hypertension (vasoreactivity) in response to iNO or iNO plus oxygen as compared to 100% oxygen alone.

19. Under the direction of the expert steering committee, inclusion and exclusion criteria were established that were intended to ensure the safe use of iNO during the conduct of the study. For example, patients dependent on right-to-left shunting and thereby contraindicated for iNO treatment were not included. Patients also were excluded if they had focal pulmonary infiltrates on chest radiograph, had a diagnosis of severe obstructive or restrictive pulmonary disease that significantly contributed to the patient's pulmonary hypertension, had received

treatment with iNO within 30 days prior to study initiation or were on other investigational medications, nitroglycerin, sodium nitroprusside, sildenafil, other PDE-5 inhibitors, or prostacyclin, or were pregnant.

20. Since the inclusion criteria included congenital heart disease or cardiomyopathy, many of the patients had, by design, significant childhood heart disease. This was not considered to pose a significant risk by the experts on the steering committee based on (1) the exclusion of right-to-left shunt-dependent patients, (2) prior extensive safe experience with iNO in pediatric patients with congenital heart disease or cardiomyopathy by the investigators and published in the medical literature,[7] and (3) the very different nature of non-ischemic, non-hypertensive childhood heart disease compared to the ischemic or hypertensive adult form marked by diastolic dysfunction.

21. Surprisingly and unexpectedly, severe adverse events including pulmonary edema and death were noted during the early phase of the study, and so the study was temporarily stopped. Analysis of the cases revealed that the patients suffering severe adverse events had severe left ventricular dysfunction, largely due to viral cardiomyopathy, and exhibited during their right-sided cardiac catheterizations an increased pulmonary capillary wedge pressure ("PCWP") of greater than 20 mm Hg, indicative of elevated pressures in the upper chamber of the left side of the heart (the left atrium).

22. To determine if there was a correlation between the severe adverse events and the left ventricular dysfunction of the patients that had suffered them, a protocol amendment was submitted to FDA to exclude – on an ongoing basis - patients with severe left ventricular dysfunction with a PCWP greater than 20 mm Hg from further enrollment in the study. The study was then completed. On analyzing the data from the study, the inventors concluded that a correlation did, in fact, exist between the severe adverse events that had occurred during the

7 *See* Atz AM et al. *Combined effects of nitric oxide and oxygen during acute pulmonary vasodilator testing.* J. Amer. Coll. Cardio. 33:813-819, 1999, at 814, 818.

study and the left ventricular dysfunction of the patients that had suffered them. Accordingly, INO subsequently requested that the FDA add an additional warning to the product labeling for INOmax concerning use of the drug in patients with left ventricular dysfunction. The FDA agreed and included an additional warning in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information (in the US and worldwide).[8]

23. Competent practitioners would understand that the warnings included in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information are intended as a separate warning generally applicable to all patients with left ventricular dysfunction and not limited to those patients having left ventricular dysfunction who rely on right to left shunting of blood. The latter category of patients is the subject of a separate section of the prescribing information that expressly states that INOmax is contraindicated for patients with this condition. The fact that administration of INOmax would be harmful to patients dependent on right to left shunting of blood has been well known for many years, as demonstrated by several of the references that are of record in the present case, including, e.g., Atz AM, Wessel DL. *Inhaled nitric oxide in the neonate with cardiac disease.* Sem. Perinatol. 21:441-455, 1997.

24. Furthermore, no competent practitioner would understand the separate warnings in section 5.4 and the Warnings and Precautions section of the INOmax prescribing information, or the disclosure in the present application of the potential for severe adverse events in patients with left ventricular dysfunction, as referring to patients dependent on right to left shunting of blood, since it has long been known that the use of INOmax is contraindicated in such patients. Rather, the competent practitioner would understand the warnings added at section 5.4 and within the Warnings and Precautions section of the INOmax prescribing information, and the disclosure in the present application of the potential for severe adverse events in patients with left ventricular dysfunction, as a distinct and separate warning and disclosure that administration

8 *See* Exhibit 2.

of INOmax to patients with left ventricular dysfunction generally (even those not dependent on right to left shunting of blood) may result in serious adverse events.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent that may issue on the present application.

Dated: August 17, 2012

Douglas A. Greene, M.D.

22897342.doc

EXHIBIT 1

CURRICULUM VITAE

PERSONAL DATA

Name: Douglas Alan Greene, M.D.

EDUCATION

High School: Columbia High School, South Orange, NJ, 1962

Undergraduate: Princeton University, Princeton, NJ, BA Biology(cum laude), 1962-1966

Graduate/Professional: Johns Hopkins School of Medicine, Baltimore, MD, M.D., 1966-1970

POSTDOCTORAL TRAINING

Medical Internship: Department of Medicine, Johns Hopkins, Baltimore, MD, 1970-1971

Medical Residency: Department of Medicine, Johns Hopkins, Baltimore, MD, 1971-1972

Fellowship: Medical Fellowship, Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, 1970-1972

Post-doctoral Research Fellow, Diabetes, George S. Cox Medical Research Institute; Hospital of the University of Pennsylvania, Philadelphia, PA (Dr. Albert I. Winegrad, preceptor), 1972-1975

Medical Fellowship, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA, 1972-1975

NON-ACADEMIC EMPLOYMENT

2000-2003 Executive Vice President, Clinical Sciences and Product Development (CSPD), Merck Research Laboratories, Rahway, New Jersey, and Corporate Officer, Merck, Inc. Supervised and directly managed all clinical research, regulatory affairs, clinical and non-clinical quality assurance and pharmaco-vigilance at Merck Research Laboratories.

2003-2006 Vice President, Head Corporate Regulatory Development, Sanofi-Aventis, Bridgewater, NJ. Overseeing all aspects of corporate regulatory development of all pre-clinical and clinical development projects/life-cycle products in Research & Development.

2006-2009 Senior Vice Preseident, Chief Medical Officer, Sanofi-Aventis, Bridgewater, NJ. Overseeing medical, regulatory, pharmocovigilance, risk management, education and medical communications for US region, Member US Executive Committee, Member Committee Operational de Development, International Clinical Development.

2009-present Senior Vice President, Senior Scientific Advisor, Sanofi-Aventis, Bridgewater, New Jersey. Member Corporate Portfolio Valuation Process and Drug Development Committees. The position at the interface between the Research and Development and Pharmaceutical Operations is responsible for providing key scientific and medical guidance for sanofi-aventis' scientific strategy within U.S. and global contexts to enhance the quality and effectiveness of the company's research and product portfolio, including assessment and guidance of internal R&D product pipeline and franchise portfolio and external commercial and academic innovation opportunities.

ACADEMIC APPOINTMENTS

1975-1980	Assistant Professor of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania
1980-1986	Associate Professor of Medicine, Director, General Clinical Research Center and Diabetes Research Laboratories, University of Pittsburgh, School of Medicine
1986-2000	Professor of Internal Medicine, Director, Michigan Diabetes Research and Training Center, University of Michigan School of Medicine
1991-2000	Chief, Division of Endocrinology & Metabolism, University of Michigan School of Medicine
2000-Present	Adjunct Professor, Internal Medicine, Division of Endocrinology & Metabolism, University of Michigan, School of Medicine

SELECTED SCIENTIFIC ACTIVITIES

1988-1994	Chairman, Endocrinologic and Metabolic Drug Advisory Board, Food and Drug Administration, Washington D.C (Chair, 1990-1994)
1994-2000	Chairman, Merck Scientific Board of Advisors

SELECTED SCIENTIFIC PRIZES AND AWARDS

1986	First Annual Raymond A. and Robert L. Kroc Lecturer, Eisenhower Medical Center, Palm Springs, California
1987	Moore Award, The American Association of Neuropathologists, Seattle, Washington
1987	Carol Sinicki Manuscript Award (The Diabetes Educator), American Association of Diabetes Educators, Chicago, Illinois
1988	Kellion Lecture, International Diabetes Federation, Sydney, Australia
1989	Banting and Best Lecture, Toronto General Hospital, Toronto, Canada
1994	Charles H. Best Lecturer, Toronto Diabetes Association, Toronto, Canada
1996	Invited Speaker, Seventy-fifth Anniversary Celebrating the Discovery of Insulin, Toronto, Canada
1996	First Alan Robinson Lecturer, University of Pittsburgh
1998	Outstanding Foreign Investigator Award, Japan Society of Diabetic Complications

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EXHIBIT 2

INOmax® (nitric oxide) for inhalation

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INOmax safely and effectively. See full prescribing information for INOmax.

INOmax (nitric oxide) for inhalation
Initial U.S. Approval: 1999

——————RECENT MAJOR CHANGES——————

Warnings and Precautions, Heart Failure (5.4) 8/2009

——————INDICATIONS AND USAGE——————

INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation (1.1).

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration (1.1).

Utilize additional therapies to maximize oxygen delivery (1.1).

——————DOSAGE AND ADMINISTRATION——————

Dosage: The recommended dose of INOmax is 20 ppm, maintained for up to 14 days or until the underlying oxygen desaturation has resolved (2.1).

Administration:

- INOmax must be delivered via a system which does not cause generation of excessive inhaled nitrogen dioxide (2.2).
- Do not discontinue INOmax abruptly (2.2).

——————DOSAGE FORMS AND STRENGTHS——————

INOmax (nitric oxide) is a gas available in 100 ppm and 800 ppm concentrations.

——————CONTRAINDICATIONS——————

Neonates known to be dependent on right-to-left shunting of blood (4).

——————WARNINGS AND PRECAUTIONS——————

Rebound: Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure (5.1).

Methemoglobinemia: Methemoglobin increases with the dose of nitric oxide; following discontinuation or reduction of nitric oxide, methemoglobin levels return to baseline over a period of hours (5.2).

Elevated NO_2 Levels: NO_2 levels should be monitored (5.3).

Heart Failure: In patients with pre-existing left ventricular dysfunction, inhaled nitric oxide may increase pulmonary capillary wedge pressure leading to pulmonary edema (5.4).

——————ADVERSE REACTIONS——————

Methemoglobinemia and elevated NO_2 levels are dose dependent adverse events. Worsening oxygenation and increasing pulmonary artery pressure occur if INOmax is discontinued abruptly. Other adverse reactions that occurred in more than 5% of patients receiving INOmax in the CINRGI study were: thrombocytopenia, hypokalemia, bilirubinemia, atelectasis, and hypotension (6).

To report SUSPECTED ADVERSE REACTIONS, contact INO Therapeutics at 1-877-566-9466 and http://www.inomax.com/ or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

——————DRUG INTERACTIONS——————

Nitric oxide donor agents: Nitric oxide donor compounds, such as prilocaine, sodium nitroprusside, and nitroglycerin, when administered as oral, parenteral, or topical formulations, may have an additive effect with INOmax on the risk of developing methemoglobinemia (7).

Revised: August 2009

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoxic Respiratory Failure

INOmax® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies *[see Clinical Studies (14)]*.

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOmax administration.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage

Term and near-term neonates with hypoxic respiratory failure

The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

2.2 Administration

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

Do not discontinue INOmax abruptly, as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

3 DOSAGE FORMS AND STRENGTHS

Nitric oxide is a gas available in 100 ppm and 800 ppm concentrations.

4 CONTRAINDICATIONS

INOmax is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.

5 WARNINGS AND PRECAUTIONS

5.1 Rebound

Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

5.2 Methemoglobinemia

Methemoglobinemia increases with the dose of nitric oxide. In clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide, the methemoglobin levels returned to baseline over a period of hours.

5.3 Elevated NO_2 Levels

In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

5.4 Heart Failure

Patients who had pre-existing left ventricular dysfunction treated with inhaled nitric oxide, even for short durations, experienced serious adverse events (e.g., pulmonary edema).

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from the clinical studies does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

6.1 Clinical Trials Experience

Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse reactions that occurred in at least 5% of patients receiving INOmax in the CINRGI study with event rates >5% and greater than placebo event rates. None of the differences in these adverse reactions were statistically significant when inhaled nitric oxide patients were compared to patients receiving placebo.

Table 1:
Adverse Reactions in the CINRGI Study

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 *(10%)*	13 *(13%)*
Withdrawal	9 *(10%)*	12 *(12%)*
Atelectasis	8 *(9%)*	9 *(9%)*
Hematuria	5 *(6%)*	8 *(8%)*
Hyperglycemia	6 *(7%)*	8 *(8%)*
Sepsis	2 *(2%)*	7 *(7%)*
Infection	3 *(3%)*	6 *(6%)*
Stridor	3 *(3%)*	5 *(5%)*
Cellulitis	0 *(0%)*	5 *(5%)*

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of INOmax. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to estimate their frequency reliably or to establish a causal relationship to drug exposure. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug; pulmonary edema in patients with CREST syndrome.

7 DRUG INTERACTIONS

No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

8.2 Labor and Delivery

The effect of INOmax on labor and delivery in humans is unknown.

8.3 Nursing Mothers

Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

8.4 Pediatric Use

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

8.5 Geriatric Use

Nitric oxide is not indicated for use in the adult population.

10 OVERDOSAGE

Overdosage with INOmax will be manifest by elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

11 DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:

$$\cdot\ddot{N}=\ddot{O}:$$

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide selectively dilates the pulmonary vasculature, and because of efficient scavenging by hemoglobin, has minimal effect on the systemic vasculature.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

12.2 Pharmacodynamics

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

12.3 Pharmacokinetics

The pharmacokinetics of nitric oxide has been studied in adults.

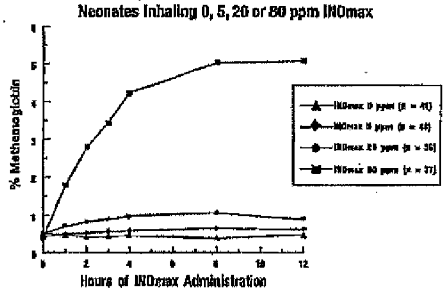
12.4 Pharmacokinetics: Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

12.5 Pharmacokinetics: Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1:
Methemoglobin Concentration – Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groups, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 35% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients, but one patient did not exceed 7% until 40 hours.

12.6 Pharmacokinetics: Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

14 CLINICAL STUDIES

14.1 Treatment of Hypoxic Respiratory Failure (HRF)

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI= mean airway pressure in cm H_2O × fraction of inspired oxygen concentration [FIO_2]× 100 divided by systemic arterial concentration in mm Hg [PaO_2]) and increases PaO_2 *[see Clinical Pharmacology (12.1)]*.

<u>NINOS Study</u>

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (RDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 2.

Table 2:
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups *[see Adverse Reactions (6.1)]*. Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

<u>CINRGI Study</u>

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 3.

Table 3:
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO*,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 58%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups *[see Adverse Reactions (6.1)]*.

14.2 Ineffective in Adult Respiratory Distress Syndrome (ARDS)

<u>ARDS Study</u>

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FIO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

16 HOW SUPPLIED/STORAGE AND HANDLING

INOmax (nitric oxide) is available in the following sizes:

Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)
Size D	Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)
Size 88	Aluminum cylinders containing 1963 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure

The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

 SPC-0303 V:4.0

Electronic Acknowledgement Receipt

EFS ID:	13526811
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	17-AUG-2012
Filing Date:	22-JUN-2010
Time Stamp:	16:44:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	transmittal0003005.pdf	50180 af466a4ec08bb57c3c241c7a5e3848b60cffd936	no	2

Warnings:

Information:

2	Oath or Declaration filed	bald041fourth.pdf	391668 2749da88c82e730680462563fb5cb986cae3a03e	no	14
Warnings:					
Information:					
3	Oath or Declaration filed	bald041third.pdf	317327 e11e8277bce2c953cdd22518b2802dfb02057fa9	no	11
Warnings:					
Information:					
4	Oath or Declaration filed	greene041fourth.pdf	870636 946e87cccafe06aa09e55bd70e32b7fb0fd66cce	no	25
Warnings:					
Information:					
5	Oath or Declaration filed	greene041third.pdf	835415 36d20a0bf7afd43e17a2cd080908900c697a1539	no	22
Warnings:					
Information:					
			Total Files Size (in bytes):	2465226	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	12/821,041	BALDASSARRE ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 8/15/12	**This patent is subject to a Terminal Disclaimer**	

Approved/Disapproved by:

ANDRE ROBINSON

U.S. Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

94169 7590 09/04/2012

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

EXAMINER	
ARNOLD, ERNST V	
ART UNIT	PAPER NUMBER
1613	

DATE MAILED: 09/04/2012

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

TITLE OF INVENTION: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$870	$0	$0	$870	12/04/2012

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

94169 7590 09/04/2012

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

TITLE OF INVENTION: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$870	$0	$0	$870	12/04/2012

EXAMINER	ART UNIT	CLASS-SUBCLASS
ARNOLD, ERNST V	1613	424-718000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 ______________________

2 ______________________

3 ______________________

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies ______________________

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____________ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature ______________________ Date ______________________

Typed or printed name ______________________ Registration No. ______________________

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

94169 7590 09/04/2012
Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

EXAMINER	
ARNOLD, ERNST V	
ART UNIT	PAPER NUMBER
1613	

DATE MAILED: 09/04/2012

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	**Application No.** 12/821,041	**Applicant(s)** BALDASSARRE ET AL.	
	Examiner ERNST ARNOLD	**Art Unit** 1613	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *8/15/12*.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *38-49 and 53-70*.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
   1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 2/17/11, 3/14/11
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

DETAILED ACTION

Claims 1-37 and 50-52 have been cancelled. Claims 38-49 and 53-70 are pending and under examination.

Withdrawn rejections:

Applicant's amendments and arguments filed 8/15/12 are acknowledged and have been fully considered. The Examiner has re-weighed all the evidence of record. Any rejection and/or objection not specifically addressed below is herein withdrawn. Claims 38-49 and 53-70 were rejected under 35 U.S.C. 103(a) as being unpatentable over Davidson et al. (Pediatrics 1998, 101 (3) pp 325-334) and The Neonatal Inhaled Nitric Oxide Study Group (The New England Journal of Medicine 1997, 336(9), pp597-604) and Macrae (Semin Neonatal 1997, 2, 49-58) and Miller et al. (Achives of Disease in Childhood 1994, 70, F47-F49) and Weinberger et al. (Toxicology Sciences 2001, 59, 5-16) and Hurford et al. (Nitric Oxide: Biology and Pathobiology 2000 Academic Press, Chapter 56, pages 931-945) and Kazerooni et al. (Cardiopulmonary Imaging 2004, Lippincott Williams & Wilkins, pp 234-235) and Wheeler et al. (Pediatric Critical Care Medicine 2007, Springer, page 278) and Moss et al. (Moss And Adams' Heart Disease in Infants, Children, and Adolescents, 2007, vol. 1, page 991 in part) and Bocchi et al. The American Journal of Cardiology 1994, 74, pp: 70-72. 4 pages) and Fraisse et al. (Cardiol Young 2004; 14: 277-283 IDS filed on 12/27/11) and Loh et al. (Circulation 1994, 90; 2780-2785; of record) and Atz et al. (Seminars in Perinatology 1997, 21(5), pp 441-455; of record) and Ichinose et al. (Circulation 2004; 109:3106-3111: IDS filed on 1/10/12). Applicant's amendments and arguments are sufficient to overcome the rejection and it is withdrawn by the Examiner.

Terminal Disclaimer

The terminal disclaimer filed on 8/15/12 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of copending applications 12/820866 and 12/821020 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Allowable Subject Matter

The following is an examiner's statement of reasons for allowance: the cited art of record does not teach or suggest, alone or in combination, the patient population of a term or near term neonate in need of the administration of 20 ppm iNO and determining the PCWP as greater than or equal to 20 mm Hg in the method as instantly claimed to reduce the risk of occurrence of pulmonary edema.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Claims 38-49 and 53-70 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ERNST ARNOLD whose telephone number is (571)272-8509. The examiner can normally be reached on M-F 7:15-4:45.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Kwon can be reached on 571-272-0581. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ernst V Arnold/
Primary Examiner, Art Unit 1613

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BIB DATA SHEET

CONFIRMATION NO. 3219

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
12/821,041	06/22/2010 RULE	424	1613	26047-0003005

APPLICANTS
James S. Baldassarre, Doylestown, PA;
Ralf Rosskamp, Chester, NJ;

** **CONTINUING DATA** *************************
This application is a CON of 12/494,598 06/30/2009 ABN

** **FOREIGN APPLICATIONS** *************************

** **IF REQUIRED, FOREIGN FILING LICENSE GRANTED** ** ** SMALL ENTITY **
06/30/2010

		STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /ERNST V ARNOLD/ Examiner's Signature	☐ Met after Allowance Initials	PA	0	30 ~~20~~	4

ADDRESS

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440
UNITED STATES

TITLE

Methods of Treating Term and Near-Term Neonates Having Hypoxic Respiratory Failure Associated with Clinical or Echocardiographic Evidence of Pulmonary Hypertension

FILING FEE RECEIVED		
1282	FEES: Authority has been given in Paper No.__________ to charge/credit DEPOSIT ACCOUNT No.__________ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other __________ ☐ Credit

BIB (Rev. 05/07).

Issue Classification

Application/Control No.	Applicant(s)/Patent Under Reexamination
12821041	BALDASSARRE ET AL.
Examiner	**Art Unit**
ERNST ARNOLD	1613

ORIGINAL

CLASS	SUBCLASS
424	718

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
128	200.24				
423	405				

INTERNATIONAL CLASSIFICATION

CLAIMED					NON-CLAIMED				
A	0	1	N	59 / 00 (2006.01.01)	A	6	1	M	16 / 00 (2006.01.01)
A	6	1	K	33 / 00 (2006.01.01)					
C	0	1	B	21 / 24 (2006.01.01)					

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	38	27	57												
2	39	4	58												
3	40	5	59												
6	41	10	60												
7	42	19	61												
8	43	15	62												
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14	46	20	65												
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18	49	28	68												
23	53	29	69												
24	54	30	70												
25	55														
26	56														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 30	
/ERNST ARNOLD/ Primary Examiner.Art Unit 1613 (Primary Examiner)	8/28/12 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office Part of Paper No. 20120828

EAST Search History

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S32	0	423/405.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; UPAD	OR	OFF	2012/08/28 11:59
S33	0	423/718.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; UPAD	OR	OFF	2012/08/28 12:00
S34	1	"12821020"	US-PGPUB; USPAT; UPAD	OR	OFF	2012/08/28 12:07
S35	0	128/200.24.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; UPAD	OR	OFF	2012/08/28 12:12

8/ 28/ 2012 2:51:21 PM
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Index of Claims

Application/Control No.	Applicant(s)/Patent Under Reexamination
12821041	BALDASSARRE ET AL.
Examiner	**Art Unit**
ERNST ARNOLD	1613

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

CLAIM		DATE							
Final	Original	08/28/2012							
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Index of Claims	Application/Control No. 12821041	Applicant(s)/Patent Under Reexamination BALDASSARRE ET AL.
	Examiner ERNST ARNOLD	Art Unit 1613

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

CLAIM		DATE							
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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12821041	BALDASSARRE ET AL.
	Examiner	**Art Unit**
	ERNST V ARNOLD	1616

SEARCHED

Class	Subclass	Date	Examiner
424	718 text limited	8/28/12	eva
423	405 text limited	8/28/12	eva
128	200.24 text limited	8/28/12	eva

SEARCH NOTES

Search Notes	Date	Examiner
inventor name EAST/PALM	8/11/10	eva
EAST 424/718 text limted all databases	8/11/10	eva
google	8/10/10	eva
various disucssions with QAS Bennett Celsa and Jean Vollano concering proper incorporation by reference as well as patentabilityn	6/18/11	eva
updated IDS	2/9/12	eva
consultation QAS Jean Vollano	2/2/12	eva
updated IDS	6/14/12	eva
Consultation 103 rejection SPE Brian Kwon	6/6/12	eva
consultation SPE BKwon on new matter and patentability	8/14/12	eva
search update EAST all databases	8/28/12	eva

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner
USPGPUB TEXT SEARCH	EAST	8/28/12	eva

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12821041
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	1							☐

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

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	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	2	Bhagavan, et al., Potential role of ubiquinone (coenzyme Q10) in pediatric cardiomyopathy, Clinical Nutrition (2005) 24, 331-338, pp. 331-338	☐
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	Attorney Docket Number	I001-0002USC4

	23	Lipshultz, Establishing norms for echocardiographic measurement of cardiovascular structures and function in children, J Appl Physiol 99: 386-388, 2005	☐
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	32	NIH Clinical Center, Department Policy and Procedure Manual for the Critical Care Therapy and Respiratory Care Section; Nitric Oxide Therapy, 2000, sections 3.1-3.1.2 & 5.2.3	☐
	33	Notification of Reason for Rejection, mailed 7/30/2010, from Japanese Patent Application No. 2009-157623 (cites foreign references).	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(**Not for submission under 37 CFR 1.99)**

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Filing Date	2010-06-22
First Named Inventor	James S. Baldassarre
Art Unit	1616
Examiner Name	Ernst V. Arnold
Attorney Docket Number	I001-0002USC4

	34	Translated copy of the Japanese Office Action mailed February 15, 2011 for Japanese Patent Application No. 2009-157623, a counterpart foreign application for US Patent Application No. 12/494,598	☐
	35	Pazopanib Plus Lapatinib Compared to Lapatinib Alone in Subjects With Inflammatory Breast Cancer, April 22, 2010, p. 4, ClinicalTrials.gov, <<http://clinicaltrials.gov/ct2/show/NCT00558103>>	☐
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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

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Art Unit	1616	
Examiner Name	Ernst V. Arnold	
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	45	Wilkinson, et al., Epidemiological and outcomes research in children with pediatric cardiomyopathy; discussions from the international workshop on primary and idiopathic cardiomyopathies in children, Progress in Pediatric Cardiology 25 (2008) 23-25	☐
	46	~~http://www.cc.nih.gov/ccmd/clinical_services.html~~	☐
	47	~~www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm073087.pdf>>~~	☐
	48	~~Federal Regulations 21 CFR Part 312. <<http://www.gcrc.uci.edu/rsa/aer.cfm>>~~	☐

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EXAMINER SIGNATURE

Examiner Signature	/Christopher P. Rogers, Reg. No. 36,334/ /Ernst Arnold/	Date Considered	2011-03-14 06/18/2011

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

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	1					

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	12821041	12821041 - GAU: 1613
	Filing Date	2010-06-22	
	First Named Inventor	James S. Baldassarre	
	Art Unit	1616	
	Examiner Name	Ernst V. Arnold	
	Attorney Docket Number	I001-0002USC4	

	1	AU 2009202685 Office Action dated 06/17/10 (3 pages)	☐
	2	AU 2009202685 Office Action Response dated 07/29/2010, 19 pages	☐
	~~3~~	~~Bates, Inhaled Nitric Oxide: A Selective Pulmonary Vasodilator,~~	☐
	4	Branson, Inhaled Nitric Oxide in Adults, The Science Journal of the American Association for Respiratory Care 1997 Open Forum Abstracts, December 7, 1997, 2 pages, retrieved at <<http://www.rcjournal.com/abstracts/1997/?id=A00000929>> on 12/22/2010	☐
	5	Braunwald, Heart Failure, chapter 233 of Harrison's Principles of Internal Medicine, 14th Edition, 1998, pp. 1287-1291 & 1360	☐
	6	Clark, et al., Low-Dose Nitric Oxide Therapy for Persistent Pulmonary Hypertension of the Newborn, New England Journal of Medicine, Vol 342, No 7, pp. 469-474 2000 /EA/ 8/30/12	☐
	7	Comparison of Supplemental Oxygen and Nitric Oxide for Inhalation in the Evaluation of the Reactivity of the Pulmonary Vasculature During Acute Pulmonary Vasodilator Testing, http://clinicaltrials.gov/archive/NCT00626028/2009_01_12 January 12, 2009	☐
	8	Cox, et al., Factors Associated With Establishing a Causal Diagnosis for Chidren With Cardiology, Pediatrics, Vol 118, No 4, October 4, 2006, pp 1519-1531, published online October 2, 2006	☐
	9	Cuthbertson et al., "UK guidelines for thee use of inhaled nitric oxide therapy in adults ICUs*", Intensive Care Med (1997), 23, Springer-Verlag, 1997, pp#1212-pp#1218	☐
	10	EP 09251949 Office Action dated 10/11/2010, 5 pages	☐
	11	Guideline for Industry; Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, March 1995, 17 pages	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	12821041 12821041 - GAU: 1613
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	12	Headrick, Hemodynamic monitoring of the critically ill neonate, J Perinat Neonatal Nurs 1992; 5(4): 58-67	☐
	13	INO Therapeutics, LLC, "INOflo for Inhalation 800ppm", package leaflet, 2010, 2	☐
	14	JP 2009157623 Office Action dated 02/23/2010, 3 pages	☐
	15	JP 2009157623 Office Action dated 07/30/2010, 6 pages	☐
	16	JP 2009157623 Office Action response filed 06/18/2010, 37 pages (no translation)	☐
	17	JP 2009157623 request for accelerated exam filed 01/15/2010 (60 pages)	☐
	18	JP 2009157623 response filed 11/30/2010, 58 pages	☐
	19	Letter of Acceptance for AU 2010202422, dated 10/7/2010	☐
	20	Letter of acceptance of AU application 2009202685, dated 08/10/2010, 3 pages	☐
	21	Lipschultz, The incidence of pediatric cardiomyopathy in two regions of the United States, New England Journal of Medicine, April 24, 2003. <<http://www.nejm.org/doi/full/10.1056/NEJMoa021715>>	☐
	22	NIH Clinical Center Services, retrieved at <http://www.cc.nih.gov/ccmd/clinical_services.html>> on 08/18/2010	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	12821041 12821041 - GAU: 1613
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	23	Notification of Reason for Rejection, mailed 7/30/2010, from Japanese Patent Application No. 2009-157623 (cites foreign references).	☐
	24	Office Action for AU 2010202422 dated 07/09/2010, 3 pages	☐
	25	Office Action from AU 2009202685 dtd 03/15/2010	☐
	26	Office Action from AU 2010206032 dated 08/16/2010 (3 pages)	☐
	27	Office Action Response for AU 2009202685 to 03/15/2010 OA, filed 06/08/2010 (16 pages)	☐
	28	Office Action Response for JP2007157623 filed on 11/12/2009 (no English translation)	☐
	29	Office Action Response to AU 2010202422 OA dated 07/09/2010, response filed 09/01/2010	☐
	30	PCT/US2010/038652 Search Report dated 07/29/2010, 16 pages	☐
	31	Response filed 08/18/2010 to EP Search Report dated 05/10/10 for EP09251949	☐
	32	Search Report from EP 09251949 dated 05/10/10	☐
	33	Towbin, et al., Incidence, Causes, and Outcomes of Dilated Cardiomyopathy in Children, JAMA, October 18, 2006 - Vol 296, No. 15, pp. 1867-1876	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	12821041 12821041 - GAU: 1613
	Filing Date	2010-06-22
	First Named Inventor	James S. Baldassarre
	Art Unit	1616
	Examiner Name	Ernst V. Arnold
	Attorney Docket Number	I001-0002USC4

	34	Yoshida, Kiyoshi, " Well-illustrated Diagnostics and Treatment of Heart Failure" Professor of Kawasaki Medical University, cardiovascular internal medicine CIRCULATION Up-to-Date Vol. 2, No. 4, 2007(343), pp. 23-28	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/Christopher P. Rogers, Reg. No. 36,334/ /Ernst Arnold/	Date Considered	2011-02-17 06/18/2011

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

EFS Web 2.1.17 ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.A./

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	0	424/7118.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2012/08/28 11:59
L2	3	424/718.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2012/08/28 11:59
L3	0	423/405.CCLS. AND (((nitric adj oxide) or (nitrogen adj monoxide)) and (edema or pulmonary) and (pcwp or (capillary with wedge) or ((ateriole or artery) with pressure))).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2012/08/28 11:59

8/28/2012 12:00:53 PM
C:\Users\earnold\Documents\EAST\Workspaces\12821020.wsp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: James S. Baldassarre et al.	Art Unit :	1613
Serial No.	: 12/821,041	Examiner :	Ernst V. Arnold
Filed	: June 22, 2010	Confirmation No.:	3219
		Notice of Allowance Date:	September 4, 2012
Title	: METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE		

MAIL STOP ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NOTICE OF ALLOWANCE
AND
COMMENTS ON STATEMENT OF REASONS FOR ALLOWANCE

In response to the Notice of Allowance mailed September 4, 2012, enclosed is a completed issue fee transmittal form PTOL-85b.

Comments on Statement of Reasons for Allowance

Applicant notes that the Examiner's statement of reasons for allowance provided on page 3 of the Notice of Allowability mailed September 4, 2012, describes just some of many reasons that the present claims are allowable over the cited art of record.

The large entity issue fee of $1740 and publication fee of $300 are being paid concurrently with this filing.

CERTIFICATE OF (A) MAILING BY FIRST CLASS MAIL OR (B) TRANSMISSION
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Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

If there are any other necessary charges, or any credits, please apply them to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: September 5, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

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PART B – FEE(S) TRANSMITTAL

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	06/22/2010	James S. Baldassarre	26047-0003005	3219

TITLE OF INVENTION: **METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE**

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$1740	$300	$2040	12/04/12

EXAMINER	ART UNIT	CLASS-SUBCLASS
ARNOLD, ERNST V.	1613	427-718000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

[ ] Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

[ ] "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. **Fish & Richardson P.C.**
2.
3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

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(A) NAME OF ASSIGNEE	(B) RESIDENCE (CITY and STATE OR COUNTRY)
INO Therapeutics LLC	**Hampton, NJ**

Please check the appropriate assignee category or categories (will not be printed on the patent): [ ] individual [X] corporation or other private group entity [ ] government

4a. The following fee(s) are enclosed:
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5. **Change in Entity Status** (from status indicated above)
[ ] a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. [X] b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.
NOTE: The issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant, a registered agent or; or the assignee or other party in interest as shown by the records of the Untied States Patent and Trademark Office.

(Authorized Signature) /Janis K. Fraser/ (Date) September 5, 2012

Typed or Printed Name Janis K. Fraser, Ph.D., J.D. Registration No. 34,819

TRANSMIT THIS FORM WITH FEE(S)

SUBSTITUTE PTOL 85 (Rev. 12/04)

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Nancy Bechet
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl issue fee	1501	1	1740	1740
Publ. Fee- early, voluntary, or normal	1504	1	300	300

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**2040**

Electronic Acknowledgement Receipt

EFS ID:	13657883
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Jodi Budge
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	05-SEP-2012
Filing Date:	22-JUN-2010
Time Stamp:	11:38:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32340 198afa76db7d77afbf19f2ae52f586d960593176	no	2
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		Total Files Size (in bytes):	139081		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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New International Application Filed with the USPTO as a Receiving Office
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/821,041	10/23/2012	8293284	26047-0003005	3219

94169 7590 10/03/2012

Fish & Richardson PC
P.O.Box 1022
minneapolis, MN 55440

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

James S. Baldassarre, Doylestown, PA;
Ralf Rosskamp, Chester, NJ;

IR103 (Rev. 10/09)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	James S. Baldassarre et al.	Art Unit	:	1613
Patent No.	:	8,293,284	Examiner	:	Ernst V. Arnold
Issue Date	:	October 23, 2012	Conf. No.	:	3219
Serial No.	:	12/821,041			
Filed	:	June 22, 2010			

Title : METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE

Attn.: Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL OF REQUEST FOR CERTIFICATE OF CORRECTION

Applicant hereby requests that a certificate of correction be issued for the above patent in accordance with the attached request.

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Respectfully submitted,

Date: December 19, 2012

/Janis K. Fraser/
Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Customer Number 94169
Fish & Richardson P.C.
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

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December 19, 2012
Date of Deposit or Transmission

/Nancy Bechet/
Signature

Nancy Bechet
Typed or Printed Name of Person Signing Certificate

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. .: 8,293,284
APPLICATION NO .: 12/821,041
DATED .: OCTOBER 23, 2012
INVENTOR(S) .: JAMES S. BALDASSARRE

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

First page, left column, FOREIGN PATENT DOCUMENTS, line 1:

Delete "7/2000" and insert -- 7/2006 -- therefor.

First page, right column, OTHER PUBLICATIONS, line 17:

Delete "(Achives" and insert -- (Archives -- therefor.

First page, right column, OTHER PUBLICATIONS, line 38:

Delete "Hyptertension" and insert -- Hypertension -- therefor.

First page, right column, OTHER PUBLICATIONS, line 56:

Delete "Hypertemsion" and insert -- Hypertension -- therefor.

MAILING ADDRESS OF SENDER:

Janis K. Fraser, Ph.D., J.D.
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440-1022

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

Page 2 of 2

PATENT NO. .: 8,293,284
APPLICATION NO .: 12/821,041
DATED .: OCTOBER 23, 2012
INVENTOR(S) .: JAMES S. BALDASSARRE

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 12, line 9:

Delete "Table 5" and insert -- Table 7 -- therefor.

Column 12, line 50:

Delete "Table 5" and insert -- Table 7 -- therefor.

Column 12, line 51:

Delete the table heading "TABLE 5" and insert -- TABLE 7 -- therefor.

MAILING ADDRESS OF SENDER:

Janis K. Fraser, Ph.D., J.D.
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440-1022

Electronic Patent Application Fee Transmittal

Application Number:	12821041
Filing Date:	22-Jun-2010
Title of Invention:	METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE
First Named Inventor/Applicant Name:	James S. Baldassarre
Filer:	Janis K. Fraser/Nancy Bechet
Attorney Docket Number:	26047-0003005

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of correction	1811	1	100	100
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**100**

Electronic Acknowledgement Receipt

EFS ID:	14508417
Application Number:	12821041
International Application Number:	
Confirmation Number:	3219
Title of Invention:	METHODS OF REDUCING THE RISK OF OCCURRENCE OF PULMONARY EDEMA IN TERM OR NEAR-TERM NEONATES IN NEED OF TREATMENT WITH INHALED NITRIC OXIDE
First Named Inventor/Applicant Name:	James S. Baldassarre
Customer Number:	94169
Filer:	Janis K. Fraser/Nancy Bechet
Filer Authorized By:	Janis K. Fraser
Attorney Docket Number:	26047-0003005
Receipt Date:	19-DEC-2012
Filing Date:	22-JUN-2010
Time Stamp:	09:36:27
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment Type	Deposit Account
Payment was successfully received in RAM	$100
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Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30448 ef1bf4ad3767e6b776df9275aca42217e66153eb	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	144844	

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,293,284 B2
APPLICATION NO. : 12/821041
DATED : October 23, 2012
INVENTOR(S) : James S. Baldassarre

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

First page, left column, FOREIGN PATENT DOCUMENTS, line 1:
Delete "7/2000" and insert -- 7/2006 -- therefor.

First page, right column, OTHER PUBLICATIONS, line 17:
Delete "(Achives" and insert -- (Archives -- therefor.

First page, right column, OTHER PUBLICATIONS, line 38:
Delete "Hyptertension" and insert -- Hypertension -- therefor.

First page, right column, OTHER PUBLICATIONS, line 56:
Delete "Hypertemsion" and insert -- Hypertension -- therefor.

Column 12, line 9:
Delete "Table 5" and insert -- Table 7 -- therefor.

Column 12, line 50:
Delete "Table 5" and insert -- Table 7 -- therefor.

Column 12, line 51:
Delete the table heading "TABLE 5" and insert -- TABLE 7 -- therefor.

Signed and Sealed this
Fifth Day of February, 2013

Teresa Stanek Rea
Acting Director of the United States Patent and Trademark Office