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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/256,396	11/29/2011	Lenny Dang	C2081-7013US	9930

37462 7590 10/28/2013

LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

EXAMINER	
POHNERT, STEVEN C	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
10/28/2013	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):

docketing@LALaw.com
gengelson@LALaw.com

Notice of Abandonment	Application No. 13/256,396	Applicant(s) DANG ET AL.
	Examiner STEVEN POHNERT	Art Unit 1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

This application is abandoned in view of:

1. ☒ Applicant's failure to timely file a proper reply to the Office letter mailed on *11 January 2013*.
   (a) ☐ A reply was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply (including a total extension of time of _____ month(s)) which expired on _____.
   (b) ☐ A proposed reply was received on _____, but it does not constitute a proper reply under 37 CFR 1.113 (a) to the final rejection.
   (A proper reply under 37 CFR 1.113 to a final rejection consists only of: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114).
   (c) ☒ A reply was received on *10 July 2013* but it does not constitute a proper reply, or a bona fide attempt at a proper reply, to the non-final rejection. See 37 CFR 1.85(a) and 1.111. (See explanation in box 7 below).
   (d) ☐ No reply has been received.

2. ☐ Applicant's failure to timely pay the required issue fee and publication fee, if applicable, within the statutory period of three months from the mailing date of the Notice of Allowance (PTOL-85).
   (a) ☐ The issue fee and publication fee, if applicable, was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the statutory period for payment of the issue fee (and publication fee) set in the Notice of Allowance (PTOL-85).
   (b) ☐ The submitted fee of $_____ is insufficient. A balance of $_____ is due.
   The issue fee required by 37 CFR 1.18 is $_____. The publication fee, if required by 37 CFR 1.18(d), is $_____.
   (c) ☐ The issue fee and publication fee, if applicable, has not been received.

3. ☐ Applicant's failure to timely file corrected drawings as required by, and within the three-month period set in, the Notice of Allowability (PTO-37).
   (a) ☐ Proposed corrected drawings were received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply.
   (b) ☐ No corrected drawings have been received.

4. ☐ The letter of express abandonment which is signed by the attorney or agent of record, the assignee of the entire interest, or all of the applicants.

5. ☐ The letter of express abandonment which is signed by an attorney or agent (acting in a representative capacity under 37 CFR 1.34(a)) upon the filing of a continuing application.

6. ☐ The decision by the Board of Patent Appeals and Interference rendered on _____ and because the period for seeking court review of the decision has expired and there are no allowed claims.

7. ☒ The reason(s) below:

The response of 7/10/2013 did not amend the claims or provide arguments to overcome the issues of record.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

Petitions to revive under 37 CFR 1.137(a) or (b), or requests to withdraw the holding of abandonment under 37 CFR 1.181, should be promptly filed to minimize any negative effects on patent term.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) C2081-7013US
Application Number 13/256,396	Filed March 12, 2010
For METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	
Art Unit 1634	Examiner S. C. Pohnert

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☒ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1,400.00
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50/2762 .

☒ Payment made via EFS-Web.

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.

I am the

☐ applicant.

☒ attorney or agent of record. Registration Number 54,301 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Catherine M. McCarty/	July 10, 2013
Signature	Date
Catherine M.McCarty	(617) 395-7015
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of 1 forms are submitted.

Electronic Patent Application Fee Transmittal

Application Number:	13256396
Filing Date:	29-Nov-2011
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Filer:	Catherine M. McCarty/Kelly Burke
Attorney Docket Number:	C2081-7013US

Filed as Large Entity

U.S. National Stage under 35 USC 371 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:				
Extension - 3 months with $0 paid	1253	1	1400	1400

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1400**

Electronic Acknowledgement Receipt	
EFS ID:	16278099
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	37462
Filer:	Catherine M. McCarty
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	10-JUL-2013
Filing Date:	29-NOV-2011
Time Stamp:	19:41:22
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1400
RAM confirmation Number	6633
Deposit Account	502762
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. 1.492 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

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	Extension of Time	Three_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a_1.pdf	20726 07850f1cd9660255743d328c777d347b16383b6d	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30702 aa7a098a059bc2628bbf5b0ff278bbe2e16c3f78	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	51428		

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Dang *et al.*	Art Unit	: 1634
Serial No.	: 13/256,396	Examiner	: Steven C. Pohnert
Filed	: March 12, 2010	Conf. No.	: 9930
Title	: METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS		

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with §1.6(a)(4), on the 27th day of June 2013.

/Asimina T. Georges Evangelinos/
Asimina T. Georges Evangelinos (Reg. No. 66,888)

Commissioner for Patents

INTERVIEW SUMMARY

Madam:

Applicants thank the Examiner for the courtesy of the telephonic interview with Applicants' representative Catherine M. McCarty on May 21, 2013. Applicants agree with the Examiner's characterization of the interview as provided in the Interview Summary mailed on May 29, 2013.

This Interview Summary is being timely filed within one month of the mailing date of the Examiner's Interview Summary form, which was May 29, 2013. No fees are believed to be due. However, any necessary charges, or any credits, should be applied to Deposit Account No. 50-2762, referencing Attorney Docket No. C2081-7013US.

Respectfully submitted,
Lenny Dang, et al.

By: /Asimina T. Georges Evangelinos/
Asimina T. Georges Evangelinos, Reg. No. 66,888
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Riverfront Office Park
One Main Street
Cambridge, MA 02142
Tel.: (617) 395-7067
Fax: (617) 395-7070
Attorney for Applicant

Electronic Acknowledgement Receipt

EFS ID:	16169691
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	37462
Filer:	Asimini T. Georges Evangelinos
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	27-JUN-2013
Filing Date:	29-NOV-2011
Time Stamp:	14:13:21
Application Type:	U.S. National Stage under 35 USC 371

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	Applicant summary of interview with examiner	T2021-7013US_Response_to_Interview_Summary_1.pdf	17795 c7b66b88c595d2a1dff382b121e31dcd39d775ab	no	1

Warnings:

Information:

Total Files Size (in bytes):	17795

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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
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/256,396	11/29/2011	Lenny Dang	C2081-7013US	9930

37462 7590 05/29/2013

LANDO & ANASTASI, LLP
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CAMBRIDGE, MA 02142

EXAMINER	
POHNERT, STEVEN C	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
05/29/2013	ELECTRONIC

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docketing@LALaw.com
gengelson@LALaw.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	13/256,396	DANG ET AL.	
	Examiner	Art Unit	
	STEVEN POHNERT	1634	

All participants (applicant, applicant's representative, PTO personnel):

(1) *STEVEN POHNERT*. (3)_____.

(2) *Catyh McCarty*. (4)_____.

Date of Interview: *21 May 2013*.

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: *93 and 97*.

Identification of prior art discussed: *none*.

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...)

Applicant's representative contacted the examiner about the 101 rejection of record. The examiner indicated that his current understanding of the 101 guidance in view of the Supreme Courts Prometheus decision is for a claim to overcome the rejection the claim would either have a novel or non-obvious reagent or treatment. The examiner further indicated that he had heard suggestions that limiting claims to a specific population, may overcome the 101 issues, but most recent direction suggested that would not be sufficient. The examiner indicated if he were the representative he would look into arguments that imaging a whole human for 2HG for the detection of cancer is non-obvious. The examiner apologized for his inability to give a specific route to overcome the 101, but indicated he had not been given any specific guidance other than novel or non-obvious reagents and treatments. There were no agreements on patentability. The examiner did indicate he would be open to further interviews of discussion upon request and scheduling availability. .

Applicant recordation instructions: 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

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

/Steven C Pohnert/ Primary Examiner, Art Unit 1634	

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.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/256,396	11/29/2011	Lenny Dang	C2081-7013US	9930

37462 7590 01/11/2013

LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

EXAMINER	
POHNERT, STEVEN C	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
01/11/2013	ELECTRONIC

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docketing@LALaw.com
gengelson@LALaw.com

Office Action Summary	Application No.	Applicant(s)	
	13/256,396	DANG ET AL.	
	Examiner	Art Unit	
	STEVEN POHNERT	1634	

-- 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 *20 December 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) *41-99* is/are pending in the application.

5a) Of the above claim(s) *41-92 and 94-95* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *93 and 96-99* is/are rejected.

8)☒ Claim(s) *93 and 96-99* is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *30 September 2011* 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).

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) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *12/20/2012*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of group 17, claims 93 (in part) and 96-99 in the reply filed on 11/29/2012 is acknowledged. The traversal is on the ground(s) that the response asserts nothing in Balss teaches or suggests evaluating a subject or selecting a subject based on IDH1 or IDH2 allele with 2HG neoactivity. The response further asserts Balss is silent with regard to 2HG neoactivity. This is not found persuasive because the specification lacks a limiting definition of 2HG neoactivity. Thus the broadest reasonable interpretation of the claims appears to merely require the detection of mutant IDH1 or IDH2 or 2HG which is obvious or anticipated over the teachings of Balss and Struys (FEBS(2004) volume 557, pages 115-120) which teaches detection of 2HG in subjects without2-hydroxyglutaric aciduria.

Claims 41-92 and 94-95 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 11/29/2012.

Claims 93 and 96-99 are being examined.

Priority

The instant application was filed 11/29/2011 and is a national stage entry of PCT/US10/27253 filed 3/12/2010 and claims priority to provisional application 61/160,253 filed 3/12/2010; provisional 61/160,664 filed 3/16/2009; provisional 61/173,518 filed 4/28/2009; provisional 61/180,609 filed 5/22/2009; provisional

61/220,543 filed 6/25/2009; provisional 61/227,649 filed 7/22/2009; provisional 61/229,689 filed 7/29/2009: provisional 61/253,820 filed 10/21/2009:and provisional 61/266,929 filed 12/4/2009.

Drawings

2. The specification indicates there is a red line in figure 28 and thus the figures are in color. Color photographs and color drawings are not accepted unless a petition filed under 37 CFR 1.84(a)(2) is granted. Any such petition must be accompanied by the appropriate fee set forth in 37 CFR 1.17(h), three sets of color drawings or color photographs, as appropriate, and, unless already present, an amendment to include the following language as the first paragraph of the brief description of the drawings section of the specification:

> The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Color photographs will be accepted if the conditions for accepting color drawings and black and white photographs have been satisfied. See 37 CFR 1.84(b)(2).

Figure 27 provides a nucleic acid sequence but does not identify it by a SEQ ID NO. 37 CFR 1.821 (d) requires , “Where the description or claims of a patent application discuss a sequence that is set forth in the “Sequence Listing ” in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by “SEQ ID NO: ” in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.” The claims/specification should be be completely reviewed and amended to be consistent with 37 CFR 1.821 (d).

Application data sheet

The ADS filed on 9/13/2011 does not comply with 37 CFR 1.76(b)(7) as it does not provide assignee information for the instant application, but Agios Pharmaceuticals is an Assignee of record.

37 CFR 1.76. Application Data Sheet
(a) Application data sheet . An application data sheet is a sheet or sheets, that may be voluntarily submitted in either provisional or nonprovisional applications, which contains bibliographic data, arranged in a format specified by the Office. An application data sheet must be titled "Application Data Sheet " and must contain all of the section headings listed in paragraph (b) of this section, with any appropriate data for each section heading. If an application data sheet is
provided, the application data sheet is part of the provisional or nonprovisional application for which it has been submitted.
(b) Bibliographic data . Bibliographic data as used in paragraph (a) of this section includes:
(1) Applicant information . This information includes the name, residence, mailing address, and citizenship of each applicant (§ 1.41(b)). The name of each applicant must include the family name, and at least one given name without abbreviation together with any other given name or initial. If the applicant is not an inventor, this information also includes the applicant 's authority (§ § 1.42, 1.43, and 1.47) to apply for the patent on behalf of the inventor.
(2) Correspondence information . This information includes the correspondence address, which may be indicated by reference to a customer number, to which correspondence is to be directed (see § 1.33(a)).
(3) Application information . This information includes the title of the invention, a suggested classification, by class and subclass, the Technology Center to which the subject matter of the invention is assigned, the total number of drawing sheets, a suggested drawing figure for publication (in a nonprovisional application), any docket number assigned to the application, the type of application (e.g., utility, plant, design, reissue, provisional), whether the application
discloses any significant part of the subject matter of an application under a secrecy order pursuant to § 5.2 of this chapter (see § 5.2(c)), and, for plant applications, the Latin name of the genus and species of the plant claimed, as well as the variety denomination. The suggested classification and
Technology Center information should be supplied for provisional applications whether or not claims are present. If claims are not present in a provisional application, the suggested classification and Technology Center should be based upon the disclosure.
(4) Representative information . This information includes the registration number of each practitioner having a power of attorney in the application (preferably by reference

to a customer number). Providing this information in the application data sheet does not constitute a power of attorney in the application (see § 1.32).
(5) Domestic priority information . This information includes the application number, the filing date, the status (including patent number if available), and relationship of each application for which a benefit is claimed under 35 U.S.C. 119(e), 120, 121, or 365(c). Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and § 1.78(a)(2) or § 1.78(a)(5), and need not otherwise be made part of the specification.
(6) Foreign priority information . This information includes the application number, country, and filing date of each foreign application for which priority is claimed, as well as any foreign application having a filing date before that of the application for which priority is claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and § 1.55(a).
(7) Assignee information . This information includes the name (either person or juristic entity) and address of the assignee of the entire right, title, and interest in an application. Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of this chapter to have an assignment recorded by the Office.
(c) Supplemental application data sheets . Supplemental application data sheets:
(1) May be subsequently supplied prior to payment of the issue fee either to correct or update information in a previously submitted application data sheet, or an oath or declaration under § 1.63 or § 1.67, except that inventorship changes are governed by § 1.48, correspondence changes are governed by § 1.33(a), and citizenship changes are governed by § 1.63 or § 1.67; and
(2) Must be titled "Supplemental Application Data Sheet, " include all of the section headings listed in paragraph (b) of this section, include all appropriate data for each section heading, and must identify the information that is being changed, preferably with underlining for insertions, and strike-through or brackets for text removed.
(d) Inconsistencies between application data sheet and other documents . For inconsistencies between information that is supplied by both an application data sheet under this section and other documents.
(1) The latest submitted information will govern notwithstanding whether supplied by an application data sheet, an amendment to the specification, a designation of a correspondence address, or by a § 1.63 or § 1.67 oath or declaration, except as provided by paragraph (d)(3) of this section;
(2) The information in the application data sheet will govern when the inconsistent information is supplied at the same time by an amendment to the specification, a designation of correspondence address, or a § 1.63 or § 1.67 oath or declaration, except as provided by paragraph
(d)(3) of this section;
(3) The oath or declaration under § 1.63 or § 1.67 governs inconsistencies with the application data sheet in the naming of inventors (§ 1.41 (a)(1)) and setting forth their citizenship (35 U.S.C. 115);

(4) The Office will capture bibliographic information from the application data sheet (notwithstanding whether an oath or declaration governs the information). Thus, the Office shall generally, for example, not look to an oath or declaration under § 1.63 to see if the bibliographic information contained therein is consistent with the bibliographic information captured from an application data sheet (whether the oath or declaration is submitted prior to or subsequent to the application data sheet). Captured bibliographic information derived from an application data sheet containing errors may be corrected if applicant submits a request therefor and a supplemental application data sheet.

Claim Objections

3. Claims 93, 96-99 are objected to because of the following informalities:

Claim 93 is objected to as it recites "2HG," "IDH1," IDH2," but does not recite the full terminology for the acronym. Claims are more concise when the first time an acronym is presented the full terminology is also presented. Finally an acronym may have alternative meanings to an artisan.

Claims 96-99 are objected to as they depend from claim 93.

Claims 96-97 and 99 are objected to as it recites "2HG," but does not recite the full terminology for the acronym. Claims are more concise when the first time an acronym is presented the full terminology is also presented. Finally an acronym may have alternative meanings to an artisan. This objection may be overcome by putting the full terminology in the claims the first time an acronym occurs.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

4. The following is a quotation of 35 U.S.C. 112(a):
(a) IN GENERAL.—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 or joint inventor of carrying out the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), first paragraph:

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.

5. Claims 93 and 96-99 rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), 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 or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

The instant claims are drawn to a method of evaluating a subject for the presence or susceptibility to cancer by analyzing the subject or a sample from the subject for one or more of : a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

Thus the claims encompass the presence, distribution, or level of mutant IDH1 enzyme or mutant IDH2 enzyme, RNA, or protein in any subject of any species. The claims further set forth the functional limitation that either mutant of which has neoactivity.

Regarding the requirement for adequate written description of chemical entities, Applicant's attention is directed to the MPEP §2163. In particular, Regents of the University of California v. Eli Lilly & Co., 119 F.3d 1559, 1568 (Fed. Cir. 1997), cert. denied, 523 U.S. 1089, 118 S. Ct. 1548 (1998), holds that an adequate written description requires a precise definition, such as by structure, formula, chemical name, or physical properties, "not a mere wish or plan for obtaining the claimed chemical invention." Eli Lilly, 119 F.3d at 1566. The Federal Circuit has adopted the standard set forth in the Patent and Trademark Office (PTO) Guidelines for Examination of Patent Applications under the 35 U.S.C. 112.I "Written Description" Requirement ("Guidelines"), 66 Fed. Reg. 1099 (Jan. 5, 2001), which state that the written description requirement can be met by "showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics," including, inter alia, "functional characteristics when coupled with a known or disclosed correlation between function and structure..." Enzo Biochem, Inc. v. Gen-Probe Inc., 296 F.3d 316, 1324-25 (Fed. Cir. 2002) (quoting Guidelines, 66 Fed. Reg. at 1106). Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. MPEP §2163. However, if a biomolecule is described only by a functional characteristic, without any disclosed correlation between function and structure of the sequence, it is “not sufficient characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence.” MPEP §2163.

The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. Univ. of Rochester v. G.D. Searle, 69 USPQ2d 1886, 1892 (CAFC 2004). A description of what a material does, rather than of what it is, usually does not suffice to provide an adequate written description of the invention. Univ. of Cal. V. Eli Lilly, 119 F.3d 1559, 1568 (Fed. Cir. 1997). Furthermore, to the extent that a functional description can meet the requirement for an adequate written description, it can do so only in accordance with PTO guidelines stating that the requirement can be met by disclosing "sufficiently detailed, relevant identifying characteristics," including "functional characteristics when coupled with a known or disclosed correlation between function and structure." Univ. of Rochester v. G.D. Searle, 69 USPQ2d 1427, 1432 (DC WNY 2003).

The specification teaches, " As used herein, a subject can be a human or non-human subject. Non-human subjects include non-human primates, rodents, e.g., mice or rats, or other non-human animals." Review of the specification failed to reveal a limiting definition of, “2HG neoactivity.” Thus the claims broadly encompass the detection of any RNA, DNA, or protein mutation of any IDH1 or IDH2 gene from any species. This is an enormous genus of protein, RNA and DNA.

May et al (Science (1988) volume 241, page 1441) teaches there are millions of known taxonomic species (table 3). May further teaches there are at least 4,500 known mammalian species (table 3).

Benner et al (Trends in Genetics (2001) volume 17, pages 414-418) teaches that, “Here, the ‘homology-implies-equivalency’ assumption is restricted to a subset of

homologs that diverged in the most-recent common ancestor of the species sharing the homologs. This strategy is useful, of course. But it is likely to be far less general than is widely thought. Two species living in the same space, almost by axiom, cannot have identical strategies for survival. This, in turn, implies that two orthologous proteins might not contribute to fitness in exactly the same way in two species" (see page 414, 3rd column last full paragraph). Benner specifically describes that although the leptin gene homologs have been found in mice and humans, their affect is different (see page 414, 3rd column last paragraph-3rd column page 415). Benner specifically teaches that the leptin gene in mice plays a major role in obesity, but no such effect has been demonstrated in humans due perhaps to the different evolutionary forces. Benner thus teaches that the activity and function of genes in different species is unpredictable.

Thus the claims in light of the specification encompass any enzyme that can broadly be interpreted as an IDH1 or IDH2 with any activity that can broadly be interpreted as an undefined 2HG neoactivity. This is an enormous genus of mutations in any gene that can broadly be identified as IDH1 or IDh2 from any species. The teachings of May suggests this encompasses millions of DNA, RNA, and protein sequences.

The teachings of the specification are limited to mutations in the human ICDH1 gene encoding codon 132. Thus while the claims encompass an enormous genus the specification appears to provide mutations of a single codon in a single species, which would not be predictive of all species based on the teachings of Benner. Thus the claims lack adequate written description.

6. The following is a quotation of 35 U.S.C. 112(b):

(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

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.

7. Claims 93 and 96-99 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, "If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2." Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

Claim 93 is indefinite because it lacks a positive active step relating back to the preamble. The preamble recites a method of method of evaluating a subject for the presence or susceptibility to a cancer, however the last positive active step is drawn to analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding

a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity;. Therefore it is unclear as to whether the method is drawn to method of evaluating a subject for the presence or susceptibility to a cancer or analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity. The claims encompass the detection of 2HG, which appears to be 2 hydroxyglutarate, a known metabolite of most known forms of aerobic respiration or any mutant RNA, DNA or protein broadly encompassed by the recitation IDH1 or IDH2. However, the claims to fail to set forth what reference sequence the mutation is relative to. Thus it is unclear how the method results in evaluating the presence or susceptibility to cancer based on the presence, level or distribution of a metabolite that appears to be required of aerobic life or a mutation relative to any IDH1 or IDH2 sequence. Further the claim recites, “2HG neoactivity.” While, review of the specification teaches, “Neoactivity, as used herein, means an activity that arises as a result of a mutation, e.g., a point mutation, e.g., a substitution, e.g., in the active site of an enzyme” The claims are drawn to “2HG neaoctivity.” Thus while the specification attempts to describe neoactivity relative to an enzyme the claims are drawn to neoactivity relative to a metabolite, thus it is unclear if

2HG neoactivity requires binding of 2HG to the enzyme, specific level of catalysis of 2HG, etc.

Claims 96-99 are rejected as they depend from claim 93.

Claim Rejections - 35 USC § 101

8. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Based upon an analysis with respect to the claim as a whole, claim(s) 93 and 96-99 is/are determined to be directed to a law of nature/natural principle. The rationale for this determination is explained below:

The claims are drawn to a method for A method of evaluating a subject for the presence or susceptibility to a cancer. The method comprises the step analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer..

The unpatentability of laws of nature was confirmed by the U.S. Supreme Court in Mayo Collaborative Services v. Prometheus Laboratories, Inc., No. 10-1150 (March

20, 2012).). "[L]aws of nature, natural phenomena, and abstract ideas" are not patentable. "Phenomena of nature, though just discovered, mental processes, and abstract intellectual concepts are not patentable, as they are the basic tools of scientific and technological work." *Gottschalk* v. *Benson*, 409 U. S. 63, 67 (1972). The Supreme Court does acknowledge that it is possible to transform an unpatentable law of nature, but one must do more than simply state the law of nature while adding the words "apply it." See, *e.g., Benson*, *supra*, at 71–72.

The memo of July 3, 2012 for interim procedure for subject matter for process claims involving laws of nature set forth 3 inquiries to determine subject eligibility.

The first inquiry is the claimed invention directed to a process, defined as an act or series of acts or steps. In the instant case the claims are drawn to a method comprising the step analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer. Thus the method passes the first inquiry.

The second inquiry set forth is does the claim focus on use of a law of nature, natural phenomenon, or naturally occurring relation or correlation. In the instant claims the claims appear to be drawn to a method of evaluating the presence or susceptibility to a cancer. Thus the claim is drawn a naturally recurring 2HG presence, distribution or level or IDH1 or IDH2 mutants with presence or susceptibility to cancer . Thus the claimed method passes the second inquiry.

The third inquiry set forth is does the claim include additional elements/steps or a combination of elements/steps that integrate the natural principle into the claimed invention such that the natural principle is practically applied, and are sufficient to ensure the claims amounts to significantly more than the natural principle itself. In the instant claims do not provide additional elements so that it amounts to more than application of the natural principle. The instant case merely requires the detection of the naturally occurring sequence or metabolites with the presence or susceptibility to cancer. Thus the claims are not patent eligible.

As the claims encompass a step of analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme,

either of which has 2HG neoactivity; thereby evaluating the subject for such cancer. The correlation between a 2HG presence, distribution or level; or IDH1 or IDH2 mutations with cancer is considered to be a law of nature. Therefore the claims simply describe a relation which is set forth in natural law. The additional steps in the claimed method (i.e., analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer) are not themselves natural laws, but neither are they sufficient to transform the nature of the claims. Rather these additional steps consist of well-understood, routine, conventional activity already engaged in by the scientific community. The additional steps, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. The Court has made clear that to transform an unpatentable law of nature into a patent-eligible *application* of such a law, one must do more than simply state the law of nature while adding the words "apply it." Essentially, appending conventional steps specified at a high level of generality, to laws of nature, natural phenomena, and abstract ideas cannot make those laws, phenomena, and ideas patent-eligible. As applicable in the instant set of claims, the step of testing does not add any additional features.

The Courts decision rested upon an examination of the particular claims in light of the Court's precedents, specifically *Bilski, Flook* and *Diehr*. The Court repeated the long standing exceptions (laws of nature, natural phenomena, and abstract ideas) to categories of patent eligibility defined in 35 U .S.C. § 101. In conducting the analysis, the Court addressed the "machine-or-transformation" test explained in *Bilski* with a reminder that the test is an *"important and useful clue"* to patentability but that it does not trump the "law of nature" exclusion. A claim that recites a law of nature or natural correlation, with additional steps that involve well-understood, routine, conventional activity previously engaged in by researchers in the field is not patent-eligible, regardless of whether the steps result in a transformation. On the other hand, reaching back to *Neilson,* the Court pointed to an eligible process that included not only a law of nature (hot air promotes ignition) but also several unconventional steps (involving a blast furnace) that confined the claims to a particular, useful application of the principle.

The Court also summarized their holding by stating “[t]o put the matter more succinctly, the claims inform a relevant audience about certain laws of nature; any additional steps consist of well understood, routine, conventional activity already engaged in by the scientific community; and those steps, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.”

For these reasons the claims are rejected under section 101 as being directed to non-statutory subject matter.

Claim Rejections - 35 USC § 102

9. 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 –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (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.

10. Claim 93 rejected under 35 U.S.C. 102(a) as being anticipated by Balss (Acata Neuropathol (2008) volume 116, pages 597-602).

As noted in the MPEP 2111.02, "If the body of a claim fully and intrinsically sets forth all of the limitations of the claimed invention, and the preamble merely states, for example, the purpose or intended use of the invention, rather than any distinct definition of any of the claimed invention's limitations, then the preamble is not considered a limitation and is of no significance to claim construction." Further, a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. See *In re Hirao*, 535 F.2d 67, 190 USPQ 15 (CCPA 1976) and *Kropa v. Robie*, 187 F.2d 150, 152, 88 USPQ 478, 481 (CCPA 1951). Accordingly, the claim language of " to a method of evaluating the presence or susceptibility to a cancer" merely sets forth the intended use or purpose of the claimed methods, but does not limit the scope of the claims.

Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim language that does not limit a claim to a particular structure. However, examples of claim language, although not exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited.'" Id.<

Thus claim 93 merely requires analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the

presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

Balss teaches detection in mutations in codon 132 of IDH1 in a sample from a subject. The mutations of Balss have 2HG activity.

11. Claim 93 rejected under 35 U.S.C. 102(b) as being anticipated by Jennings et al (Biochemistry (1997)volume 36, pages 13743-13747).

As noted in the MPEP 2111.02, "If the body of a claim fully and intrinsically sets forth all of the limitations of the claimed invention, and the preamble merely states, for example, the purpose or intended use of the invention, rather than any distinct definition of any of the claimed invention's limitations, then the preamble is not considered a limitation and is of no significance to claim construction." Further, a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. See *In re Hirao*, 535 F.2d 67, 190 USPQ 15 (CCPA 1976) and *Kropa v. Robie*, 187 F.2d 150, 152, 88 USPQ 478, 481 (CCPA 1951). Accordingly, the claim language of " to a method of evaluating the presence or susceptibility to a cancer" merely sets forth the intended use or purpose of the claimed methods, but does not limit the scope of the claims.

Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim language that does not limit a claim to a particular structure. However, examples of claim language, although not

exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited.'" Id.<

Thus claim 93 merely requires analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

Jennings teaches detection in mutations in codon 132 of isocitrate dehydrogenase in a sample from a subject. The mutations of Jennings have 2HG activity.

12. Claim 93, 96-97 and 99 are rejected under 35 U.S.C. 102(b) as being anticipated by Struys (FEBS letters 92004) volume 557, pages 115-120).

As noted in the MPEP 2111.02, "If the body of a claim fully and intrinsically sets forth all of the limitations of the claimed invention, and the preamble merely states, for example, the purpose or intended use of the invention, rather than any distinct definition of any of the claimed invention's limitations, then the preamble is not considered a limitation and is of no significance to claim construction." Further, a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. See *In re Hirao*, 535 F.2d 67, 190 USPQ 15 (CCPA 1976) and *Kropa v. Robie*, 187 F.2d 150, 152, 88 USPQ 478, 481 (CCPA 1951). Accordingly, the claim language of " to a method of evaluating the presence or susceptibility to a cancer" merely sets forth the intended use or purpose of the claimed methods, but does not limit the scope of the claims.

Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim language that does not limit a claim to a particular structure. However, examples of claim language, although not

exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited.'" Id.<

Thus claim 93 merely requires analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

With regards to claims 93, 96-97 and 99, Struys teaches the detection of 2HG concentrations in samples isolated from control cell lines by mass spectroscopy. Struys thus anticipates the active step of the claims.

Claim Rejections - 35 USC § 103

13. 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.

14. Claims 93 and 96-99 are rejected under 35 U.S.C. 103(a) as being unpatentable over Holmes et al (Journal of Pharmaceutical and Biomedical Analysis (1997) volume 15, pages 1647-1659).

As noted in the MPEP 2111.02, "If the body of a claim fully and intrinsically sets forth all of the limitations of the claimed invention, and the preamble merely states, for example, the purpose or intended use of the invention, rather than any distinct definition of any of the claimed invention's limitations, then the preamble is not considered a limitation and is of no significance to claim construction." Further, a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. See *In re Hirao*, 535 F.2d 67, 190 USPQ 15 (CCPA 1976) and *Kropa v. Robie*, 187 F.2d 150, 152, 88 USPQ 478, 481 (CCPA 1951). Accordingly, the claim language of " to a method of evaluating the presence or

susceptibility to a cancer" merely sets forth the intended use or purpose of the claimed methods, but does not limit the scope of the claims.

Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim language that does not limit a claim to a particular structure. However, examples of claim language, although not exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited.'" Id.<

Thus claim 93 merely requires analyzing the subject or a sample from the subject for one or more of: a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria; b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which

has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

Holmes teaches the use of non-invasive NMR spectroscopy to detect 2 hydroxyglutarate in the urine of a subject with 2-hydroxyglutaric aciduria. Holmes further teaches, " Many resonances in normal urine [14], blood plasma [11], cerebrospinal fluid [12] and seminal fluid [13] have been assigned previously."

Thus while Holmes does not specifically teach the detection of 2HG in samples from subjects not having or not diagnosed with 2-hydroxyglutaric aciduria, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made, that the resonances of normal urine, plasma, cerebrospinal fluid and seminal fluid would analyzed the presence of 2HG in the normal samples. The artisan would have a reasonable expectation of success as the artisan is merely using known techniques to detect know metabolites in known samples.

Summary

No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. 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.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

Notice of References Cited	Application/Control No. 13/256,396	Applicant(s)/Patent Under Reexamination DANG ET AL.	
	Examiner STEVEN POHNERT	Art Unit 1634	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-			
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	E	US-			
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	N					
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	Q					
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	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	May et al (Science (1988) volume 241, page 1441)
	V	Benner et al (Trends in Genetics (2001) volume 17, pages 414-418)
	W	Jennings et al (Biochemistry (1997)volume 36, pages 13743-13747)
	X	Struys et a; (FEBS letters 92004) volume 557, pages 115-120)

*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. 13/256,396	Applicant(s)/Patent Under Reexamination DANG ET AL.	
	Examiner STEVEN POHNERT	Art Unit 1634	Page 2 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
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	K	US-			
	L	US-			
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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Holmes et al (Journal of Pharmaceutical and Biomedical Analysis (1997) volume 15, pages 1647-1659)
	V	
	W	
	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.

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	1	13/256396	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 07:08
S2	1	S1 and neoactivity	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 07:08
S3	4	2hg near2 neoactivity	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 08:49
S4	1	13/256396	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 08:49
S5	1	S3 and S4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 08:49
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S7	1	S4 and (red or blue or yellow or color)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 09:10
S8	1	S4 and (subject)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 09:41
S9	1	13/256396	US-PGPUB; USPAT; USOCR;	OR	ON	2013/01/08 12:49

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S11	621	2hg or hydroxyglutarate	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:10
S12	36	S11 same (nmr or mri or (magnetic near2 resonance))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:11
S13	32	S12 and @pd<"20080313"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:11
S14	129	hydroxyglutarate and (nmr or mri or (magnetic near2 resonance))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:13
S15	32	S13 and @pd<"20080313"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:13
S16	64	S14 and @pd<"20080313"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:13
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S19	1	((LENNY) near2 (DANG)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41

S20	4	((VALERIA) near2 (FANTIN)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41
S21	34	((STEFAN) near2 (GROSS)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41
S22	1	(("HYUN GYUNG") near2 (JANG)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41
S23	11	((SHENGFANG) near2 (JIN)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41
S24	101	((FRANCESCO) near2 (SALITURO)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:41
S25	71	((JEFFREY) near2 (SAUNDERS)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:42
S26	2	((SHINSAN) near2 (SU)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:42
S27	1	((KATHARINE) near2 (YEN)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2013/01/08 14:42
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S31	9000	idh or odh1 or idh2 or "Cytosolic NADP-isocitrate dehydrogenase" or IDCD or IDH or IDP or IDPC or "NADP(+)-specific ICDH, Oxalosuccinate decarboxylase" or PICD	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:45
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S33	1	S30 and S31	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:45
S34	748	2hg or hydroxyglutarate or (hydroxy near2 glutarate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:46

S35	48	S34.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:46
S36	1	S30 and S35	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:46
S37	235	S34 and (nmr or mri or (nuclear near2 magnet$4))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:47
S38	45	S34 same (nmr or mri or (nuclear near2 magnet$4))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:47
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S43	0	S34 and S42	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/01/08 14:50

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13256396	DANG ET AL.
	Examiner	Art Unit
	STEVEN POHNERT	1634

SEARCHED			
Class	Subclass	Date	Examiner
	none		

SEARCH NOTES		
Search Notes	Date	Examiner
Searched EAST and PLus	1/8/2013	SCP
inventor search EAST and PALM	1/8/2013	SCP
Assignee search EAST	1/8/2013	SCP
consulted Kevin Orwig claim interpretation and 112 issues	1/8/2013	SCP

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SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/256,396	11/29/2011	435	1634	C2081-7013US

APPLICANTS

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Shinsan Su, Newton, MA;
Katharine Yen, Wellesley, MA;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/US10/27253 03/12/2010
which claims benefit of 61/160,253 03/13/2009
and claims benefit of 61/160,664 03/16/2009
and claims benefit of 61/173,518 04/28/2009
and claims benefit of 61/180,609 05/22/2009
and claims benefit of 61/220,543 06/25/2009
and claims benefit of 61/227,649 07/22/2009
and claims benefit of 61/229,689 07/29/2009
and claims benefit of 61/253,820 10/21/2009
and claims benefit of 61/266,929 12/04/2009

**** FOREIGN APPLICATIONS ***************************

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Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /STEVEN C POHNERT/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		MA	49	59	2

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UNITED STATES

TITLE

METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

FILING FEE RECEIVED	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time)

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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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13256396
Filing Date	2010-03-12
First Named Inventor	Leonard L. Dang
Art Unit	1634
Examiner Name	S. C. Pohnert
Attorney Docket Number	C2081-7013US

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/S.P./	1	20060281122		2006-12-14	Bryant et al.	
/S.P./	2	20080300208		2008-12-04	Einat et al.	

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/Steven Pohnert/ (01/08/2013)

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	Application Number	13256396
	Filing Date	2010-03-12
	First Named Inventor	Leonard L. Dang
	Art Unit	1634
	Examiner Name	S. C. Pohnert
	Attorney Docket Number	C2081-7013US

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.	T5
/S.P./	1	BLEEKER et al., "IDH1 mutations at residue p.R132 (IDH1 (R132)) occur frequently in high-grade 18-22 gliomas but not in other solid tumors." Hum Muta1., January 2009, Vol 30, No 1, pp 7-11; Abstract.	☐
/S.P./	2	DANG et al., "Cancer-associated IDH1 mutations produce 2-hydroxyglutarate." Nature, 10 29-32 December 2009, Vol 462, No 7274, pp 739-744.	☐
/S.P./	3	International Preliminary Report on Patentability for PCT/US2010/027253 mailed 09/13/11.	☐
/S.P./	4	International Search Report for PCT/US2010/027253 mailed 08/19/10.	☐
/S.P./	5	POLLARD et al, "Cancer. Puzzling patterns of predisposition." Science. 10 April 2009, Vol 324, 1-5,15-16, 18-22,35-38 No 5924, pp 192-194.	☐
/S.P./	6	THOMPSON, "Metabolic Enzymes as Oncogenes or Tumor Suppressors." The New England 18-22 Journal of Medicine, 19 February 2009, Vol 360, No 8, pp 813-815; pg 813, pg 815, col 1; Fig 1.	☐
/S.P./	7	WATANABE et al., "IDH1 Mutations Are Early Events in the Development of Astrocytomas and Oligodendrogliomas". American Journal of Pathology, April 2009 (published online 26 February (2009), Vol 174, No 4, pp 1149-1153; Abstract, pg 1150, col 1.	☐
/S.P./	8	Written Opinion for PCT/US2010/027253 mailed 08/19/10.	☐
/S.P./	9	YAN et al., "IDH1 and IDH2 Mutations in Gliomas." The New England Journal of Medicine, 19 18-22 February 2009, Vol 360, No. 8, pp 765-73.	☐
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13256396
	Filing Date	2010-03-12
	First Named Inventor	Leonard L. Dang
	Art Unit	1634
	Examiner Name	S. C. Pohnert
	Attorney Docket Number	C2081-7013US

EXAMINER SIGNATURE			
Examiner Signature	/Steven Pohnert/ (01/08/2013)	Date Considered	

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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).

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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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Signature	/Peter Korakas/	Date (YYYY-MM-DD)	2012-12-20
Name/Print	Peter Korakas	Registration Number	66513

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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.

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.

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	1	HAI ET AL: "IDH1 and IDH2 Mutations in Gliomas", NEW ENGLAND JOURNAL OF MEDICINE, MASSACHUSETTS MEDICAL SOCIETY, BOSTON, MA, US, vol. 360, no. 8, 19 February 2009 (2009-02-19), pages 765-773	☐
	2	Supplimentary European Search Report for EP 10751525 Mailed December 14, 2012.	☐
	3	ZHAO ET AL: "Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha", SCIENCE, vol. 324, no. 5924, 10 April2009 (2009-04-10), pages 261-265	☐

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Signature	/Peter Korakas/	Date (YYYY-MM-DD)	2013-01-10
Name/Print	Peter Korakas	Registration Number	66513

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I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 10, 2013
Electronic Signature for Peter Korakas: /Peter Korakas/

Docket No.: C2081-7013US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard L. Dang et al.

Application No.: 13/256,396 | Confirmation No.: 9930

Filed: March 12, 2010 | Art Unit: 1634

For: METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS | Examiner: S. C. Pohnert

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

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

Dear Madam:

Pursuant to 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Supplemental Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits as far as is known to the undersigned (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patents and U.S. patent applications. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 C.F.R. § 1.56(a) exists. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 50/2762, under Order No. C2081-7013US.

Dated: January 10, 2013

Respectfully submitted,

Electronic signature: /Peter Korakas/
Peter Korakas
Registration No.: 66,513
Catherine M. McCarty
Registration No.: 54,301
LANDO & ANASTASI LLP
Riverfront Office Park
One Main Street
Suite 1100
Cambridge, Massachusetts 02142
(617) 395-7000
Attorneys for Applicant

Electronic Acknowledgement Receipt

EFS ID:	14660717
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	37462
Filer:	Peter Korakas
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	10-JAN-2013
Filing Date:	29-NOV-2011
Time Stamp:	14:12:02
Application Type:	U.S. National Stage under 35 USC 371

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	Attorney Docket Number	C2081-7013US

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.	T5
	1	BLEEKER et al., "IDH1 mutations at residue p.R132 (IDH1 (R132)) occur frequently in high-grade 18-22 gliomas but not in other solid tumors." Hum Muta1., January 2009, Vol 30, No 1, pp 7-11; Abstract.	☐
	2	DANG et al., "Cancer-associated IDH1 mutations produce 2-hydroxyglutarate." Nature, 10 29-32 December 2009, Vol 462, No 7274, pp 739-744.	☐
	3	International Preliminary Report on Patentability for PCT/US2010/027253 mailed 09/13/11.	☐
	4	International Search Report for PCT/US2010/027253 mailed 08/19/10.	☐
	5	POLLARD et al, "Cancer. Puzzling patterns of predisposition." Science. 10 April 2009, Vol 324, 1-5,15-16, 18-22,35-38 No 5924, pp 192-194.	☐
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*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	13256396
	Filing Date	2010-03-12
	First Named Inventor	Leonard L. Dang
	Art Unit	1634
	Examiner Name	S. C. Pohnert
	Attorney Docket Number	C2081-7013US

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	/Peter Korakas/	Date (YYYY-MM-DD)	2012-12-20
Name/Print	Peter Korakas	Registration Number	66513

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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.**

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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: December 20, 2012
Electronic Signature for Peter Korakas: /Peter Korakas/

Docket No.: C2081-7013US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard L. Dang et al.

Application No.: 13/256,396 | Confirmation No.: 9930

Filed: March 12, 2010 | Art Unit: 1634

For: METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS | Examiner: S. C. Pohnert

INFORMATION DISCLOSURE STATEMENT (IDS)

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

Dear Madam:

Pursuant to 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits as far as is known to the undersigned (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patents and U.S. patent applications. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 C.F.R. § 1.56(a) exists. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 50/2762, under Order No. C2081-7013US.

Dated: December 20, 2012

Respectfully submitted,

Electronic signature: /Peter Korakas/
Peter Korakas
Registration No.: 66,513
LANDO & ANASTASI LLP
Riverfront Office Park
One Main Street
Suite 1100
Cambridge, Massachusetts 02142
(617) 395-7000
Attorney for Applicant

Electronic Acknowledgement Receipt

EFS ID:	14527719
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	37462
Filer:	Peter Korakas
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	20-DEC-2012
Filing Date:	29-NOV-2011
Time Stamp:	16:48:51
Application Type:	U.S. National Stage under 35 USC 371

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	Non Patent Literature	C2081-7013WO_-_NPL_-_BLEEKER_-_IDH1_mutations_at_residue_pR132_IDH1_R132_occur_freque_3.pdf	37666 10fa71360d5125914b5fe62c265e43877def6fcb	no	1
Warnings:					
Information:					

2	Non Patent Literature	C2081-7013WO_-_NPL_-_DANG_-_Cancer-associated_IDH1_mutations_produce_2-hydroxyglutarate_4.pdf	630240 48dc294db81a58a9d8b90c27c76b18ed2288d471	no	6
Warnings:					
Information:					
3	Non Patent Literature	C2081-7013WO_-_C2081-7013WO_IPRP_5.pdf	66934 8f7cb845124def91a0ad6ac99aebc5f21c9c6532	no	2
Warnings:					
Information:					
4	Non Patent Literature	C2081-7013WO_-_C2081-7013WO_ISR_6.pdf	202934 c434c23569a6441069e56bd985ae0461f787a5d0	no	4
Warnings:					
Information:					
5	Non Patent Literature	C2081-7013WO_-_NPL_-_POLLARD_-_Cancer_Puzzling_patterns_of_predisposition_7.pdf	432420 094c4e577fd84ac3345e06552576dc095872d70b	no	3
Warnings:					
Information:					
6	Non Patent Literature	C2081-7013WO_-_NPL_-_THOMPSON_-_Metabolic_Enzymes_as_Oncogenes_or_Tumor_Suppressors_8.pdf	424539 259d21b659a8ac4b1ab4af793a82617b472b1bed	no	3
Warnings:					
Information:					
7	Non Patent Literature	C2081-7013WO_-_NPL_-_Watanabe_-_IDH1_Mutations_Are_Early_Events_in_the_Development_of_Astr_9.pdf	502096 a2d39e7d4a9a8a4746eb11cdf7c61d785994a82a	no	5
Warnings:					
Information:					
8	Non Patent Literature	C2081-7013WO_-_C2081-7013US_Written_Opinion_10.pdf	492698 6d0b0a3ad6a14570baaf1d9f8831156ca0ac38fd	no	8
Warnings:					
Information:					
9	Non Patent Literature	C2081-7013WO_-_NPL_-_YAN_-_IDH1_and_IDH2_Mutations_in_Gliomas_11.pdf	654142 8f486b37c885b6219343f4e752c0363cbcb35eeb	no	9
Warnings:					
Information:					
10	Information Disclosure Statement (IDS) Form (SB08)	C2081-7013US_-_Information_Disclosure_Statement_Fillable_PDF_1.pdf	613319 3e44e939df543f91684bc70ff36ffeaf5433633f	no	5
Warnings:					
Information:					

11	Transmittal Letter	Information_Disclosure_Statement.pdf	21183 c0f70e3cb1091567af120e1625eaef49f47d16b0	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	4078171	

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.

PATENT COOPERATION TREATY

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

MCCARTY, Catherine, M.
Lando & Anastasi, LLP
One Main Street, Eleventh Floor
Cambridge, MA 02142
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 22 September 2011 (22.09.2011)	
Applicant's or agent's file reference C2081-7013WO	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/027253	12 March 2010 (12.03.2010)	13 March 2009 (13.03.2009)

Applicant
AGIOS PHARMACEUTICALS, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Yukari Nakamura

e-mail: pt07.pct@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference C2081-7013WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/027253	International filing date *(day/month/year)* 12 March 2010 (12.03.2010)	Priority date *(day/month/year)* 13 March 2009 (13.03.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant AGIOS PHARMACEUTICALS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	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 Article 35(2) with regard to novelty, inventive step or 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

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 13 September 2011 (13.09.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Yukari Nakamura e-mail: pt07.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference C2081-7013WO	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 10/27253	International filing date *(day/month/year)* 12 March 2010 (12.03.2010)	(Earliest) Priority Date *(day/month/year)* 13 March 2009 (13.03.2009)
Applicant AGIOS PHARMACEUTICALS, 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.

☐ 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:

   ☒ 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.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, 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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/27253

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
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:

3. ☒ Claims Nos.: 6-14, 17, 23-28, 33-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Group I: Claims 1-5, 15-16, 18-22, 29-32, and 35-39, drawn to methods of treating and diagnosing cancer associated with mutant IDH having 2HG neoactivity, and methods of screening for compounds that inhibit mutant IDH having 2HG neoactivity.

Group II: Claim 40, drawn to a pharmaceutical composition.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the invention listed as Group I is a mutant IDH having 2HG neoactivity. This special technical feature is not shared by the invention listed as Group II. The special technical feature of Group II are the compounds recited therein. This special technical feature is not shared by the invention listed as Group I.

********** See Supplemental Box to continue **********

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 additional fees, this Authority did not invite payment of additional fees.

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.:
Claims 1-5, 15-16, 18-22, 29-32, 35-39

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/27253

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/32, A61P 35/00 (2010.01)
USPC - 435/26, 435/190, 536/24.5
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)
USPC-- 435/26, 435/190, 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-- 435/4, 514/44A

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (PGPB,USPT,USOC,EPAB,JPAB), PubMed, Google/Scholar: isocitrate dehydrogenase, Oxalosuccinate decarboxylase, IDCD, mutant, cancer, proliferation, apoptosis, (2-hydroxyglutarate OR 2HG OR "2-HG" OR 2-hydroxyglutaric), gain-of-function
Reactome [<http://brie8.cshl.org/cgi-bin/frontpage?DB=gk_central>: oxoglutarate, hydroxyglutarate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WATANABE et al., IDH1 Mutations Are Early Events in the Development of Astrocytomas and Oligodendrogliomas. American Journal of Pathology, April 2009 (published online 26 February 2009), Vol 174, No 4, pp 1149-1153; Abstract, pg 1150, col 1	18-22 ---------- 1-5, 15-16
Y	US 2008/0300208 A1 (PAZ et al.) 04 December 2008 (04.12.2008) Abstract, para [0022], [0057], [0065], [0156], [0162], [0177]	1-5,15-16, 35-38
X -- Y --- A	THOMPSON, Metabolic Enzymes as Oncogenes or Tumor Suppressors. The New England Journal of Medicine, 19 February 2009, Vol 360, No 8, pp 813-815; pg 813, pg 815, col 1; Fig 1	18-22 -------- 35-39 -------- 29-32
Y	US 2006/0281122 A1 (BRYANT et al.) 14 December 2006 (14.12.2006) claim 27	39
X	BLEEKER et al., IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat., January 2009, Vol 30, No 1, pp 7-11; Abstrac	18-22
X	YAN et al., IDH1 and IDH2 Mutations in Gliomas. The New England Journal of Medicine, 19 February 2009, Vol 360, No 8, pp 765-73	18-22
A, P	DANG et al., Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 10 December 2009, Vol 462, No 7274, pp 739-744.	29-32
A,P	POLLARD et al., Cancer. Puzzling patterns of predisposition. Science. 10 April 2009, Vol 324, No 5924, pp 192-194	1-5, 15-16, 18-22, 35-38

☐ Further documents are listed in the continuation of Box C. ☐

* 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 application or patent 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
06 August 2010 (08.06.2010)	19 AUG 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

********** Supplemental Box **********

In continuation of Box III:

The special technical feature of the invention listed as Group I is a mutant IDH having 2HG neoactivity. This special technical feature is not shared by the invention listed as Group II. The special technical feature of Group II are the compounds recited therein. This special technical feature is not shared by the invention listed as Group I.

Regarding the treatment methods of Group I, while the compounds of Group II may be used in the treatment methods of Group I, it is readily apparent that the treatment methods of Group I may employ a therapeutic agent not limited by any special technical feature to the compounds of Group II. According to PCT Rule 13.2, unity of invention exists only when the same or corresponding technical feature is shared by all claimed inventions. Thus, the inventions listed at Groups I and II do not relate to a single general inventive concept because they lack the same or corresponding special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

Furthermore, regarding the claims in Group I having candidate compound screening methods, the technical feature of the candidate compound screening methods of Group I resides in the step of observing the ability of the candidate compounds to modulate the 2HG neoactivity of mutant IDH in a screening assay. Neither the same nor a corresponding special technical feature is present in any of the compounds of Group II. No manufacturing relationship exists between the screening method and the claimed compounds. Further, these screening methods are not a method of using the claimed compounds of Group II. In the absence of any teaching as to the structure required for a compound to act as an inhibitor of the 2HG neoactivity of mutant IDH, there is no single general concept that links the screening methods to the claimed compounds.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. With out a shared special technical feature, the inventions of Groups I and II lack unity with one another.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: CATHERINE M. MCCARTY
LANDO & ANASTASI, LLP
ONE MAIN STREET, ELEVENTH FLOOR
CAMBRIDGE, MA 02142

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	19 AUG 2010
Applicant's or agent's file reference C2081-7013WO	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 10/27253	12 March 2010 (12.03.2010)	13 March 2009 (13.03.2009)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/32, A61P 35/00 (2010.01)
USPC - 435/26, 435/190, 536/24.5

Applicant AGIOS PHARMACEUTICALS, 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 or 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 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 notified 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/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	06 August 2010(08.06.2010)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/27253

Box No. I Basis of this 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 43*bis*.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:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/27253

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. 6-14, 17, 23-28, 33-34

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 6-14, 17, 23-28, 33-34 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 6-14, 17, 23-28, 33-34 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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 said claims Nos. 6-14, 17, 23-28, 33-34

☐ 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 Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/27253

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

Group I: Claims 1-5, 15-16, 18-22, 29-32, and 35-39, drawn to methods of treating and diagnosing cancer associated with mutant IDH having 2HG neoactivity, and methods of screening for compounds that inhibit mutant IDH having 2HG neoactivity.

Group II: Claim 40, drawn to a pharmaceutical composition.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the invention listed as Group I is a mutant IDH having 2HG neoactivity. This special technical feature is not shared by the invention listed as Group II. The special technical feature of Group II are the compounds recited therein. This special technical feature is not shared by the invention listed as Group I.

Regarding the treatment methods of Group I, while the compounds of Group II may be used in the treatment methods of Group I, it is readily apparent that the treatment methods of Group I may employ a therapeutic agent not limited by any special technical feature to the compounds of Group II. According to PCT Rule 13.2, unity of invention exists only when the same or corresponding technical feature is shared by all claimed inventions. Thus, the inventions listed at Groups I and II do not relate to a single general inventive concept because they lack the same or corresponding special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

Furthermore, regarding the claims in Group I having candidate compound screening methods, the technical feature of the candidate compound screening methods of Group I resides in the step of observing the ability of the candidate compounds to modulate the 2HG neoactivity of mutant IDH in a screening assay. Neither the same nor a corresponding special technical feature is present in any of the compounds of Group II. No manufacturing relationship exists between the screening method and the claimed compounds. Further, these screening methods are not a method of using the claimed compounds of Group II. In the absence of any teaching as to the structure required for a compound to act as an inhibitor of the 2HG neoactivity of mutant IDH, there is no single general concept that links the screening methods to the claimed compounds.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. With out a shared special technical feature, the inventions of Groups I and II lack unity with one another.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-5, 15-16, 18-22, 29-32 and 35-39

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/27253

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)	Claims	1-5, 15-16, 18-22,29-32, 35-39	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	29-32	YES
	Claims	1-5, 15-16, 18-22, 35-39	NO
Industrial applicability (IA)	Claims	1-5, 15-16, 18-22,29-32, 35-39	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 18-22 lack an inventive step under PCT Article 33(3) as being obvious over the article entitled "IDH1 Mutations Are Early Events in the Development of Astrocytomas and Oligodendrogliomas" by Watanabe et al. (hereinafter "Watanabe").

As to claims 18 and 21, Watanabe discloses a method comprising analyzing the subject or a sample from the subject for b) the presence, distribution, or level of a mutant IDH1 enzyme (pg 1150, col 1, sections "Tumor Samples" and "SSCP Analysis and Direct DNA Sequencing for IDH1 Mutations").
Watanabe discloses analyzing the subject or a sample from the subject not for the purposes of evaluating a subject for the presence or susceptibility to a cancer, as required by the claim, but for the purpose of characterizing samples from patients with cancer (Abstract, "We assessed IDH1 mutations in 321 gliomas of various histological types and biological behaviors"). However, it would have been obvious to one of ordinary skill in the art to modify the procedure described by Watanabe (pg 1150, col 1, sections "Tumor Samples" and "SSCP Analysis and Direct DNA Sequencing for IDH1 Mutations") such as to evaluate a subject or the presence or susceptibility to a cancer.
Furthermore, Watanabe does not disclose that said IDH1mutant enzyme has 2HG neoactivity. However, this limitation is inherently present in Watanabe's disclosure, because 1) Watanabe discloses that an IDH1 mutant which presence correlates with cancer is the Arg132His IDH1 mutant: "IDH1 encodes isocitrate dehydrogenase 1, which participates in the citric acid cycle and was recently reported to be mutated in 12% of glioblastomas. We assessed IDH1 mutations in 321 gliomas of various histological types and biological behaviors. A total of 130 IDH1 mutations was detected, and all were located at amino acid residue 132. Of these, 91% were G to A mutations Arg132His)" (Abstract). This is the same mutant that according to Applicant has said 2HG neoactivity (instant application, pg 5to pg 6, "In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDHI associated with 2HG neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C)". As a structure of a protein determines its function, the functional limitation that said IDH1mutant enzyme has 2HG neoactivity is inherently present in the disclosure of Watanabe.

As to claim 19, Watanabe further discloses that the cancer is an astrocytic tumor (Abstract, "IDH1 mutations were frequent in low-grade diffuse astrocytomas (88%)").

As to claim 20, Watanabe further discloses that the cancer is a glioblastoma (Abstract, "IDH1 mutations were frequent ... in secondary glioblastomas that developed through progression from low-grade diffuse or anaplastic astrocytoma (82%)").

As to claim 22, Watanabe further discloses evaluation of a tissue by DNA sequencing (pg 1150, col 1, "Direct DNA Sequencing for IDH1 Mutations").

Claims 1-5, 15-16 lack an inventive step under PCT Article 33(3) as being obvious over US 2008/0300208 A1 to Paz et al. (hereinafter "Paz") in view of Watanabe, as above.

As to claims 1-2, 5, 15-16, Paz discloses a method of treating a subject having a cell proliferation-related disorder (Abstract, "uses for the IDH gene and/or polypeptide and/or modulators thereof in the diagnosis and treatment of apoptosis-related diseases"), the method comprising administering to the subject in need thereof a therapeutically effective amount of a nucleic acid based inhibitor, which targets mRNA encoding IDH (para [0057], "... a pharmaceutical composition for treating an apoptosis-related disease, such as a cancer, comprising ... (a) an antisense oligonucleotide complementary to the entire or a portion of a DNA molecule encoding said IDH polypeptide, said oligonucleotide being capable of inhibiting the expression of said polypeptide").
Paz does not expressly disclose that the IDH gene encodes a mutant of IDH having 2HG neoactivity and is responsible for the elevated levels of 2HG causing said cell proliferation-related disorder in a subject. However, Paz does disclose that "the IDH gene apparently plays a role in preventing apoptosis, and the inhibition of its expression or neutralization of its expression products promotes cell death. IDH molecules useful in the apoptosis-preventing aspect of the invention may have the nucleic acid sequence of the IDH gene (para [0065]). Furthermore, the disclosure of Paz does encompass inhibiting IDH mutant (para [0022], "IDH gene"-the isocitrate dehydrogenase I coding sequence open reading frame, as shown in FIG. 1 (SEQ ID NO: I), or the Isocitrate dehydrogenase 2 coding sequence open reading frame, as shown in FIG. 2 (SEQ ID NO:3), or any homologous sequence thereof preferably having at least 70% identity, more preferable 80% identity, even more preferably 90% or 95% identity").

---------- SEE SUPPLEMENTAL BOX TO CONTINUE ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/27253

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V(2) Citations and Explanations:

Furthermore, Watanabe expressly provides a motivation to design a nucleic acid inhibitor specific for a mutant form of IDH, because Watanabe discloses that isocitrate dehydrogenase 1 "was recently reported to be mutated in 12% of glioblastomas. We assessed IDH1 mutations in 321 gliomas of various histological types and biological behaviors. A total of 130 IDH1 mutations was detected, and all were located at amino acid residue 132. Of these, 91% were G to A mutations (Arg132His)" (Abstract). Thus, it would have been obvious to one of ordinary skill in the art to combine the teaching of Paz that a subject having a cell proliferation- related disorder (Abstract) may be treated by administering to said subject an antisense oligonucleotide (para [0057]) capable of inhibiting expression of the wild type IDH1 as well as IDH1 mutants (para [0022]) with the teaching of Watanabe that IDH1 is mutated at the position 132 in 91% of examined gliomas samples (Abstract) by designing an antisense oligonucleotide that would preferentially inhibit expression of said mutant, thereby treating said subject having cell proliferation-related disorder.
Neither Paz nor Watanabe discloses that said Arg132His IDH1 mutant has 2HG neoactivity. However, this limitation is inherently present in the disclosure of Watanabe, because this is the same mutant that according to Applicant has said 2HG neoactivity (instant application, pg 5 to pg 6, "In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDHI associated with 2HG neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C)".
Albeit the combination of Paz and Watanabe does not disclose that said Arg132His IDH1 mutant possesses said 2HG neoactivity that is the underlying cause of the cell proliferation-related disorder, inhibiting expression of said Arg132His IDH1 mutant necessarily leads to decreasing of the elevated levels of 2HG in the subject, thereby treating said cell proliferation-related disorder. Therefore, the functional limitation that a mutant IDH has 2HG neoactivity is inherently present in the disclosure of Paz and Watanabe and as such does not have patentable weight.

As to claim 3, the combination of Paz and Watanabe further discloses that the cancer is an astrocytic tumor (Watanabe, Abstract, "IDH1 mutations were frequent in low-grade diffuse astrocytomas (88%)").

As to claim 4, the combination of Paz and Watanabe further discloses that the cancer is a glioblastoma (Watanabe, Abstract, "IDH1 mutations were frequent ... in secondary glioblastomas that developed through progression from low-grade diffuse or anaplastic astrocytoma (82%)").

Claims 35-38 lack an inventive step under PCT Article 33(3) as being obvious over Paz, as above, in view of the article entitled "Metabolic Enzymes as Oncogenes or Tumor Suppressors" by Thompson (hereinafter "Thompson").

As to claim 35, Paz discloses a method of evaluating a candidate compound for the ability to inhibit a neoactivity of a IDH gene (para [0065], "... the IDH gene apparently plays a role in preventing apoptosis, and the inhibition of its expression or neutralization of its expression products promotes cell death"; para [0177], "[t]he IDH gene may be used in a screening assay for identifying and isolating compounds which inhibit or stimulate apoptosis, and in particular, Fas-induced and drug-induced apoptosis"), the method comprising:
contacting the candidate compound with a IDH having a neoactivity; and
evaluating the ability of the candidate compound to modulate the neoactivity, thereby evaluating the candidate compound (para [0084], "[i]n a cell-based embodiment ..., there is provided a process for obtaining a compound which modulates apoptosis in a cell comprising: a) providing cells which express the human IDH polypeptide; b) contacting said cells with said compound; and c) determining the ability of said compound to modulate apoptosis in the cells").
Paz does not expressly disclose that an IDH mutant is used in said screening assay. However, the disclosure of Paz does encompass using an IDH mutant in said analysis: "IDH gene"-the isocitrate dehydrogenase I coding sequence open reading frame, as shown in FIG. 1 (SEQ ID NO: I), or the isocitrate dehydrogenase 2 coding sequence open reading frame, as shown in FIG. 2 (SEQ ID NO:3), or any homologous sequence thereof preferably having at least 70% identity..." (para [0022]).
A motivation to use a mutant form of IDH in a screening assay of Paz is provided by Thompson that discloses that "70% or more of low-grade gliomas bear mutations in one of two NADP+-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)" (pg 813, col 1), and that "in stage II or III astrocytomas or oligodendrogliomas, more than 70% of tumors had mutations in either IDH1, the gene for the form of NADP+-dependent isocitrate dehydrogenase associated with cytosol or peroxisomes, or IDH2, the mitochondria-associated form of the enzyme. On the basis of the analysis of the mutational status of other genes implicated in the pathogenesis of gliomas, these studies provide compelling evidence that IDH1 mutations occur at an early stage in the development of gliomas. These mutations of enzymes that are involved in the metabolism of citrate might share a mechanism that promotes tumorigenesis (Fig. 1)" (pg 813, col 2).
Neither Paz nor Thompson discloses that said IDH mutant has 2HG neoactivity. However, said limitation is inherently present in the disclosure of Paz and Thompson as follows:
Thompson suggests that "[t]he frequency of somatic mutations affecting this single codon in the absence of any other mutation that would cause gene inactivation suggests that the mutations in IDH1 and IDH2 do not result in a simple loss of function. Furthermore, the affected residue in mutated IDH1, arginine 132, appears to contribute to the regulation of IDH1 activity" (pg 814 col 2 to pg 815, col 1) and that "the mutant enzyme might have a gain of function under certain metabolic conditions" (pg 815, col 1).
Furthermore, the R132 IDH1 mutant is the same mutant that Applicant showed has said 2HG neoactivity (instant application, pg 5to pg 6, "In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDHI associated with 2HG neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C)").
It would have been obvious to one of ordinary skill in the art to combine the teaching of Paz disclosing a method of evaluating a candidate compound for the ability to inhibit a neoactivity of a IDH gene (Abstract, para [0065], [0177]) with the teaching of Thompson disclosing that IDH1 is mutated at the position 132 in 70% of examined tumor samples (pg 813, col 2) by screening antisense oligonucleotides that would preferentially inhibit expression of said mutant, thereby evaluating the candidate compound.
Thus, by using the Arg132His IDH1 mutant described by Thompson in a cell-based assay described by Paz (para [0084]), one of ordinary skill in the art would have been able to perform the claimed evaluating of a candidate compound for its ability to inhibit a neoactivity of a mutant IDH. As to the functional limitation regarding 2HG neoactivity, it is inherently present in the disclosure of Paz and Thompson and therefore does not have patentable weight.
---------- SEE SUPPLEMENTAL BOX TO CONTINUE ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/27253

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box No. V(2) Citations and Explanations and the preceding SUPPLEMENTAL BOX:

As to claim 36, Paz discloses a method of evaluating a candidate compound for the ability to inhibit a neoactivity of the translation of an RNA encoding an IDH (para [0156], "A) The Effect of IDH Antisense Fragment on FAS Induced Apoptosis in HeLa Cells was Tested by Loss of Function (LOF) Assays. See FIGS. 7, 8 and 10"; para [0162], " B) The Effect ofIDH siRNA on Doxorubicin Induced Apoptosis in HeLa Cells was Tested by a Loss of Function (LOF) Assay See FIG. 10"), the method comprising:
contacting the candidate compound with a system comprising a cell (para [0163], "HeLa cells were transiently transfected with an IDH siRNA (5' AAATCGTGATGCCACCAACGAC'3); and evaluating the ability of the candidate compound to inhibit the translation of the RNA or inhibit a mutant IDH having 2HG neoactivity, thereby evaluating the candidate compound (para [0163], "48 hours after transfection, cells were treated with doxorubicin (500 ng/ml for 16 h). Apoptosis was measured using an Annexin-V-FITC kit... The results are presented as fold of apoptosis in IDH siRNA transfected cells over apoptosis in LUC transfected cells (control)").
Paz does not expressly disclose that an IDH mutant is used in said screening assay. However, the disclosure of Paz does encompass inhibiting an IDH mutant (para [0022], "IDH gene"-the isocitrate dehydrogenase I coding sequence open reading frame, as shown in FIG. 1 (SEQ ID NO: I), or the isocitrate dehydrogenase 2 coding sequence open reading frame, as shown in FIG. 2 (SEQ ID NO:3), or any homologous sequence thereof preferably having at least 70% identity...").
A motivation to use a mutant form of IDH in a screening assay is provided by Thompson that discloses that " 70% or more of low-grade gliomas bear mutations in one of two NADP+-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)" (pg 813, col 1), and that "in stage II or III astrocytomas or oligodendrogliomas, more than 70% of tumors had mutations in either IDH1, the gene for the form of NADP+ -dependent isocitrate dehydrogenase associated with cytosol or peroxisomes, or IDH2, the mitochondria-associated form of the enzyme. On the basis of the analysis of the mutational status of other genes implicated in the pathogenesis of gliomas, these studies provide compelling evidence that IDH1 mutations occur at an early stage in the development of gliomas. of gliomas. These mutations of enzymes that are involved in the metabolism of citrate might share a mechanism that promotes tumorigenesis (Fig. 1)" (pg 813, col 2).
Although Thompson suggests that "[t]he frequency of somatic mutations affecting this single codon in the absence of any other mutation that would cause gene inactivation suggests that the mutations in IDH1 and IDH2 do not result in a simple loss of function. Furthermore, the affected residue in mutated IDH1, arginine 132, appears to contribute to the regulation of IDH1 activity" (pg 814 col 2 to pg 815, col 1) and that "the mutant enzyme might have a gain of function under certain metabolic conditions" (pg 815, col 1), neither Paz nor Thompson recognizes that said IDH mutant has the 2HG neoactivity. However, this limitation is inherently present in the disclosure of Thompson, because the R132 IDH1 mutant is the same mutant that Applicant showed has said 2HG neoactivity (Instant application, pg 5to pg 6, "In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDHI associated with 2HG neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C)".
Thus, it would have been obvious to one of ordinary skill in the art to combine the teaching of Paz disclosing a method of evaluating a candidate compound for the ability to inhibit a neoactivity of a IDH gene (Abstract, para [0065], [0177]) with the teaching of Thompson disclosing that IDH1 is mutated at the position 132 in 70% of examined tumor samples (pg 813, col 2) by screening antisense oligonucleotides that would preferentially inhibit expression of said mutant, thereby evaluating the candidate compound.
The combination of Paz and Thompson does not disclose that said Arg132His IDH1 mutant possesses said 2HG neoactivity. However, by using the Arg132His IDH1 mutant described by Thompson in a cell-based assay described by Paz (para [0084]), one of ordinary skill in the art would have been able to perform the claimed evaluating of a candidate compound for its ability to inhibit a neoactivity of a mutant IDH. Therefore, the functional limitation that a mutant IDH has 2HG neoactivity does not have patentable weight.

As to claim 37, Thompson further discloses that the mutant IDH is a mutant IDH1 (pg 813, col 1, "70% or more of low-grade gliomas bear mutations in one of two NADP+-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)").

As to claim 38, Thompson further discloses that the mutant IDH is a mutant IDH2 (pg 813, col 1, "70% or more of low-grade gliomas bear mutations in one of two NADP+-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)").

---------- SEE the following SUPPLEMENTAL BOX TO CONTINUE ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/27253

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V(2) Citations and Explanations and the preceding SUPPLEMENTAL BOX:

Claim 39 lacks an inventive step under PCT Article 33(3) as being obvious over US 2006/0281122 A1 to Bryant et al. (hereinafter "Bryant") in view of Thompson, as above.

As to claim 39, Bryant discloses a method of selecting a payment class for treatment of a subject having a cell proliferation related disorder (Bryant, claim 27, "a method of deciding whether to pay for the treatment of cancer...") the method comprising:
providing an evaluation of whether the subject is positive for a predictive marker of cancer, and (1) if the subject is positive, then selecting a first payment class (Bryant, claim 27, "a) obtaining the informative expression level of a predictive marker or a predictive marker set selected from or derived from Table 1A, Table 1B, Table 2A, Table 2B, and Table 3; b) authorizing payment if the informative expression level identifies a responsive patient").
Bryant does not disclose that said method is for selecting a payment class for treatment with an inhibitor of a mutant IDH having 2HG neoactivity, and that said method comprises:
providing an evaluation of whether the subject is positive for increased levels of a mutant IDHI or mutant IDH2 having 2HG neoactivity, and (1) if the subject is positive, then selecting a first payment class.
Thompson discloses that a presence of a mutant IDHI or mutant IDH2 is indicative of the subject having or being predisposed to having a cancer (pg 813, col 1, "70% or more of low-grade gliomas bear mutations in one of two NADP+-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)"; pg 813, col 2, "compelling evidence that IDH1 mutations occur at an early stage in the development of gliomas. These mutations of enzymes that are involved in the metabolism of citrate might share a mechanism that promotes tumorigenesis (Fig. 1)").
Although Thompson suggests that "[t]he frequency of somatic mutations affecting this single codon in the absence of any other mutation that would cause gene inactivation suggests that the mutations in IDH1 and IDH2 do not result in a simple loss of function. Furthermore, the affected residue in mutated IDH1, arginine 132, appears to contribute to the regulation of IDH1 activity" (pg 814 col 2 to pg 815, col 1) and that "the mutant enzyme might have a gain of function under certain metabolic conditions" (pg 815, col 1), Thompson does not disclose that said IDH mutant has the 2HG neoactivity. However, this limitation is inherently present in Thompson, because the R132 IDH1 mutant is the same mutant that Applicant showed has said 2HG neoactivity (instant application, pg 5to pg 6, "In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDHI associated with 2HG neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C)"). Thus, it would have been obvious to one of ordinary skill in the art to combine the teaching of Bryant that discloses a method of selecting a payment class for treatment of a subject having a cell proliferation related disorder (Bryant, claim 27) with the teaching of Thompson that discloses that the R132H IDH1 mutant is a marker of glyomas (pg 813, col 2), thereby achieving cost-efficient treatment of the subject.

Claims 29-32 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed subject matter.

The best prior art on record, Thompson, suggests that "[t]he frequency of somatic mutations affecting this single codon in the absence of any other mutation that would cause gene inactivation suggests that the mutations in IDH1 and IDH2 do not result in a simple loss of function. Furthermore, the affected residue in mutated IDH1, arginine 132, appears to contribute to the regulation of IDH1 activity" (pg 814 col 2 to pg 815, col 1) and that "the mutant enzyme might have a gain of function under certain metabolic conditions" (pg 815, col 1). However, there is no prior art on record that discloses or fairly suggests that said gain of function by the mutant IDH is the ability to produce 2HG.

The earliest art on record, article entitled "Cancer-associated IDH1 mutations produce 2-hydroxyglutarate" by Dang et al., that discloses that said gain of function by the mutant IDH is the ability to produce 2HG (Abstract, "cancer-associated IDH1 mutations result in a new ability of the enzyme to catalyze the NADPH-dependent reduction of a-ketoglutarate to R(2)-2-hydroxyglutarate (2HG)") was published after priority date at issue, and therefore is not prior art.

To summarize, albeit Thompson's suggestion that "the mutant enzyme might have a gain of function under certain metabolic conditions" (pg 815, col 1) would have provided a motivation for one of ordinary skill in the art to search for said new function, it would have required undue experimentation to do so. Therefore, there is no prior art on record that teaches or fairly suggests a method of evaluation a subject for the presence or susceptibility to a cancer by analyzing the subject or a sample from the subject for presence, distribution, or level of 2HG.

Claims 1-5, 15-16, 18-22 and 35-39 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PLUS Search Results for S/N 13256396, Searched Thu Dec 13 09:04:05 EST 2012

The Patent Linguistics Utility System (PLUS) is a USPTO automated search system for U.S. Patents from 1971 to the present PLUS is a query-by-example search system which produces a list of patents that are most closely related linguistically to the application searched. This search was prepared by the staff of the Scientific and Technical Information Center, SIRA.

20120121515 99
20040067234 51
20080300208 51
20120295286 47
6204039 44
20040132145 44
20040214270 44
20080312331 44
20090093030 44
20120202883 43
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20080309265 40
20090042265 40
20100112651 40
6312924 30
6251605 27
6355473 27

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: November 29, 2012
Electronic Signature for Peter Korakas: /Peter Korakas/

Docket No.: C2081-7013US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard L. Dang et al.

Application No.: 13/256,396 | Confirmation No.: 9930

Filed: March 12, 2010 | Art Unit: 1634

For: METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS | Examiner: S. C. Pohnert

RESPONSE TO RESTRICTION REQUIREMENT

In response to the Restriction Requirement mailed October 29, 2012, Applicants elect Group 17, claim(s) 93 (in part) and 96-99, drawn to method of evaluating a subject for presence or susceptibility by 2HG. The above election is made without prejudice to the filing of one or more divisional applications directed to non-elected subject matter.

Applicants makes this election with traverse. Applicants submit that nothing in Balss et al. (Acata Neuropathol 116:597-602 (2008)) teaches or suggests evaluating a subject or selecting a subject based on an IDH1 or IDH2 allele having 2HG neoactivity or a mutant IDH1 enzyme or IDH2 enzyme having 2HG neoactivity as recited in claims 41 and 93. In fact, Balss is silent with regard to 2HG neoactivity. Accordingly, the pending claims do have a special technical feature over the prior art, and therefore should be found to have unity of invention. Applicants submit that groups 1-17 of the restriction requirement should be examined simultaneously.

Conclusion

In view of the foregoing, consideration and favorable action is respectfully requested. Applicants believe this response is timely filed. If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicants hereby request any necessary extension of time. Please apply any charges or deficiencies to Deposit Account No. 50-2762, referencing Attorney Docket No. C2081-7013US.

Dated: November 29, 2012

Respectfully submitted,

Electronic signature: /Peter Korakas/
Peter Korakas
Registration No.: 66,513
LANDO & ANASTASI LLP
Riverfront Office Park
One Main Street
Suite 1100
Cambridge, Massachusetts 02142
(617) 395-7000
Attorney for Applicant

Electronic Acknowledgement Receipt

EFS ID:	14337670
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	37462
Filer:	Peter Korakas
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	29-NOV-2012
Filing Date:	29-NOV-2011
Time Stamp:	18:37:14
Application Type:	U.S. National Stage under 35 USC 371

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 to Election / Restriction Filed	C2081-7013US_Response_to_RR_1.PDF	21592 bbb5a44e6ec940bcc86d9aa7b053621594e211f1	no	2
Warnings:					
Information:					

Total Files Size (in bytes):	21592

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

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 NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/256,396	11/29/2011	Lenny Dang	C2081-7013US	9930

37462 7590 10/29/2012

LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

EXAMINER	
POHNERT, STEVEN C	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
10/29/2012	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):

docketing@LALaw.com
gengelson@LALaw.com

Office Action Summary	Application No.	Applicant(s)	
	Examiner	Art Unit	

-- 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 1 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 September 2011*.

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) *41-99* 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) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *41-99* 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 _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Election/Restrictions

1. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group 1, claim(s) 41(in part)-42(in part) ,57-62 drawn to methods of evaluating a subject based on a mutant protein or RNA.

Group 2, claim(s) 41(in part), 42 (in part) 43, , 54-56 drawn to methods of evaluating subjects based on 2HG.

Group 3, claim(s) 41(in part), 42 (in part) 44-53, , drawn to methods of evaluating a subject based on 2HG in a sample from a subject.

Group 4, claim(s) 41(in part), 57-62, drawn to methods of evaluating a subject by DNA sequencing.

Group 5, claim(s) 41 (in part) and 63, drawn to methods of evaluating a subject comprising diagnosing cancer.

Group 6, claim(s) 41 (in part) and 64, drawn to drawn to methods of evaluating a subject comprising diagnosing precancerous condition.

Group 7, claim(s) 41 (in part) and 65, drawn to drawn to methods of evaluating a subject wherein subject does not have 2-hydroxygluatric aciduria.

Group 8, claim(s) 41 (in part), 66-79, drawn to methods of evaluating a subject wherein subject has IDH1 neoactive mutant.

Group 9, claim(s) 41 (in part) and 80, drawn to method of evaluating a subject wherein the subject has leukemia.

Group 10, claim(s) 41 (in part) and 81, drawn to method of evaluating a subject wherein the subject has AML.

Group 11, claim(s) 41 (in part) and 82, drawn to method of evaluating a subject wherein the subject has myelodisplasia.

Group 12, claim(s) 41 (in part) and 83, drawn to method of evaluating a subject wherein subject has myelodisplastic syndrome.

Group 13, claim(s) 41 (in part) , drawn to method of evaluating a subject comprising recommending treatment.

Group 14, claim(s) 41 (in part) and 84-89, drawn to memorializing a result.

Group 15, claim(s) 41 (in part) and 90-92, drawn to method of evaluating a patient by select a payment class.

Group 16, claim(s) 93 (in part) 94-95, drawn to method of evaluating a subjects for presence or susceptibility of cancer recited in claims 94-95.

Group 17, claim(s) 93 (in part) 96-99, drawn to method of evaluating a subjects for presence or susceptibility by 2HG.

2. The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Balss (Acata Neuropathol (2008) volume 116, pages 597-602) teaches detection in mutations in codon 132 of IDH1. Thus independent claims 41 and 93 lack a special technical feature over the prior art and unity of invention.
3. This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species are as follows:

If applicant elects group1 applicant must elect RNA or protein.

If applicant elects groups 4 or 8 applicant must elect a specific mutation.

I applicant elects group 16 applicant must elect b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of

which has 2HG neoactivity; c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; ord) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; thereby evaluating the subject for such cancer.

Additionally if applicant elects a group 16 applicant must further elect a specific cancer.

Applicant is required, in reply to this action, to elect a single species to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise require all the limitations of an allowed generic claim.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention or species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the

restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention or species.

Should applicant traverse on the ground that the inventions have unity of invention (37 CFR 1.475(a)), applicant must provide reasons in support thereof. Applicant may submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. Where such evidence or admission is provided by applicant, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone

number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. 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.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

Notice of References Cited	Application/Control No. 13/256,396	Applicant(s)/Patent Under Reexamination DANG ET AL.	
	Examiner STEVEN POHNERT	Art Unit 1634	Page 1 of 1

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-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		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	Balss (Acata Neuropathol (2008) volume 116, pages 597-602)
	V	
	W	
	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.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/256,396	11/29/2011	Lenny Dang	C2081-7013US

CONFIRMATION NO. 9930

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

PUBLICATION NOTICE

Title:METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

Publication No.US-2012-0121515-A1
Publication Date:05/17/2012

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

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/256,396	Lenny Dang	C2081-7013US

37462
LANDO & ANASTASI, LLP
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CAMBRIDGE, MA 02142

INTERNATIONAL APPLICATION NO.	
PCT/US10/27253	
I.A. FILING DATE	PRIORITY DATE
03/12/2010	03/13/2009

CONFIRMATION NO. 9930
371 ACCEPTANCE LETTER

OC000000052482575

Date Mailed: 02/10/2012

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

11/29/2011	11/29/2011
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Copy of the International Application filed on 09/13/2011
- Copy of the International Search Report filed on 09/13/2011
- Preliminary Amendments filed on 09/13/2011
- Biochemical Sequence Diskette filed on 11/29/2011
- Oath or Declaration filed on 11/29/2011
- Biochemical Sequence Listing filed on 11/29/2011
- U.S. Basic National Fees filed on 09/13/2011
- Priority Documents filed on 09/13/2011
- Specification filed on 09/13/2011
- Claims filed on 09/13/2011
- Abstracts filed on 09/13/2011
- Drawings filed on 09/13/2011

FORM PCT/DO/EO/903 (371 Acceptance Notice)

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

RODERICK M JONES

Telephone: (571) 272-9083

FORM PCT/DO/EO/903 (371 Acceptance Notice)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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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
13/256,396	11/29/2011		3618	C2081-7013US	59	2

CONFIRMATION NO. 9930

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

FILING RECEIPT

OC000000052482574

Date Mailed: 02/10/2012

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)

Lenny Dang, Boston, MA;
Valeria Fantin, La Jolla, CA;
Stefan Gross, Brookline, MA;
Hyun Gyung Jang, Arlington, MA;
Shengfang Jin, Newton, MA;
Francesco G. Salituro, Marlborough, MA;
Jeffrey O. Saunders, Concord, MA;
Shinsan Su, Newton, MA;
Katharine Yen, Wellesley, MA;

Power of Attorney: The patent practitioners associated with Customer Number 37462

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US10/27253 03/12/2010
which claims benefit of 61/160,253 03/13/2009
and claims benefit of 61/160,664 03/16/2009
and claims benefit of 61/173,518 04/28/2009
and claims benefit of 61/180,609 05/22/2009
and claims benefit of 61/220,543 06/25/2009
and claims benefit of 61/227,649 07/22/2009
and claims benefit of 61/229,689 07/29/2009
and claims benefit of 61/253,820 10/21/2009
and claims benefit of 61/266,929 12/04/2009

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 02/08/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/256,396**

Projected Publication Date: 05/17/2012

Non-Publication Request: No

Early Publication Request: No
Title

METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

Preliminary Class

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.

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/256,396

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	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	380
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	120
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	250
TOTAL CLAIMS (37 CFR 1.16(i))	59 minus 20 =	* 39			OR	x 60 =	2340
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 250 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 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).						930
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	4020

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))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	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 found in the appropriate box in column 1.

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217-9197 (toll free).
Reviewer: Anne Corrigan
Timestamp: [year=2012; month=2; day=7; hr=12; min=32; sec=5; ms=746; ]
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Docket No.: C2081-7013US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Lenny Dang et al.
Serial No:	13/256,396
Confirmation No:	9930
Filed:	September 13, 2011
Title:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATED-RELATED DISORDERS

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with §1.6(a)(4), on the 29th day of November, 2011.

/Peter Korakas/
Peter Korakas, Reg. No. 66,513

Commissioner for Patents

PRELIMINARY AMENDMENT AND RESPONSE TO THE NOTIFICATION OF MISSING REQUIREMENTS DATED SEPTEMBER 29, 2011

Prior to examination, please amend the above-identified application as follows:

Amendments to the Specification

Please insert the electronic and paper copy of the sequence listing submitted herewith into the application, following the drawings.

REMARKS

Oath or Declaration: The Notification of Missing Requirements mailed September 29, 2011, indicates that the oath or declaration is missing. Applicant submits herewith a properly signed declaration in compliance with 37 C.F.R §1.63.

Fees: With this reply, Applicant is paying the surcharge of $130 by way of deposit account authorization.

Sequence Listing: The Notification also requires a Sequence Listing in computer readable form. Applicant submits herewith a Sequence Listing in computer readable form; a statement to fulfill the requirements of 37 C.F.R. §§ 1.821-1.825; and requests that the paper copy be entered into the application by way of amendment.

Applicants state that the content of the sequence listing information recorded in the computer readable form is identical to the paper copy of the sequence listing. No new matter has been added. The amendments to the specification insert the Sequence Listing into the application.

It is understood that this perfects the application and no additional papers or filing fees are required. Please apply any other charges or credits to Deposit Account No. 50-2762, referencing Attorney Docket No. C2081-7013US.

Respectfully submitted,

/Peter Korakas/

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Catherine M. McCarty, Reg. No. 54,301
LANDO & ANASTASI, LLP
One Main Street
Cambridge, Massachusetts 02142
United States of America
Telephone: 617-395-7000
Facsimile: 617-395-7070

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the application entitled

METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

the specification of which is attached hereto unless the following is checked:

[X] was filed on **September 13 2011**, as Application No.**13/256,396** , bearing attorney docket **No. C2081-7013US**.

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, and amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign or PCT International Application(s) and any priority claims under 35 U.S.C. §119(a)-(d) and §365(a),(b):

			Priority Claimed
PCT/US2010/027253	PCT	12-03-2010	[X] [ ]
(Number)	(Country-if PCT, so indicate)	(DD/MM/YY Filed)	YES NO

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

61/266,929 (Application Number)	2009-12-04 (filing date)
61/253,820 (Application Number)	2009-10-21 (filing date)
61/229,689 (Application Number)	2009-07-29 (filing date)
61/227,649 (Application Number)	2009-07-22 (filing date)
61/220,543 (Application Number)	2009-06-25 (filing date)
61/180,609 (Application Number)	2009-05-22 (filing date)
61/173,518 (Application Number)	2009-04-28 (filing date)
61/160,664 (Application Number)	2009-03-16 (filing date)
61/160,253 (Application Number)	2009-03-13 (filing date)

I hereby appoint the following Registered Practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All Registered Practitioners of Lando & Anastasi, LLP associated with Customer Number 37462.

Address all telephone calls to Catherine M. McCarty at telephone no. (617) 395-7000.

Address all correspondence to: the Correspondence Address associated with Customer Number 37462.

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 application or any patent issued thereon.

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Inventor's signature 27 oct 11 Date

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Post Office Address: Same

Inventor's signature 21 oct 11 Date

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Inventor's signature 10/31/2011 Date

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Inventor's signature **Date**

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Jeffrey O Saunders 27. Oct. 2011

Inventor's signature **Date**

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Post Office Address: Same

Shinsan Su 10/28/2011

Inventor's signature **Date**

Full name of first or joint inventor: Shinsan Su
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Post Office Address: Same

10/31/11

Inventor's signature **Date**

Full name of first or joint inventor: Katharine Yen

Citizenship: US

Residence: ~~6 Shirley Road Wellesley, MA 02482~~ 7 Norwich Rd Wellesley, MA 02481

Post Office Address: ~~Same~~ KEY

SEQUENCE LISTING

<110> Dang, Lenny
Fantin, Valeria
Gross, Stefan
Jang Gyung, Hyun
Jin, Shengfang
Salituro G., Francesco
Saunders O., Jeffrey
Su, Shinsan
Yen, Katherine

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ggaggcttca tctgggcctg taaaaactat gatggtgacg tgcagtcgga ctctgtggcc 1080

caagggtatg gctctctcgg catgatgacc agcgtgctgg tttgtccaga tggcaagaca 1140

gtagaagcag aggctgccca cgggactgta acccgtcact accgcatgta ccagaaagga 1200

caggagacgt ccaccaatcc cattgcttcc atttttgcct ggaccagagg gttagcccac 1260

agagcaaagc ttgataacaa taaagagctt gccttctttg caaatgcttt ggaagaagtc 1320

tctattgaga caattgaggc tggcttcatg accaaggact tggctgcttg cattaaaggt 1380

ttacccaatg tgcaacgttc tgactacttg aatacatttg agttcatgga taaacttgga 1440

gaaaacttga agatcaaact agctcaggcc aaactttaag ttcatacctg agctaagaag 1500

gataattgtc ttttggtaac taggtctaca ggtttacatt tttctgtgtt acactcaagg 1560

ataaaggcaa aatcaatttt gtaatttgtt tagaagccag agtttatctt ttctataagt 1620

ttacagcctt tttcttatat atacagttat tgccaccttt gtgaacatgg caagggactt 1680

ttttacaatt tttattttat tttctagtac cagcctagga attcggttag tactcatttg 1740

tattcactgt cacttttttct catgttctaa ttataaatga ccaaaatcaa gattgctcaa 1800

aagggtaaat gatagccaca gtattgctcc ctaaaatatg cataaagtag aaattcactg 1860

ccttcccctc ctgtccatga ccttgggcac agggaagttc tggtgtcata gatatcccgt 1920

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tttgtcctct ccatttttct ctttctcctc ctggcctggc ctgaatatta tactactcta 2160

aatagcatat ttcatccaag tgcaataatg taagctgaat ctttttgga cttctgctgg 2220

cctgttttat ttcttttata taaatgtgat ttctcagaaa ttgatattaa acactatctt 2280

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<212> PRT
<213> Homo sapiens

<400> 10

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Ser Arg Pro Ala Trp Ala Pro Ala Ala Leu Thr Ala Pro Thr Ser Gln
            20                  25                  30

Glu Gln Pro Arg Arg His Tyr Ala Asp Lys Arg Ile Lys Val Ala Lys
        35                  40                  45

Pro Val Val Glu Met Asp Gly Asp Glu Met Thr Arg Ile Ile Trp Gln
    50                  55                  60

Phe Ile Lys Glu Lys Leu Ile Leu Pro His Val Asp Ile Gln Leu Lys
65                  70                  75                  80

Tyr Phe Asp Leu Gly Leu Pro Asn Arg Asp Gln Thr Asp Asp Gln Val
                85                  90                  95

Thr Ile Asp Ser Ala Leu Ala Thr Gln Lys Tyr Ser Val Ala Val Lys
            100                 105                 110

Cys Ala Thr Ile Thr Pro Asp Glu Ala Arg Val Glu Glu Phe Lys Leu
        115                 120                 125

Lys Lys Met Trp Lys Ser Pro Asn Gly Thr Ile Arg Asn Ile Leu Gly
    130                 135                 140

Gly Thr Val Phe Arg Glu Pro Ile Ile Cys Lys Asn Ile Pro Arg Leu
145                 150                 155                 160
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Val Pro Gly Trp Thr Lys Pro Ile Thr Ile Gly Arg His Ala His Gly
                165                 170                 175

Asp Gln Tyr Lys Ala Thr Asp Phe Val Ala Asp Arg Ala Gly Thr Phe
            180                 185                 190

Lys Met Val Phe Thr Pro Lys Asp Gly Ser Gly Val Lys Glu Trp Glu
        195                 200                 205

Val Tyr Asn Phe Pro Ala Gly Gly Val Gly Met Gly Met Tyr Asn Thr
    210                 215                 220

Asp Glu Ser Ile Ser Gly Phe Ala His Ser Cys Phe Gln Tyr Ala Ile
225                 230                 235                 240

Gln Lys Lys Trp Pro Leu Tyr Met Ser Thr Lys Asn Thr Ile Leu Lys
                245                 250                 255

Ala Tyr Asp Gly Arg Phe Lys Asp Ile Phe Gln Glu Ile Phe Asp Lys
            260                 265                 270

His Tyr Lys Thr Asp Phe Asp Lys Asn Lys Ile Trp Tyr Glu His Arg
        275                 280                 285

Leu Ile Asp Asp Met Val Ala Gln Val Leu Lys Ser Ser Gly Gly Phe
    290                 295                 300

Val Trp Ala Cys Lys Asn Tyr Asp Gly Asp Val Gln Ser Asp Ile Leu
305                 310                 315                 320

Ala Gln Gly Phe Gly Ser Leu Gly Leu Met Thr Ser Val Leu Val Cys
                325                 330                 335

Pro Asp Gly Lys Thr Ile Glu Ala Glu Ala Ala His Gly Thr Val Thr
            340                 345                 350

Arg His Tyr Arg Glu His Gln Lys Gly Arg Pro Thr Ser Thr Asn Pro
        355                 360                 365

Ile Ala Ser Ile Phe Ala Trp Thr Arg Gly Leu Glu His Arg Gly Lys
    370                 375                 380

Leu Asp Gly Asn Gln Asp Leu Ile Arg Phe Ala Gln Met Leu Glu Lys
385                 390                 395                 400

Val Cys Val Glu Thr Val Glu Ser Gly Ala Met Thr Lys Asp Leu Ala
                405                 410                 415
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Gly Cys Ile His Gly Leu Ser Asn Val Lys Leu Asn Glu His Phe Leu
420 425 430

Asn Thr Thr Asp Phe Leu Asp Thr Ile Lys Ser Asn Leu Asp Arg Ala
435 440 445

Leu Gly Arg Gln
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<212> DNA
<213> Homo sapiens

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gacaaaagga tcaaggtggc gaagcccgtg gtggagatgg atggtgatga gatgacccgt 180

attatctggc agttcatcaa ggagaagctc atcctgcccc acgtggacat ccagctaaag 240

tattttgacc tcgggctccc aaaccgtgac cagactgatg accaggtcac cattgactct 300

gcactggcca cccagaagta cagtgtggct gtcaagtgtg ccaccatcac ccctgatgag 360

gcccgtgtgg aagagttcaa gctgaagaag atgtggaaaa gtcccaatgg aactatccgg 420

aacatcctgg gggggactgt cttccgggag cccatcatct gcaaaaacat cccacgccta 480

gtccctggct ggaccaagcc catcaccatt ggcaggcacg cccatggcga ccagtacaag 540

gccacagact ttgtggcaga ccgggccggc actttcaaaa tggtcttcac cccaaaagat 600

ggcagtggtg tcaaggagtg ggaagtgtac aacttccccg caggcggcgt gggcatgggc 660

atgtacaaca ccgacgagtc catctcaggt tttgcgcaca gctgcttcca gtatgccatc 720

cagaagaaat ggccgctgta catgagcacc aagaacacca tactgaaagc ctacgatggg 780

cgtttcaagg acatcttcca ggagatcttt gacaagcact ataagaccga cttcgacaag 840

aataagatct ggtatgagca ccggctcatt gatgacatgg tggctcaggt cctcaagtct 900

tcgggtggct ttgtgtgggc ctgcaagaac tatgacggag atgtgcagtc agacatcctg 960

gcccagggct ttggctccct tggcctgatg acgtccgtcc tggtctgccc tgatgggaag 1020

acgattgagg ctgaggccgc tcatgggacc gtcacccgcc actatcggga gcaccagaag 1080

ggccggccca ccagcaccaa ccccatcgcc agcatctttg cctggacacg tggcctggag 1140

caccgggga agctggatgg gaaccaagac ctcatcaggt ttgcccagat gctggagaag 1200

gtgtgcgtgg agacggtgga gagtggagcc atgaccaagg acctggcggg ctgcattcac 1260

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atcaagagca acctggacag agccctgggc aggcagtag 1359

<210> 12
<211> 1740
<212> DNA
<213> Homo sapiens

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gcagagcctc aggctcgcgg ccggcctggg cgccggcggc cctgacagcc cccacctcgc 180

aagagcagcc gcggcgccac tatgccgaca aaaggatcaa ggtggcgaag cccgtggtgg 240

agatggatgg tgatgagatg acccgtatta tctggcagtt catcaaggag aagctcatcc 300

tgccccacgt ggacatccag ctaaagtatt ttgacctcgg gctcccaaac cgtgaccaga 360

ctgatgacca ggtcaccatt gactctgcac tggccaccca gaagtacagt gtggctgtca 420

agtgtgccac catcacccct gatgaggccc gtgtggaaga gttcaagctg aagaagatgt 480

ggaaaagtcc caatggaact atccggaaca tcctgggggg gactgtcttc cgggagccca 540

tcatctgcaa aaacatccca cgcctagtcc ctggctggac caagcccatc accattggca 600

ggcacgccca tggcgaccag tacaaggcca cagactttgt ggcagaccgg gccggcactt 660

tcaaaatggt cttcacccca aaagatggca gtggtgtcaa ggagtgggaa gtgtacaact 720

tccccgcagg cggcgtgggc atgggcatgt acaacaccga cgagtccatc tcaggttttg 780

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acaccatact gaaagcctac gatgggcgtt tcaaggacat cttccaggag atctttgaca 900

agcactataa gaccgacttc gacaagaata agatctggta tgagcaccgg ctcattgatg 960

acatggtggc tcaggtcctc aagtcttcgg gtggctttgt gtgggcctgc aagaactatg 1020

acggagatgt gcagtcagac atcctggccc agggctttgg ctcccttggc ctgatgacgt 1080

ccgtcctggt ctgccctgat gggaagacga ttgaggctga ggccgctcat gggaccgtca 1140

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tcaggtttgc ccagatgctg gagaaggtgt gcgtggagac ggtggagagt ggagccatga 1320

ccaaggacct ggcgggctgc attcacggcc tcagcaatgt gaagctgaac gagcacttcc 1380

tgaacaccac ggacttcctc gacaccatca agagcaacct ggacagagcc ctgggcaggc 1440

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tcctgagcgc ggcagagggt gagcctcaca gcccctctct ggaggccttt ctaggggatg 1560

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ggcaggagca gtgcgtttta cctcagccag tcagtatgtt ttgcatactg taatttatat 1680

tgcccttgga acacatggtg ccatatttag ctactaaaaa gctcttcaca aaaaaaaaaa 1740

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<212> PRT
<213> Homo sapiens

<400> 13

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Glu Met Thr Arg Ile Ile Trp Glu Leu Ile Lys Glu Lys Leu Ile Phe
            20                  25                  30

Pro Tyr Val Glu Leu Asp Leu His Ser Tyr Asp Leu Gly Ile Glu Asn
        35                  40                  45

Arg Asp Ala Thr Asn Asp Gln Val Thr Lys Asp Ala Ala Glu Ala Ile
    50                  55                  60

Lys Lys His Asn Val Gly Val Lys Cys Ala Thr Ile Thr Pro Asp Glu
65                  70                  75                  80

Lys Arg Val Glu Glu Phe Lys Leu Lys Gln Met Trp Lys Ser Pro Asn
                85                  90                  95

Gly Thr Ile Arg Asn Ile Leu Gly Gly Thr Val Phe Arg Glu Ala Ile
            100                 105                 110

Ile Cys Lys Asn Ile Pro Arg Leu Val Ser Gly Trp Val Lys Pro Ile
        115                 120                 125

Ile Ile Gly Arg His Ala Tyr Gly Asp Gln Tyr Arg Ala Thr Asp Phe
    130                 135                 140

Val Val Pro Gly Pro Gly Lys Val Glu Ile Thr Tyr Thr Pro Ser Asp
145                 150                 155                 160

Gly Thr Gln Lys Val Thr Tyr Leu Val His Asn Phe Glu Glu Gly Gly
                165                 170                 175

Gly Val Ala Met Gly Met Tyr Asn Gln Asp Lys Ser Ile Glu Asp Phe
            180                 185                 190

Ala His Ser Ser Phe Gln Met Ala Leu Ser Lys Gly Trp Pro Leu Tyr
        195                 200                 205
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Leu Ser Thr Lys Asn Thr Ile Leu Lys Lys Tyr Asp Gly Arg Phe Lys
210 215 220

Asp Ile Phe Gln Glu Ile Tyr Asp Lys Gln Tyr Lys Ser Gln Phe Glu
225 230 235 240

Ala Gln Lys Ile Trp Tyr Glu His Arg Leu Ile Asp Asp Met Val Ala
245 250 255

Gln Ala Met Lys Ser Glu Gly Gly Phe Ile Trp Ala Cys Lys Asn Tyr
260 265 270

Asp Gly Asp Val Gln Ser Asp Ser Val Ala Gln Gly Tyr Gly Ser Leu
275 280 285

Gly Met Met Thr Ser Val Leu Val Cys Pro Asp Gly Lys Thr Val Glu
290 295 300

Ala Glu Ala Ala His Gly Thr Val Thr Arg His Tyr Arg Met Tyr Gln
305 310 315 320

Lys Gly Gln Glu Thr Ser Thr Asn Pro Ile Ala Ser Ile Phe Ala Trp
325 330 335

Thr Arg Gly Leu Ala His Arg Ala Lys Leu Asp Asn Asn Lys Glu Leu
340 345 350

Ala Phe Phe Ala Asn Ala Leu Glu Glu Val Ser Ile Glu Thr Ile Glu
355 360 365

Ala Gly Phe Met Thr Lys Asp Leu Ala Ala Cys Ile Lys Gly Leu Pro
370 375 380

Asn Val Gln Arg Ser Asp Tyr Leu Asn Thr Phe Glu Phe Met Asp Lys
385 390 395 400

Leu Gly Glu Asn Leu Lys Ile Lys Leu Ala Gln Ala Lys Leu
405 410

<210> 14
<211> 19
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<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 14
gguuucugca gagucuacu 19

<210> 15
<211> 19
<212> RNA
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<220>
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<400> 15
aguagacucu gcagaaacc 19

<210> 16
<211> 19
<212> RNA
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<220>
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<400> 16
cucuucgcca gcauaucau 19

<210> 17
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<220>
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<400> 17
augauaugcu ggcgaagag 19

<210> 18
<211> 19
<212> RNA
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ggcaggcgau aaacuacau 19

<210> 19
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<220>
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<400> 19
auguaguuua ucgccugcc 19

<210> 20
<211> 19
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<220>
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<400> 20
gcgauaaacu acauucagu 19

<210> 21
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<220>
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<400> 21
acugaaugua guuuaucgc 19

<210> 22
<211> 19
<212> RNA
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<220>
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<400> 22
gaaaucuauu cacugucaa 19

<210> 23
<211> 19
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<220>
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<400> 23
uugacaguga auagauuuc 19

<210> 24
<211> 19
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<220>
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oligonucleotide

<400> 24
guucuguggu agagaugca 19

<210> 25
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<400> 25
ugcaucucua ccacagaac 19

<210> 26
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<400> 26
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<210> 27
<211> 19
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<400> 27
ugucauuuca ucuccuugc 19

<210> 28
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<400> 28
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<210> 29
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<210> 30
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<400> 30
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<210> 31
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auucguguca uuucaucuc 19

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gaugaaauga cacgaauca 19

<210> 33
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<400> 33
ugauucgugu cauuucauc 19

<210> 34
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<210> 35
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<400> 35
ucaauuccca aaugauucg 19

<210> 36
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<400> 36
gggaauugau uaaagagaa 19

<210> 37
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<400> 37
uucucuuuaa ucaauuccc 19

<210> 38
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<220>
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<400> 38
ccuacgugga auuggaucu 19

<210> 39

<211> 19
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<210> 40
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<400> 40
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<400> 44
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<210> 45
<211> 19
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<210> 46
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<400> 46
ggaugcugca gaagcuaua 19

<210> 47
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<400> 47
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<210> 48
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<400> 48

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<210> 50
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<400> 50
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<210> 51
<211> 19
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<400> 51
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<210> 52
<211> 19
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gcauaauguu ggcgucaaa 19

<210> 53
<211> 19
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<400> 53
uuugacgcca acauuaugc 19

<210> 54
<211> 19
<212> RNA
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<400> 54
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<210> 55
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<210> 56
<211> 19
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oligonucleotide

<400> 56
guugaggagu ucaaguuga 19

<210> 57
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<400> 57
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<210> 58
<211> 19
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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 58
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<210> 59
<211> 19
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<400> 59
uuuguuucaa cuugaacuc 19

<210> 60
<211> 19
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<400> 60
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<210> 61
<211> 19
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<400> 61
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<210> 62
<211> 19
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caaaugugga aaucaccaa 19

<210> 63
<211> 19
<212> RNA
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<400> 63
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<210> 64
<211> 19
<212> RNA
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<210> 65
<211> 19
<212> RNA
<213> Artificial Sequence

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<210> 66
<211> 19
<212> RNA
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<400> 66
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<210> 67
<211> 19
<212> RNA
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oligonucleotide

<400> 67
acccagaaua uuucguaug 19

<210> 68
<211> 19

<212> RNA
<213> Artificial Sequence

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oligonucleotide

<400> 68
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ccaccaacga ccaagtcacc aaggatgctg cagaagctat aaagaagcat aatgttggcg      180

tcaaatgtgc cactatcact cctgatgaga agagggttga ggagttcaag ttgaaacaaa      240

tgtggaaatc accaaatggc accatacgaa atattctggg tggcacggtc ttcagagaag      300

ccattatctg caaaaatatc ccccggcttg tgagtggatg ggtaaaacct atcatcatag      360

gtcgtcatgc ttatggggat caagtaagtc atgttggcaa taatgtgatt ttgcatgbtg      420

gcccagaaat ttccaacttg tatgtgtttt attcttatct tttggtatct acacccatta      480

agcaaggta                                                              489
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Electronic Patent Application Fee Transmittal

Application Number:	13256396
Filing Date:	
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Filer:	Peter Korakas/Kristi Travers
Attorney Docket Number:	C2081-7013US

Filed as Large Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Oath/decl > 30 months from priority date	1617	1	130	130
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**130**

Electronic Acknowledgement Receipt	
EFS ID:	11497746
Application Number:	13256396
International Application Number:	
Confirmation Number:	9930
Title of Invention:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS
First Named Inventor/Applicant Name:	Lenny Dang
Customer Number:	94970
Filer:	Peter Korakas
Filer Authorized By:	
Attorney Docket Number:	C2081-7013US
Receipt Date:	29-NOV-2011
Filing Date:	
Time Stamp:	16:26:30
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$130
RAM confirmation Number	3335
Deposit Account	502762
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. 1.492 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)	
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	Applicant Response to Pre-Exam Formalities Notice	Amendment.pdf	27072 88fa3e78e914b5eba7a4631efd0b185a10b380cd	no	3
Warnings:					
Information:					
2	Oath or Declaration filed	Declaration.pdf	1192793 cc913283c62bb29df4ed8d314de4cda793ae4f06	no	5
Warnings:					
Information:					
3	Sequence Listing	Sequence_listing.pdf	146089 49a9a5359ce9c75e864426d41a81c81c25291b90	no	176
Warnings:					
Information:					
4	Computer Listing (text file)	1189804_1.TXT	211410	no	0
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30057 5573ce7d45745d4fb4a4873a565f0a0ad9841edb	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1607421		

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.

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 13256396 Document Date: 11/29/2011

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Sequence Listing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

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

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/256,396	Lenny Dang	C2081-7013US

94970
LANDO & ANASTASI, LLP
C2081
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

INTERNATIONAL APPLICATION NO.	
PCT/US10/27253	
I.A. FILING DATE	PRIORITY DATE
03/12/2010	03/13/2009

CONFIRMATION NO. 9930
371 FORMALITIES LETTER

Date Mailed: 09/29/2011

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated Office (37 CFR 1.494):

- Priority Document
- Copy of the International Application filed on 09/13/2011
- Copy of the International Search Report filed on 09/13/2011
- Preliminary Amendments filed on 09/13/2011
- U.S. Basic National Fees filed on 09/13/2011
- Priority Documents filed on 09/13/2011
- Specification filed on 09/13/2011
- Claims filed on 09/13/2011
- Abstracts filed on 09/13/2011
- Drawings filed on 09/13/2011

The applicant needs to satisfy supplemental fees problems indicated below.

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.
- To avoid abandonment, a surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.492(h) of $130 for a non-small entity, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fees required for this application is $**130** for a Large Entity:

- $**130** Surcharge.
- This application clearly fails to comply with the requirements of 37 CFR. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", **as**

FORM PCT/DO/EO/905 (371 Formalities Notice)

well as an amendment specifically directing its entry into the application. Applicant must also provide a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

- A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

For questions regarding compliance to 37 CFR 1.821-1.825 requirements, please contact:

- **For Rules Interpretation, call (571) 272-0623**
- **For Patentin Software Program Help, call Patent EBC at 1-866-217-9197 or directly at 703-305-3028 / 703-308-6845 between the hours of 6 a.m. and 12 midnight, Monday through Friday, EST.**
- **Send e-mail correspondence for Patentin Software Program Help @ ebc@uspto.gov**

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

RODERICK M JONES

Telephone: (571) 272-9083

FORM PCT/DO/EO/905 (371 Formalities Notice)

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/256,396

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	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	380
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	120
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	250
TOTAL CLAIMS (37 CFR 1.16(i))	59 minus 20 =	* 39			OR	x 60 =	2340
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 250 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 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).						930
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	4020

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))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	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 found in the appropriate box in column 1.

PTO-1390 (Rev. 09-08)
Approved for use through 3/31/2010. OMB 0651-0021
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
C2081-7013US	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US2010/027253	12 March 2010 (12.03.2010)	13 March 2009 (13.03.2009)

TITLE OF INVENTION
METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

APPLICANT(S) FOR DO/EO/US
DANG, Lenny; FANTIN, Valeria; GROSS, Stefan; JANG, Hyun Gyung; JIN, Shengfang; SALITURO, Francesco G....

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☑ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☑ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A preliminary amendment.
14. ☑ An Application Data Sheet under 37 CFR 1.76.
15. ☐ A substitute specification.
16. ☐ A power of attorney and/or change of address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821- 1.825.
18. ☐ A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/US2010/027253	C2081-7013US

20. Other items or information:

The following fees have been submitted	CALCULATIONS	PTO USE ONLY
21. ☑ Basic national fee (37 CFR 1.492(a)) **$330**	$ 330	
22. ☑ Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** All other situations **$220**	$ 220	
23. ☑ Search fee (37 CFR 1.492(b)) If the written opinion of the ISA/US or the International preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority ..... **$100** International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB ..... **$430** All other situations **$540**	$ 100	
TOTAL OF 21, 22 and 23 =	650	
☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). The fee is **$270** for each additional 50 sheets of paper or fraction thereof.		

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round **up** to a whole number)	RATE	
249 - 100 =	149 /50 =	3	x $**270**	$ 810

Surcharge of **$130.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	59 - 20 =	39	x $ **52**	$ 2028	
Independent claims	2 - 3 =	0	x **$220**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$390**	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 3488	
☐ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by ½.					
			SUBTOTAL =	$ 3488	
Processing fee of **$130.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$ 0	
			TOTAL NATIONAL FEE =	$ 3488	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0	
			TOTAL FEES ENCLOSED =	$ 3488	
			Amount to be refunded:	$	
			Amount to be charged	$	

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 502762 in the amount of $ 3488 to cover the above fees.

c. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. _____.

d. ☐ Fees are to be charged to a credit card. **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. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended paying fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer No. 37462

/Peter Korakas/
SIGNATURE

Peter Korakas
NAME

66,513
REGISTRATION NUMBER

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.

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Applicant Information:

Applicant 1 Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Lenny		Dang	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Boston	State/Province	MA	Country of Residence [i]	US

Citizenship under 37 CFR 1.41(b) [i]	US

Mailing Address of Applicant:

Address 1	30 Union Park Street, #201		
Address 2			
City	Boston	State/Province	MA
Postal Code	02118	Country [i]	US

Applicant 2 Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Valeria		Fantin	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Cambridge	State/Province	MA	Country of Residence [i]	US

Citizenship under 37 CFR 1.41(b) [i]	AR

Mailing Address of Applicant:

Address 1	195 Binney Street , Apt. 4515		
Address 2			
City	Cambridge	State/Province	MA
Postal Code	02142	Country [i]	US

Applicant 3 Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Stefan		Gross	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brookline	State/Province	MA	Country of Residence [i]	US

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

Citizenship under 37 CFR 1.41(b) i	US				
Mailing Address of Applicant:					
Address 1	14 Park Street #1				
Address 2					
City	Brookline	**State/Province**	MA		
Postal Code	02446	**Country**i	US		

Applicant 4 Remove

Applicant Authority ◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118		
Prefix	**Given Name**	**Middle Name**	**Family Name**	**Suffix**
	Hyun Gyung		Jang	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Arlington	**State/Province**	MA	**Country of Residence** i	US
Citizenship under 37 CFR 1.41(b) i	KR				
Mailing Address of Applicant:					
Address 1	6 William Street				
Address 2					
City	Arlington	**State/Province**	MA		
Postal Code	02476	**Country**i	US		

Applicant 5 Remove

Applicant Authority ◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118		
Prefix	**Given Name**	**Middle Name**	**Family Name**	**Suffix**
	Shengfang		Jin	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Newton	**State/Province**	MA	**Country of Residence** i	US
Citizenship under 37 CFR 1.41(b) i	US				
Mailing Address of Applicant:					
Address 1	6 Audubon Drive				
Address 2					
City	Newton	**State/Province**	MA		
Postal Code	02467	**Country**i	US		

Applicant 6 Remove

Applicant Authority ◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118		
Prefix	**Given Name**	**Middle Name**	**Family Name**	**Suffix**
	Francesco	G.	Salituro	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Marlborough	**State/Province**	MA	**Country of Residence** i	US
Citizenship under 37 CFR 1.41(b) i	US				

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

Mailing Address of Applicant:			
Address 1	25 Baker Drive		
Address 2			
City	Marlborough	State/Province	MA
Postal Code	01752	Country[i]	US

Applicant 7 — Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jeffrey	O.	Saunders	

Residence Information (Select One)	◉ US Residency	○ Non US Residency	○ Active US Military Service		
City	Concord	State/Province	MA	Country of Residence [i]	US
Citizenship under 37 CFR 1.41(b) [i]	US				

Mailing Address of Applicant:			
Address 1	117 Seymour Street		
Address 2			
City	Concord	State/Province	MA
Postal Code	01742	Country[i]	US

Applicant 8 — Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Shinsan		Su	

Residence Information (Select One)	◉ US Residency	○ Non US Residency	○ Active US Military Service		
City	Newton	State/Province	MA	Country of Residence [i]	US
Citizenship under 37 CFR 1.41(b) [i]	US				

Mailing Address of Applicant:			
Address 1	346 Hartman Road		
Address 2			
City	Newton	State/Province	MA
Postal Code	02459	Country[i]	US

Applicant 9 — Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Katharine		Yen	

Residence Information (Select One)	◉ US Residency	○ Non US Residency	○ Active US Military Service		
City	Wellesley	State/Province	MA	Country of Residence [i]	US
Citizenship under 37 CFR 1.41(b) [i]	US				

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

Mailing Address of Applicant:			
Address 1	6 Shirley Road		
Address 2			
City	Wellesley	State/Province	MA
Postal Code	02482	Country[i]	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	94970		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS		
Attorney Docket Number	C2081-7013US	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S. C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	94970		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	a 371 of international	PCT/US2010/027253	2010-03-12
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61266929	2009-12-04
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61253820	2009-10-21
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61229689	2009-07-29
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61227649	2009-07-22
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61220543	2009-06-25
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61180609	2009-05-22
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61173518	2009-04-28
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61160664	2009-03-16
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/027253	non provisional of	61160253	2009-03-13

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

PTO/SB/14 (11-08)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Remove

Application Number	Country [i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
			(•) Yes ( ) No

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

Assignee 1 Remove

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1			
Address 2			
City		**State/Province**	
Country [i]		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the **Add** button. Add

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Peter Korakas/			Date (YYYY-MM-DD)	2011-09-13
First Name	Peter	Last Name	Korakas	Registration Number	66513

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	C2081-7013US
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS	

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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.**

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 records.

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.

ATTORNEY DOCKET NO: C2081-7013US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Lenny Dang et al.
Serial No:	Not Yet Assigned
Confirmation No:	Not Yet Assigned
Filed:	September 13, 2011
Title:	METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION RELATED DISORDERS
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with §1.6(a)(4), on the 13th day of September, 2011.

/Peter Korakas/
Peter Korakas, Reg. No. 66,513

Commissioner for Patents

PRELIMINARY AMENDMENT

Sir:

Applicants request that the application be amended as follows before examination:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 9 of this paper.

Amendments to the Specification

Please replace the paragraph below "CLAIM OF PRIORITY" on page 1 of the application with the following paragraph:

This application is a national stage application under 35 U.S.C. §371 of International Application No. PCT/US2010/027253, filed March 12, 2010, published as International Publication No. WO 2010/105243 on September 16, 2010 which claims priority to U.S.S.N. 61/160253, filed March 13, 2009; U.S.S.N. 61/160664, filed March 16, 2009; U.S.S.N. 61/173518, filed April 28, 2009; U.S.S.N. 61/180609, filed May 22, 2009; U.S.S.N. 61/220543, filed June 25, 2009; U.S.S.N. 61/227649, filed July 22, 2009; U.S.S.N. 61/229689, filed July 29, 2009; U.S.S.N. 61/253820, filed October 21, 2009; and U.S.S.N. 61/266929, filed December 4, 2009, the contents of each of which are incorporated herein by reference.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1-40. (Canceled)

41. (New) A method of evaluating a subject comprising,
analyzing a parameter related to the IDH1 or IDH2 neoactivity phenotype of said subject, wherein analyzing comprises performing a test, on said subject, or on a sample from said subject, and
responsive to said analysis, selecting said subject as having an IDH1 or IDH2 allele having 2HG neoactivity,
thereby evaluating the subject.

42. (New) The method of claim 41, wherein analyzing comprises analyzing one or more of:

a) the presence of 2HG;

b) the presence of 2HG neoactivity from an IDH1 or IDH2 mutant protein; or

c) the presence of RNA corresponding to an IDH1 or IDH2 mutant protein having 2HG neoactivity.

43. (New) The method of claim 41, wherein analyzing comprises analyzing the presence 2HG.

44. (New) The method of claim 41, wherein a sample, from said subject, is analyzed.

45. (New) The method of claim 41, wherein said sample is a tumor sample, cancer cell sample, or precancerous cell sample.

46. (New) The method of claim 45, wherein said sample is analyzed for the presence or level of 2HG.

47. (New) The method of claim 45, wherein said analysis comprises a chromatographic method.

48. (New) The method of claim 45, wherein said analysis comprises LC-MS analysis.

49. (New) The method of claim 41, comprising subjecting said subject to imaging and/or spectroscopic analysis to provide a determination of the presence, distribution, or level of 2HG.

50. (New) The method of claim 49, wherein said presence is associated with a tumor in said subject.

51. (New) The method of claim 50, wherein said tumor is a glioma.

52. (New) The method of claim 49, wherein said imaging and/or spectroscopic analysis comprises magnetic resonance-based analysis.

53. (New) The method of claim 49, wherein said imaging and/or spectroscopic analysis comprises MRI and/or MRS imaging analysis.

54. (New) The method of claim 41, wherein said subject has an increased level of 2HG as compared with a reference.

55. (New) The method of claim 54, wherein said reference is the level seen in an otherwise similar cell, tissue or product lacking an IDH1and IDH2 neoactive mutation.

56. (New) The method of claim 54, wherein said reference is the level seen in an otherwise similar cell lacking said IDH1 or IDH2 mutation, or in a tissue or product, from said subject not having said IDH1 or IDH2 mutation.

57. (New) The method of claim 41, further comprising determining that the subject has a cancer characterized by an IDH1 or IDH2 allele having 2HG neoactivity by DNA sequencing.

58. (New) The method of claim 41, further comprising confirming or determining that the subject has a cancer characterized by an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8).

59. (New) The method of claim 58, further comprising confirming or determining that the subject has a cancer characterized by an IDH1 allele having His at residue 132 (SEQ ID NO:8).

60. (New) The method of claim 58, further comprising confirming or determining that the subject has a cancer characterized by an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

61. (New) The method of claim 41, further comprising determining the identity of amino acid residue 132 (SEQ ID NO:8) in the IDH1 gene.

62. (New) The method of claim 57, further comprising confirming or determining that the subject has a cancer characterized an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10).

63. (New) The method of claim 41, further comprising diagnosing said subject as having cancer.

64. (New) The method of claim 41, further comprising diagnosing said subject as having a precancerous disorder.

65. (New) The method of claim 41, wherein said subject does not have 2-hydroxyglutaric aciduria.

66. (New) The method of claim 41, wherein said subject has an IDH1 neoactive mutant.

67. (New) The method of claim 66, wherein said neoactive mutant arises from a mutation at residue 132.

68. (New) The method of claim 67, wherein said IDH1 mutant has His, Ser, Cys, Gly, Val, Pro or Leu, at residue 132.

69. (New) The method of claim 67, wherein said IDH1 mutant has His at residue 132.

70. (New) The method of claim 67, wherein said IDH1 mutant has Ser at residue 132.

71. (New) The method of claim 67, wherein said IDH1 mutant has Cys at residue 132.

72. (New) The method of claim 67, wherein said IDH1 mutant has Gly at residue 132.

73. (New) The method of claim 67, wherein said IDH1 mutant has Val at residue 132.

74. (New) The method of claim 67, wherein said IDH1 mutant has Pro at residue 132.

75. (New) The method of claim 67, wherein said IDH1 mutant has Leu at residue 132.

76. (New) The method of claim 41, wherein said subject has an IDH2 neoactive mutant.

77. (New) The method of claim 76, wherein said neoactive mutant arises from a mutation at residue 172.

78. (New) The method of claim 76, wherein said IDH2 mutant has a Lys, Gly, Met, Trp, Thr, or Ser at residue 172.

79. (New) The method of claim 78, wherein said IDH2 mutant has a Lys at residue 172.

80. (New) The method of claim 41, wherein said subject has a leukemia.

81. (New) The method of claim 41, wherein said subject has AML.

82. (New) The method of claim 41, wherein said subject has myelodisplasia.

83. (New) The method of claim 41, wherein said subject has myelodisplastic syndrome.

84. (New) The method of claim 41, further comprising providing a recommendation for treatment of said subject.

85. (New) The method of claim 41, further comprising memorializing a result of, or ouput from, the method.

86. (New) The method of claim 84, further comprising transmitting the memorialization to a party.

87. (New) The method of claim 86, wherein said party is a healthcare provider.

88. (New) The method of claim 86, wherein said party is an entity that pays for the subject's treatment.

89. (New) The method of claim 86, wherein said party is a government or insurance company.

90. (New) The method of claim 41, further comprising, selecting a payment class for treatment with a therapeutic agent, comprising, responsive to said analysis,

performing at least one of (1) if the subject is positive for increased levels of 2HG selecting a first payment class, and (2) if the subject is a not positive for increased levels of 2HG selecting a second payment class.

91. (New) The method of claim 90, wherein said selection is memorialized.

92. (New) The method of claim 91, further comprising communicating said selection to another party.

93. (New) A method of evaluating a subject for the presence or susceptibility to a cancer comprising analyzing the subject or a sample from the subject for one or more of:

a) the presence, distribution, or level of 2HG, wherein the subject is not having or not diagnosed as having 2-hydroxyglutaric aciduria;

b) the presence, distribution, or level of a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity;

c) the presence, distribution, or level of a RNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity; or

d) the presence of DNA encoding a mutant IDH1 enzyme or mutant IDH2 enzyme, either of which has 2HG neoactivity;

thereby evaluating the subject for such cancer.

94. (New) The method of claim 93, wherein the cancer is an astrocytic tumor, an oligodendroglial tumor, an oligoastrocytic tumor, an anaplastic astrocytoma, fibrosarcoma, paraganglioma, prostate cancer, acute lymphoblastic leukemia, or acute myelogenous leukemia.

95. (New) The method of claim 93, wherein the cancer is a glioblastoma.

96. (New) The method of claim 93, the method comprising analyzing the presence, distribution, or level of 2HG.

97. (New) The method of claim 96, wherein the presence, distribution or level of 2HG is determined non-invasively by imaging or spectroscopic analysis.

98. (New) The method of claim 97, wherein the imaging or spectroscopic analysis comprises magnetic resonance imaging or magnetic resonance spectroscopy.

99. (New) The method of claim 96, wherein the presence, distribution or level of 2HG is determined by evaluating a tissue, product or bodily fluid of the subject.

REMARKS

Applicants have amended the specification to update the priority claim. Applicants have canceled former claims 1-40 and added new claims 41-99. Support for new claims 41-99 can be found throughout the specification as originally filed. This amendment and new claims 41-99 add no new matter. Applicants ask that all claims be examined in view of the amendments to the claims.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. Please charge any deficiency to Deposit Account No. 50/2762, referencing Attorney Docket No.: C2081-7013US

Respectfully submitted,

Dang et al., Applicant

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1	Transmittal of New Application	C2081-7013USNational.pdf	597853 3e03515e20dd3b7615bf8b07bda53909a82b8332	no	4
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Information:					
2	Application Data Sheet	C2081-7013USADS.pdf	1906817 5ed3bdc5021834ed74ef1e1ad69162976c2e4537	no	8
Warnings:					
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(54) **Title**: METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

(57) **Abstract**: Methods of treating and evaluating subjects having neoactive mutants are described herein.

METHODS AND COMPOSITIONS FOR CELL-PROLIFERATION-RELATED DISORDERS

CLAIM OF PRIORITY

This application claims priority to U.S.S.N. 61/160253, filed March 13, 2009; U.S.S.N. 61/160664, filed March 16, 2009; U.S.S.N. 61/173518, filed April 28, 2009; U.S.S.N. 61/180609, filed May 22, 2009; U.S.S.N. 61/220543, filed June 25, 2009; U.S.S.N. 61/227649, filed July 22, 2009; U.S.S.N. 61/229689, filed July 29, 2009; U.S.S.N. 61/253820, filed October 21, 2009; and U.S.S.N. 61/266929, filed December 4, 2009, the contents of each of which are incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods and compositions for evaluating and treating cell proliferation-related disorders, *e.g.,* proliferative disorders such as cancer.

BACKGROUND

Isocitrate dehydrogenase, also known as IDH, is an enzyme which participates in the citric acid cycle. It catalyzes the third step of the cycle: the oxidative decarboxylation of isocitrate, producing alpha-ketoglutarate (α-ketoglutarate or α-KG) and CO_2 while converting NAD+ to NADH. This is a two-step process, which involves oxidation of isocitrate (a secondary alcohol) to oxalosuccinate (a ketone), followed by the decarboxylation of the carboxyl group beta to the ketone, forming alpha-ketoglutarate. Another isoform of the enzyme catalyzes the same reaction; however this reaction is unrelated to the citric acid cycle, is carried out in the cytosol as well as the mitochondrion and peroxisome, and uses NADP+ as a cofactor instead of NAD+.

SUMMARY OF THE INVENTION

Methods and compositions disclosed herein relate to the role played in disease by neoactive products produced by neoactive mutant enzymes, e.g., mutant metabolic pathway enzymes. The inventors have discovered, *inter alia*, a neoactivity associated with IDH mutants and that the product of the neoactivity can be significantly elevated in cancer cells. Disclosed herein are methods and compositions for treating, and methods of evaluating, subjects having or at risk for a disorder, *e.g.,* a cell

proliferation-related disorder characterized by a neoactivity in a metabolic pathway enzyme, e.g., IDH neoactivity. Such disorders include *e.g.,* proliferative disorders such as cancer. The inventors have discovered and disclosed herein novel therapeutic agents for the treatment of disorders, e.g., cancers, characterized by, e.g., by a neoactivity, neoactive protein, neoactive mRNA, or neoactive mutations. In embodiments a therapeutic agent reduces levels of neoactivity or neoactive product or ameliorates an effect of a neoactive product. Methods described herein also allow the identification of a subject, or identification of a treatment for the subject, on the basis of neaoctivity genotype or phenotype. This evaluation can allow for optimal matching of subject with treatment, e.g., where the selection of subject, treatment, or both, is based on an analysis of neoactivity genotype or phenotype. E.g., methods describe herein can allow selection of a treatment regimen comprising administration of a novel compound, e.g., a novel compound disclosed herein, or a known compound, e.g., a known compound not previously recommended for a selected disorder. In embodiments the known compound reduces levels of neoactivity or neoactive product or ameliorates an effect of a neoactive product. Methods described herein can guide and provide a basis for selection and administration of a novel compound or a known compound, or combination of compounds, not previously recommended for subjects having a disorder characterized by a somatic neoactive mutation in a metabolic pathway enzyme. In embodiments the neoactive genotype or phenotype can act as a biomarker the presence of which indicates that a compound, either novel, or previously known, should be administered, to treat a disorder characterized by a somatic neoactive mutation in a metabolic pathway enzyme. Neoactive mutants of IDH1 having a neoactivity that results in the production of 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate and associated disorders are discussed in detail herein. They are exemplary, but not limiting, examples of embodiments of the invention.

While not wishing to be bound by theory it is believed that the balance between the production and elimination of neoactive product, e.g., 2HG, e.g., R-2HG, is important in disease. Neoactive mutants, to varying degrees for varying mutations, increase the level of neoactive product, while other processes, e.g., in the case of 2HG, e.g., R-2HG, enzymatic degradation of 2HG, e.g., by 2HG dehydrogenase, reduce the level of neoative product. An incorrect balance is associated with disease. In embodiments, the net result of a neoactive mutation at IDH1 or IDH2 result in increased levels, in affected cells, of neoactive product, 2HG, e.g., R-2HG,

Accordingly, in one aspect, the invention features, a method of treating a subject having a cell proliferation-related disorder, *e.g.,* a disorder characterized by unwanted cell proliferation, *e.g.,* cancer, or a precancerous disorder. The cell proliferation-related disorder is characterized by a somatic mutation in a metabolic pathway enzyme. The mutation is associated with a neoactivity that results in the production of a neoactivity product. The method comprises: administering to the subject a therapeutically effective amount of a therapeutic agent described herein, e.g., a therapeutic agent that decreases the level of neoactivity product encoded by a selected or mutant somatic allele, e.g., an inhibitor of a neoactivity of the metabolic pathway enzyme (the neoactive enzyme), a therapeutic agent that ameliorates an unwanted affect of the neoactivity product, or a nucleic acid based inhibitor, e.g., a dRNA which targets the neoactive enzyme mRNA,
to thereby treat the subject.

In an embodiment the subject is a subject not having, or not diagnosed as having, 2-hydroxyglutaric aciduria.

In an embodiment the subject has a cell proliferation-related disorder, *e.g.,* a cancer, characterized by the neoactivity of the metabolic pathway enzyme encoded by selected or mutant allele.

In an embodiment the subject has a cell proliferation-related disorder, *e.g.,* a cancer, characterized by the product formed by the neoactivity of the metabolic pathway enzyme encoded by selected or mutant allele.

In one embodiment, the metabolic pathway is selected from a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, nucleotide biosynthetic pathways, or the fatty acid biosynthetic pathway.

In an embodiment the therapeutic agent is a therapeutic agent described herein.

In an embodiment the method comprises selecting a subject on the basis of having a cancer characterized by the selected or mutant allele, the neoactivity, or an elevated level of neaoctivity product.

In an embodiment the method comprises selecting a subject on the basis of having a cancer characterized by the product formed by the neoactivity of the protein encoded by selected or mutant allele, *e.g.,* by the imaging and/or spectroscopic analysis, *e.g.,* magnetic resonance-based analysis, *e.g.,* MRI (magnetic resonance imaging) and/or MRS (magnetic resonance spectroscopy), to determine the presence,

distribution or level of the product of the neoactivity, *e.g.,* in the case of an IDH1 allele described herein, 2-hydroxyglutarate (sometimes referred to herein as 2HG), *e.g.,* R-2-hydroxyglutarate (sometimes referred to herein as R-2HG).

In an embodiment the method comprises confirming or determining, *e.g.,* by direct examination or evaluation of the subject, or sample *e.g.,* tissue, product (e.g., feces, sweat, semen, exhalation, hair or nails), or bodily fluid (*e.g.,* blood (*e.g.,* blood plasma), urine, lymph, or cerebrospinal fluid or other sample sourced disclosed herein) therefrom, (*e.g.,* by DNA sequencing, immuno analysis, or assay for enzymatic activity), or receiving such information about the subject, that the cancer is characterized by the selected or mutant allele.

In an embodiment the method comprises confirming or determining, *e.g.,* by direct examination or evaluation of the subject, the level of neoactivity or the level of the product of the neoactivity, or receiving such information about the subject. In an embodiment the presence, distribution or level of the product of the neoactivity, *e.g.,* in the case of an IDH1 allele described herein, 2HG, *e.g.,* R-2HG, is determined non-invasively, *e.g.,* by imaging methods, *e.g.,* by magnetic resonance-based methods.

In an embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.,* surgical removal or administration of a chemotherapeutic.

In another aspect, the invention features, a method of treating a subject having a cell proliferation-related disorder, *e.g.,* a precancerous disorder, or cancer. In an embodiment the subject does not have, or has not been diagnosed as having, 2-hydroxyglutaric aciduria. The cell proliferation-related disorder is characterized by a somatic allele, e.g., a preselected allele, or mutant allele, of an IDH, *e.g.,* IDH1 or IDH2, which encodes a mutant IDH, *e.g.,* IDH1 or IDH2, enzyme having a neoactivity.

In embodiments the neoactivity is alpha hydroxy neoactivity. As used herein, alpha hydroxy neoactivity refers to the ability to convert an alpha ketone to an alpha hydroxy. In embodiments alpha hydroxy neoactivity proceeds with a reductive cofactor, e.g., NADPH or NADH. In embodiments the alpha hydroxyl neoactivity is 2HG neoactivity. 2HG neoactivity, as used herein, refers to the ability to convert alpha ketoglutarate to 2-hydroxyglutarate (sometimes referred to herein as 2HG), *e.g.,* R-2-hydroxyglutarate (sometimes referred to herein as R-2HG). In embodiments 2HG neoactivity proceeds with a reductive cofactor, e.g., NADPH or NADH. In an

embodiment a neoactive enzyme, e.g., an alpha hydroxyl, e.g., a 2HG, neoactive enzyme, can act on more than one substrate, e.g., more than one alpha hydroxy substrate.

The method comprises administering to the subject an effective amount of a therapeutic agent of type described herein to thereby treat the subject.

In an embodiment the therapeutic agent: results in lowering the level of a neoactivity product, e.g., an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG.

In an embodiment the method comprises administering a therapeutic agent that lowers neoactivity, e.g., 2HG neoactivity. In an embodiment the method comprises administering an inhibitor of a mutant IDH protein, *e.g.,* a mutant IDH1 or mutant IDH2 protein, having a neoactivity, *e.g.,* alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment the therapeutic agent comprises a compound from Table 24a or Table 24b or a compound having the structure of Formula (X) or (Formula (XI) described herein.

In an embodiment the therapeutic agent comprises nucleic acid-based therapeutic agent, e.g., a dsRNA, e.g., a dsRNA described herein.

In an embodiment the the therapeutic agent is an inhibitor, *e.g.,* a polypeptide, peptide, or small molecule (*e.g.,* a molecule of less than 1,000 daltons), or aptomer, that binds to an IDH1 mutant or wildtype subunit and inhibits neoactivity, e.g., by inhibiting formation of a dimer, *e.g.,* a homodimer of mutant IDH1 subunits or a heterodimer of a mutant and a wildype subunit. In an embodiment the inhibitor is a polypeptide. In an embodiment the polypeptide acts as a dominant negative with respect to the neoactivity of the mutant enzyme. The polypeptide can correspond to full length IDH1 or a fragment thereof. The polypeptide need not be indentical with the corresponding residues of wildtype IDH1, but in embodiments has at least 60, 70, 80, 90 or 95 % homology with wildtype IDH1.

In an embodiment the therapeutic agent decreases the affinity of an IDH, e.g., IDH1 or IDH2 neoactive mutant protein for NADH, NADPH or a divalent metal ion, e.g., Mg^{2+} or Mn^{2+}, or decreases the levels or availability of NADH, NADPH or divalent metal ion, e.g., Mg^{2+} or Mn^{2+}, *e.g.,* by competing for binding to the mutant enzyme. In an embodiment the enzyme is inhibited by replacing Mg^{2+} or Mn^{2+} with Ca^{2+}.

In an embodiment the therapeutic agent is an inhibitor that reduces the level a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that reduces the level of the product of a mutant having a neoactivity of an IDH, *e.g.,* IDH1 or IDH2 mutant, *e.g.,* it reduces the level of 2HG, *e.g.,* R-2HG.

In an embodiment the therapeutic agent is an inhibitor that:

inhibits, *e.g.,* specifically, a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein, *e.g.,* 2HG neoactivity; or

inhibits both the wildtype activity and a neoactivity of an IDH, *e.g.,* IDH1 orIDH2, *e.g.,* a neoactivity described herein, e.g, 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that is selected on the basis that it:

inhibits, *e.g.,* specifically, a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein *e.g.,* 2HG neoactivity; or

inhibits both the wildtype activity and a neoactivity of an IDH1, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein, *e.g.,* 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that reduces the amount of a mutant IDH, *e.g.,* IDH1 or IDH2, protein or mRNA.

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* it binds to, the mutant IDH, *e.g.,* IDH1 or IDH2 mRNA.

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* it binds to, the mutant IDH, *e.g.,* IDH1 or IDH2, protein.

In an embodiment the therapeutic agent is an inhibitor that reduces the amount of neoactive enzyme activity, *e.g.,* by interacting with, *e.g.,* binding to, mutant IDH, *e.g.,* IDH1 or IDH2, protein. In an embodiment the inhibitor is other than an antibody.

In an embodiment the therapeutic agent is an inhibitor that is a small molecule and interacts with, *e.g.,* binds, the mutant RNA, *e.g.,* mutant IDH1 or IDH2 mRNA (e.g., mutant IDH1 mRNA).

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* binds, either the mutant IDH, *e.g.,* IDH1 or IDH2, protein or interacts directly with, *e.g.,* binds, the mutant IDH mRNA, *e.g.,* IDH1 or IDH2 mRNA.

In an embodiment the IDH is IDH1 and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDH1 associated with 2HG

neoactivity include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L, or R132V (e.g., R132H or R132C).

In an embodiment the IDH is IDH2 and the neoactivity of the IDH2 mutant is alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations in IDH2 associated with 2HG neoactivity include mutations at residue 172, e.g., R172K, R172M, R172S, R172G, or R172W.

Treatment methods described herein can comprise evaluating a neoactivity genotype or phenotype. Methods of obtaining and analyzing samples, and the in vivo analysis in subjects, described elsewhere herein, e.g., in the section entitled, "Methods of evaluating samples and/or subjects," can be combined with this method.

In an embodiment, prior to or after treatment, the method includes evaluating the growth, size, weight, invasiveness, stage or other phenotype of the cell proliferation-related disorder.

In an embodiment, prior to or after treatment, the method includes evaluating the IDH, e.g., IDH1 or IDH2, alpha hydroxyl neoactivity genotype, e.g., 2HG, genotype, or alpha hydroxy neoactivity phenotype, e.g., 2HG, e.g., R-2HG, phenotype. Evaluating the alpha hydroxyl, e.g., 2HG, genotype can comprise determining if an IDH1 or IDH2 mutation having alpha hydroxy neoactivity, e.g., 2HG neoactivity, is present, e.g., a mutation disclosed herein having alpha hydroxy neoactivity, e.g., 2HG neoactivity. Alpha hydroxy neoactivity phenotype, e.g., 2HG, e.g., R-2HG, phenotype, as used herein, refers to the level of alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, level of alpha hydroxy neoactivity, e.g., 2HG neoactivity, or level of mutant enzyme having alpha hydroxy neoactivity, e.g., 2HG neoactivity (or corresponding mRNA). The evaluation can be by a method described herein.

In an embodiment the subject can be evaluated, before or after treatment, to determine if the cell proliferation-related disorder is characterized by an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment a cancer, *e.g.,* a glioma or brain tumor in a subject, can be analyzed, *e.g.,* by imaging and/or spectroscopic analysis, *e.g.,* magnetic resonance-based analysis, *e.g.,* MRI and/or MRS, *e.g.,* before or after treatment, to determine if it is characterized by presence of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the method comprises evaluating, *e.g.,* by direct examination or evaluation of the subject, or a sample from the subject, or receiving

such information about the subject, the IDH, *e.g.,* IDH1 or IDH2, genotype, or an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG phenotype of, the subject, *e.g.,* of a cell, *e.g.,* a cancer cell, characterized by the cell proliferation-related disorder. (As described in more detail elsewhere herein the evaluation can be, *e.g.,* by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, *e.g.,* from spectroscopic analysis, *e.g.,* magnetic resonance-based analysis, *e.g.,* MRI and/or MRS measurement, sample analysis such as serum or spinal cord fluid analysis, or by analysis of surgical material, *e.g.,* by mass-spectroscopy). In embodiments this information is used to determine or confirm that a proliferation-related disorder, *e.g.,* a cancer, is characterized by an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. In embodiments this information is used to determine or confirm that a cell proliferation-related disorder, *e.g.,* a cancer, is characterized by an IDH, *e.g.,* IDH1 or IDH2, allele described herein, *e.g.,* an IDH1 allele having a mutation, *e.g.,* a His, Ser, Cys, Gly, Val, Pro or Leu (e.g., His, Ser, Cys, Gly, Val, or Leu at residue 132, more specifically, His or Cys, or an IDH2 allele having a mutation at residue 172, *e.g.,* a K, M, S, G, or W.

In an embodiment, before and/or after treatment has begun, the subject is evaluated or monitored by a method described herein, *e.g.,* the analysis of the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, *e.g.,* to select, diagnose or prognose the subject, to select an inhibitor, or to evaluate response to the treatment or progression of disease.

In an embodiment the cell proliferation-related disorder is a tumor of the CNS, *e.g.,* a glioma, a leukemia, *e.g.,* AML or ALL, e.g., B-ALL or T-ALL, prostate cancer, fibrosarcoma, paraganglioma, or myelodysplasia or myelodysplastic syndrome (e.g., B-ALL or T-ALL, prostate cancer, or myelodysplasia or myelodysplastic syndrome) and the evaluation is: evaluation of the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG; or evaluation of the presence, distribution, or level of a neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., 2HG neoactivity, of an IDH1 or IDH2, mutant protein.

In an embodiment the disorder is other than a solid tumor. In an embodiment the disorder is a tumor that, at the time of diagnosis or treatment, does not have a necrotic portion. In an embodiment the disorder is a tumor in which at least 30, 40,

50, 60, 70, 80 or 90% of the tumor cells carry an IHD, e.g., IDH1 or IDH2, mutation having 2HG neoactivity, at the time of diagnosis or treatment.

In an embodiment the cell proliferation-related disorder is a cancer, e.g., a cancer described herein, characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the tumor is characterized by increased levels of an alpha hydroxy neoactivity product, 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by unwanted (i.e., increased) levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a tumor of the CNS, *e.g.,* a glioma, *e.g.,* wherein the tumor is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. Gliomas include astrocytic tumors, oligodendroglial tumors, oligoastrocytic tumors, anaplastic astrocytomas, and glioblastomas. In an embodiment the tumor is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu (e.g., His, Ser, Cys, Gly, Val, or Leu), or any residue described in Yan *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **Fig. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132.

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation; specifically a C394A or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having a glioma, wherein the cancer is characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8), more specifically His, Ser, Cys, Gly, Val, or Leu; or His or Cys.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by an IDH1 allele described herein, *e.g.*, an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8) , more specifically His, Ser, Cys, Gly, Val, or Leu; or His or Cys.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.*, R-2HG.

In an embodiment the method comprises selecting a subject having a fibrosarcoma or paraganglioma wherein the cancer is characterized by having an IDH1 allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having a fibrosarcoma or paraganglioma, on the basis of the cancer being characterized by an IDH1 allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having a fibrosarcoma or paraganglioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.*, R-2HG.

In an embodiment the cell proliferation-related disorder is localized or metastatic prostate cancer, *e.g.*, prostate adenocarcinoma, *e.g.*, wherein the cancer is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.*, a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type.

E.g., in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**) (e.g., His, Ser, Cys, Gly, Val, or Leu). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.,* prostate adenocarcinoma, wherein the cancer is characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having prostate cancer, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a hematological cancer, *e.g.,* a leukemia, *e.g.,* AML, or ALL, wherein the hematological cancer is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type.

In an embodiment the cell proliferation-related disorder is acute lymphoblastic leukemia (*e.g.,* an adult or pediatric form), *e.g.,* wherein the acute lymphoblastic leukemia (sometimes referred to herein as ALL) is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. The ALL can be, e.g., B-ALL or T-ALL. In an embodiment the cancer is characterized by increased levels of 2 an alpha hydroxy neoactivity product, e.g., HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et a.l,* at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**), more specifically His, Ser, Cys, Gly, Val, or Leu. In an embodiment the allele encodes an IDH1 having Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 according to the sequence of SEQ ID NO:8.

In an embodiment the method comprises selecting a subject ALL, e.g., B-ALL or T-ALL, on the basis of cancer being characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is acute myelogenous leukemia (*e.g.,* an adult or pediatric form), *e.g.,* wherein the acute myelogenous leukemia (sometimes referred to herein as AML) is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys, His or Gly at residue 132, more specifically, Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML) characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys, His, or Gly at residue 132 according to the sequence of SEQ ID NO:8, more specifically, Cys at residue 132.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML) on the basis of cancer being characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys, His, or Gly at residue 132 (SEQ ID NO:8), more specifically, Cys at residue 132.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML), on the basis of the cancer being

characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the method further comprises evaluating the subject for the presence of a mutation in the NRAS or NPMc gene.

In an embodiment the cell proliferation-related disorder is myelodysplasia or myelodysplastic syndrome, *e.g.,* wherein the myelodysplasia or myelodysplastic syndrome is characterized by having an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the disorder is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et a.l*, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**), more specifically His, Ser, Cys, Gly, Val, or Leu. In an embodiment the allele encodes an IDH1 having Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys, His, or Gly at residue 132 according to the sequence of SEQ ID NO:8, more specifically, Cys at residue 132.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome on the basis of cancer being characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys, His, or Gly at residue 132 (SEQ ID NO:8), more specifically, Cys at residue 132.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a glioma, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes

Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), more specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having a glioma, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), more specifically Lys, Gly, Met, Trp, or Ser; or Lys or Met.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), more specifically Lys, Gly, Met, Trp, or Ser; or Lys or Met.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a prostate cancer, *e.g.,* prostate adenocarcinoma, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), more specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is ALL, e.g., B-ALL or T-ALL, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**). In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is AML, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), more specifically Lys, Gly, Met, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172. In an embodiment the allele encodes an IDH2 having Gly at residue 172.

In an embodiment the method comprises selecting a subject having AML, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,*

an IDH2 allele having Lys, Gly or Met at residue 172 (SEQ ID NO:10), more specifically Lys or Met.

In an embodiment the method comprises selecting a subject having AML, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, or Met at residue 172 (SEQ ID NO:10), more specifically Lys or Met.

In an embodiment the method comprises selecting a subject having AML, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is myelodysplasia or myelodysplastic syndrome, characterized by a mutation, or preselected allele, of IDH2. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), more specifically Lys, Gly, Met, Trp or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172. In an embodiment the allele encodes an IDH2 having Gly at residue 172.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome,wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, or Met at residue 172 (SEQ ID NO:10), in specific embodiments, Lys or Met.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, or Met at residue 172 (SEQ ID NO:10), in specific embodiments, Lys or Met.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment a product of the neoactivity is 2HG (*e.g.,* R-2HG) which acts as a metabolite. In another embodiment a product of the neoactivity is 2HG (*e.g.,* R-2HG) which acts as a toxin, *e.g.,* a carcinogen.

In some embodiments, the methods described herein can result in reduced side effects relative to other known methods of treating cancer.

Therapeutic agents and methods of subject evaluation described herein can be combined with other therapeutic mocalities, e.g., with art-known treatments.

In an embodiment the method comprises providing a second treatment, to the subject, *e.g.,* surgical removal, irradiation or administration of a chemotherapeutitc agent, *e.g.,* an administration of an alkylating agent. Administration (or the establishment of therapeutic levels) of the second treatment can: begin prior to the beginning or treatment with (or prior to the establishment of therapeutic levels of) the inhibitor; begin after the beginning or treatment with (or after the establishment of therapeutic levels of) the inhibitor, or can be administered concurrently with the inhibitor, *e.g.,* to achieve therapeiutc levels of both concurrently.

In an embodiment the cell proliferation-related disorder is a CNS tumor, *e.g.,* a glioma, and the second therapy comprises administration of one or more of: radiation; an alkylating agent, e.g., temozolomide, e.g., Temoader®, or BCNU; or an inhibitor of HER1/EGFR tyrosine kinase, e.g., erlotinib, e.g., Tarceva®.

The second therapy, *e.g.,* in the case of glioma, can comprise implantation of BCNU or carmustine in the brain, e.g., implantation of a Gliadel® wafer.

The second therapy, *e.g.,* in the case of glioma, can comprise administration of imatinib, e.g., Gleevec®.

In an embodiment the cell proliferation-related disorder is prostate cancer and the second therapy comprises one or more of: androgen ablation; administration of a microtubule stabilizer, e.g., docetaxol, e.g., Taxotere®; or administration of a topoisomerase II inhibitor, e.g., mitoxantrone.

In an embodiment the cell proliferation-related disorder is ALL, e.g., B-ALL or T-ALL, and the second therapy comprises one or more of:

induction phase treatment comprising the administration of one or more of: a steroid; an inhibitor of microtubule assembly, e.g., vincristine; an agent that reduces the availability of asparagine, e.g., asparaginase; an anthracycline; or an antimetabolite, e.g., methotrexate, e.g., intrathecal methotrexate, or 6-mercaptopurine;

consolidation phase treatment comprising the administration of one or more of: a drug listed above for the induction phase; an antimetabolite, e.g., a guanine analog, e.g., 6-thioguanine; an alkylating agent , e.g., cyclophosphamide; an anti-metabolite, e.g., AraC or cytarabine; or an inhibitor of topoisomerase I, e.g., etoposide; or

maintenance phase treatment comprising the administration of one or more of the drugs listed above for induction or consolidation phase treatment.

In an embodiment the cell proliferation-related disorder is AML and the second therapy comprises administration of one or more of: an inhibitor of topoisomerase II, e.g., daunorubicin, idarubicin, topotecan or mitoxantrone; an inhibitor of topoisomerase I, e.g., etoposide; or an anti-metabolite, e.g., AraC or cytarabine.

In another aspect, the invention features, a method of evaluating, e.g. diagnosing, a subject, *e.g.,* a subject not having, or not diagnosed as having, 2-hydroxyglutaric aciduria. The method comprises analyzing a parameter related to the neoactivity genotype or phenotype of the subject, e.g., analyzing one or more of:

a) the presence, distribution, or level of a neoactive product, e.g., the product of an alpha hydroxy neoactivity, e.g., 2HG, *e.g.,* R-2HG, e.g., an increased level of product, 2HG, *e.g.,* R-2HG (as used herein, an increased level of a product of an alpha hydroxy neoactivity, e.g., 2HG, *e.g.,* R-2HG, or similar term, e.g., an increased level of neoactive product or neoactivity product, means increased as compared with a reference, e.g., the level seen in an otherwise similar cell lacking the IDH mutation, e.g., IDH1 or IDH2 mutation, or in a tissue or product from a subject noth having the mutation (the terms increased and elevated as refered to the level of a product of alpha hydroxyl neoactivity as used herein, are used interchangably);

b) the presence, distribution, or level of a neoactivity, *e.g.,* alpha hydroxy neoactivity, e.g., 2HG neoactivity, of an IDH1 or IDH2, mutant protein;

c) the presence, distribution, or level of a neoactive mutant protein, e.g., an IDH, *e.g.,* an IDH1 or IDH2, mutant protein which has a neoactivity, e.g., alpha hydroxy neoactivity, *e.g.,* 2HG neoactivity, or a corresponding RNA; or

d) the presence of a selected somatic allele or mutation conferring neoactivity, e.g., an IDH, *e.g.,* IDH1 or IDH2, which encodes a protein with a neoactivity, *e.g.,* alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* an allele disclosed herein, in cells characterized by a cell proliferation-related disorder from the subject, thereby evaluating the subject.

In an embodiment analyzing comprises performing a procedure, e.g., a test, to provide data or information on one or more of a-d, e.g., performing a method which results in a physical change in a sample, in the subject, or in a device or reagent used in the analysis, or which results in the formation of an image representative of the data.

Methods of obtaining and analyzing samples, and the in vivo analysis in subjects, described elsewhere herein, e.g., in the section entitled, "Methods of evaluating samples and/or subjects," can be combined with this method. In another embodiment analyzing comprises receiving data or information from such test from another party. In an embodiment the analyzing comprises receiving data or information from such test from another party and, the method comprises, responsive to that data or information, administering a treatment to the subject.

As described herein, the evaluation can be used in a number of applications, e.g., for diagnosis, prognosis, staging, determination of treatment efficacy, patent selection, or drug selection.

Thus, in an embodiment method further comprises, *e.g.,* responsive to the analysis of one or more of a-d:

diagnosing the subject, *e.g.,* diagnosing the subject as having a cell proliferation-related disorder, *e.g.,* a disorder characterized by unwanted cell proliferation, *e.g.,* cancer, or a precancerous disorder;

staging the subject, *e.g.,* determining the stage of a cell proliferation-related disorder, *e.g.,* a disorder characterized by unwanted cell proliferation, *e.g.,* cancer, or a precancerous disorder;

providing a prognosis for the subject, *e.g.,* providing a prognosis for a cell proliferation-related disorder, *e.g.,* a disorder characterized by unwanted cell proliferation, *e.g.,* cancer, or a precancerous disorder;

determining the efficacy of a treatment, *e.g.,* the efficacy of a chemotherapeutic agent, irradiation or surgery;

determining the efficacy of a treatment with a therapeutic agent, e.g., an inhibitor, described herein;

selecting the subject for a treatment for a cell proliferation-related disorder, *e.g.,* a disorder characterized by unwanted cell proliferation, *e.g.,* cancer, or a precancerous disorder. The selection can be based on the need for a reduction in neoactivity or on the need for amelioration of a condition associated with or resulting from neoactivity. For example, if it is determined that the subject has a cell proliferation-related disorder, *e.g., e.g.,* cancer, or a precancerous disorder characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or by a mutant IDH1 or IDH2, having alpha hydroxyl neoactivity, e.g., 2HG, neaoctivity, selecting the subject for treatment with a therapeutic agent

described herein, e.g., an inhibitor (*e.g.,* a small molecule or a nucleic acid-based inhibitor) of the neoactivity of that mutant (*e.g.,* conversion of alpha-ketoglutarate to 2HG, *e.g.,* R-2HG);

correlating the analysis with an outcome or a prognosis;

providing a value for an analysis on which the evaluation is based, *e.g.,* the value for a parameter correlated to the presence, distribution, or level of an alpha hydroxyl neoactivity product, e.g., 2HG, *e.g.,* R-2HG;

providing a recommendation for treatment of the subject; or

memorializing a result of, or ouput from, the method, *e.g.,* a measurement made in the course of performing the method, and optionally transmitting the memorialization to a party, *e.g.,* the subject, a healthcare provider, or an entity that pays for the subject's treatment, *e.g.,* a government, insurance company, or other third party payer.

As described herein, the evaluation can provide information on which a number of decisions or treatments can be based.

Thus, in an embodiment the result of the evaluation, *e.g.,* an increased level of an alpha hydroxyl neoactivity product, e.g., 2HG, *e.g.,* R-2HG, the presence of an IDH, *e.g.,* IDH1 or IDH2, neoactivity, *e.g.,* alpha hydroxyl neoactivity, e.g., 2HG neoactivity, the presence of an IDH, *e.g.,* IDH1 or IDH2, mutant protein (or corresponding RNA) which has alpha hydroxyl neoactivity, e.g., 2HG neoactivity, the presence of a mutant allele of IDH, *e.g.,* IDH1 or IDH2, having alpha hydroxyl neoactivity, 2HG neoactivity, *e.g.,* an allele disclosed herein, is indicative of:

a cell proliferation-related disorder, *e.g.,* cancer, *e.g.,* it is indicative of a primary or metastatic lesion;

the stage of a cell proliferation-related disorder;

a prognosis or outcome for a cell proliferation-related disorder, *e.g.,* it is indicative of a less aggressive form of the disorder, *e.g.,* cancer. *E.g.,* in the case of glioma, presence of an alpha hydroxyl neoactivity product, e.g., 2HG, *e.g.,* R-2HG, can indicate a less aggressive form of the cancer;

the efficacy of a treatment, *e.g.,* the efficacy of a chemotherapeutic agent, irradiation or surgery;

the need of of a therapy disclosed herein, e.g., inhibition a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, neoactive mutant described herein. In an embodiment

relatively higher levels (or the presence of the mutant) is correlated with need of inhibition a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, mutant described herein; or

responsiveness to a treatment. The result can be used as a noninvasive biomarker for clinical response. *E.g.,* elevated levels can be predictive on better outcome in glioma patients (*e.g.,* longer life expectancy).

As described herein, the evaluation can provide for the selection of a subject.

Thus, in an embodiment the method comprises, *e.g.,* responsive to the analysis of one or more of a-d, selecting a subject, *e.g.,* for a treatment. The subject can be selected on a basis described herein, e.g., on the basis of:

said subject being at risk for, or having, higher than normal levels of an alpha hydroxy neoactivity product, e.g., 2-hydroxyglurarate (*e.g.,* R-2HG) in cell having a cell proliferation-related disorder, *e.g.,* a leukemia such as AML or ALL, e.g., B-ALL or T-ALL, or a tumor lesion, *e.g.,* a glioma or a prostate tumor;

said subject having a proliferation-related disorder characterized by a selected IDH, *e.g.,* IDH1 or IDH2 allele, *e.g.,* an IDH1 or IDH2 mutation, having alpha hydroxyl neoactivity, e.g., 2HG neoactivity;

said subject having a selected IDH allele, *e.g.,* a selected IDH1 or IDH2 allele; having alpha hydroxyl neoactivity, e.g., 2HG neoactivity;

said subject having a proliferation-related disorder;

said subject being in need of, or being able to benefit from, a therapeutic agent of a type described herein;

said subject being in need of, or being able to benefit from, a compound that inhibits alpha hydroxyl neoactivity, e.g., 2HG neoactivity;

said subject being in need of, or being able to benefit from, a compound that lowers the level of an alpha hydroxyl neoactivity product, e.g., 2HG, e.g., R-2HG.

In an embodiment evaluation comprises selecting the subject, *e.g.,* for treatment with an anti-neoplastic agent, on the establishment of, or determination that, the subject has increased alpha hydroxyl neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or increased alpha hydroxyl neoactivity, e.g., 2HG neoactivity, or that the subject is in need of inhibition of a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, mutant described herein.

As described herein, the evaluations provided for by methods described herein allow the selection of optimal treatment regimens.

Thus, in an embodiment the method comprises, *e.g.,* responsive to the analysis of one or more of a-d, selecting a treatment for the subject, e.g., selecting a treatment on a basis disclosed herein. The treatment can be the administration of a therapeutic agent disclosed herein. The treatment can be selected on the basis that:

it us useful in treating a disorder charcterized by one or more of alpha hydroxyl neoactivity, e.g., 2HG neoactivity, an IDH1 or IDH2, mutant protein having alpha hydroxyl neoactivity, e.g., 2HG neoactivity (or a corresponding RNA);

it is useful in treating a disorder characterized by a selected somatic allele or mutation of an IDH, *e.g.,* IDH1 or IDH2, which encodes a protein with alpha hydroxyl neoactivity, e.g., 2HG neoactivity, *e.g.,* an allele disclosed herein, in cells characterized by a cell proliferation-related disorder from the subject;

it reduces the level of an alpha hydroxyl neoactivity product, e.g., 2HG, *e.g.,* R-2HG;

it reduces the level of alpha hydroxyl neoactivity, e.g., 2HG neoactivity.

In an embodiment evaluation comprises selecting the subject, *e.g.,* for treatment.

In embodiments the treatment is the administration of a therapeutic agent described herein.

The methods can also include treating a subject, e.g, with a treatment selected in response to, or on the basis of, an evaluation made in the method.

Thus, in an embodiment the method comprises, *e.g.,* responsive to the analysis of one or more of a-d, administerin a treatment to the subject, e.g., the administration of a therapeutic agent of a type described herein.

In an embodiment the therapeutic agent comprises a compound from Table 24a or Table 24b or a compound having the structure of Formula (X) or (XI) described below.

In an embodiment the therapeutic agent comprises nucleic acid, e.g., dsRNA, e.g., a dsRNA described herein.

In an embodiment the the therapeutic agent is an inhibitor, *e.g.,* a polypeptide, peptide, or small molecule (*e.g.,* a molecule of less than 1,000 daltons), or aptomer, that binds to an IDH1 or IDH2 mutant (e.g., an aptomer that binds to an IDH1 mutant) or wildtype subunit and inhibits neoactivity, e.g., by inhibiting formation of a dimer, *e.g.,* a homodimer of mutant IDH1 or IDH2 subunits (e.g., a homodimer of mutant IDH1 subunits) or a heterodimer of a mutant and a wildype subunit. In an

embodiment the inhibitor is a polypeptide. In an embodiment the polypeptide acts as a dominant negative with respect to the neoactivity of the mutant enzyme. The polypeptide can correspond to full length IDH1 or IDH2 or a fragment thereof (e.g., the polypeptide correspondes to full length IDH1 or a fragment thereof). The polypeptide need not be indentical with the corresponding residues of wildtype IDH1 or IDH2 (e.g., wildtype IDH1), but in embodiments has at least 60, 70, 80, 90 or 95 % homology with wildtype IDH1 or IDH2 (e.g., wildtype IDH1).

In an embodiment the therapeutic agent decreases the affinity of an IDH, e.g., IDH1 or IDH2 neoactive mutant protein for NADH, NADPH or a divalent metal ion, e.g., Mg^{2+} or Mn^{2+}, or decreases the levels or availability of NADH, NADPH or divalent metal ion, e.g., Mg^{2+} or Mn^{2+}, *e.g.,* by competing for binding to the mutant enzyme. In an embodiment the enzyme is inhibited by replacing Mg^{2+} or Mn^{2+} with Ca^{2+}.

In an embodiment the therapeutic agent is an inhibitor that reduces the level a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that reduces the level of the product of a mutant having a neoactivity of an IDH, *e.g.,* IDH1 or IDH2 mutant, *e.g.,* it reduces the level of 2HG, *e.g.,* R-2HG.

In an embodiment the therapeutic agent is an inhibitor that:

inhibits, *e.g.,* specifically, a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein, *e.g.,* 2HG neoactivity; or

inhibits both the wildtype activity and a neoactivity of an IDH, *e.g.,* IDH1 orIDH2, *e.g.,* a neoactivity described herein, e.g, 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that is selected on the basis that it:

inhibits, *e.g.,* specifically, a neoactivity of an IDH, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein *e.g.,* 2HG neoactivity; or

inhibits both the wildtype activity and a neoactivity of an IDH1, *e.g.,* IDH1 or IDH2, *e.g.,* a neoactivity described herein, *e.g.,* 2HG neoactivity.

In an embodiment the therapeutic agent is an inhibitor that reduces the amount of a mutant IDH, *e.g.,* IDH1 or IDH2, protein or mRNA.

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* it binds to, the mutant IDH, *e.g.,* IDH1 or IDH2 mRNA.

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* it binds to, the mutant IDH, *e.g.,* IDH1 or IDH2, protein.

In an embodiment the therapeutic agent is an inhibitor that reduces the amount of neoactive enzyme activity, *e.g.,* by interacting with, *e.g.,* binding to, mutant IDH, *e.g.,* IDH1 or IDH2, protein. In an embodiment the inhibitor is other than an antibody.

In an embodiment the therapeutic agent is an inhibitor that is a small molecule and interacts with, *e.g.,* binds, the mutant RNA, *e.g.,* mutant IDH1 mRNA.

In an embodiment the therapeutic agent is an inhibitor that interacts directly with, *e.g.,* binds, either the mutant IDH, *e.g.,* IDH1 or IDH2, protein or interacts directly with, *e.g.,* binds, the mutant IDH mRNA, *e.g.,* IDH1 or IDH2 mRNA.

In an embodiment the therapeutic agent is administered.

In an embodiment the treatment: inhibits, *e.g.,* specifically, a neoactivity of IDH1 or IDH2 (e.g., a neoactivity of IDH1) , *e.g.,* a neoactivity described herein; or inhibits both the wildtype and activity and a neoactivity of IDH1 or IDH2 (e.g., a neoactivity of IDH1), *e.g.,* a neoactivity described herein In an embodiment, the subject is subsequently evaluated or monitored by a method described herein, *e.g.,* the analysis of the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, *e.g.,* to evaluate response to the treatment or progression of disease.

In an embodiment the treatment is selected on the basis that it: inhibits, *e.g.,* specifically, a neoactivity of IDH1 or IDH2 (e.g., a neoactivity of IDH1), *e.g.,* alpha hydroxy neoactivity, e.g., 2HG neoactivity; or inhibits both the wildtype and activity and a neoactivity of IDH1 or IDH2 (e.g., a neoactivity of IDH1), *e.g.,* a neoactivity described herein.

In an embodiment, the method comprises determining the possibility of a mutation other than a mutation in IDH1 or in IDH2. In embodiments a relatively high level of 2HG, *e.g.,* R-2HG is indicative of another mutation.

In an embodiment, which embodiment includes selecting or administering a treatment for the subject, the subject:

has not yet been treated for the subject the cell proliferation-related disorder and the selected or administered treatment is the initial or first line treatment;

has already been treated for the the cell proliferation-related and the selected or administered treatment results in an alteration of the existing treatment;

has already been treated for the the cell proliferation-related, and the selected treatment results in continuation of the existing treatment; or

has already been treated for the the cell proliferation-related disorder and the selected or administered treatment is different, *e.g.,* as compared to what was administered prior to the evaluation or to what would be administered in the absence of elevated levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment, which embodiment includes selecting or administering a treatment for the subject, the selected or administered treatment can comprise:

a treatment which includes administration of a therapeutic agent at different, *e.g.,* a greater (or lesser) dosage (*e.g.,* different as compared to what was administered prior to the evaluation or to what would be administered in the absence of elevated levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG);

a treatment which includes administration of a therapeutic agent at a different frequency, *e.g.,* more or less frequently, or not at all (*e.g.,* different as compared to what was administered prior to the evaluation or to what would be administered in the absence of elevated levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG); or

a treatment which includes administration of a therapeutic agent in a different therapeutic setting (*e.g.,* adding or deleting a second treatment from the treatment regimen) (*e.g.,* different as compared to what was administered prior to the evaluation or to what would be administered in the absence of elevated levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG).

Methods of evaluating a subject described herein can comprise evaluating a neoactivity genotype or phenotype. Methods of obtaining and analyzing samples, and the in vivo analysis in subjects, described elsewhere herein, e.g., in the section entitled, "Methods of evaluating samples and/or subjects," can be combined with this method.

In an embodiment the method comprises:

subjecting the subject (*e.g.,* a subject not having 2-hydroxyglutaric aciduria) to imaging and/or spectroscopic analysis, *e.g.,* magnetic resonance-based analysis, *e.g.,* MRI and/or MRS *e.g.,*imaging analysis, to provide a determination of the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, *e.g.,* as associated with a tumor, *e.g.,* a glioma, in the subject;

optionally storing a parameter related to the determination, *e.g.,* the image or a value related to the image from the imaging analysis, in a tangible medium; and

responsive to the determination, performing one or more of: correlating the determination with outcome or with a prognosis; providing an indication of outcome or prognosis; providing a value for an analysis on which the evaluation is based, *e.g.,* the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG; providing a recommendation for treatment of the subject; selecting a course of treatment for the subject, *e.g.,* a course of treatment described herein, *e.g.,* selecting a course of treatment that includes inhibiting a neoactivity of a mutant IDH, *e.g.,* IDH1 or IDH2, allele, *e.g.,* a neoactivity described herein; administering a course of treatment to the subject, *e.g.,* a course of treatment described herein, *e.g.,* a course of treatment that includes inhibiting a neoactivity of a mutant IDH, *e.g.,* IDH1 or IDH2, allele, *e.g.,* a neoactivity described herein; and memorializing memorializing a result of the method or a measurement made in the course of the method, *e.g.,* one or more of the above and/or transmitting memorialization of one or more of the above to a party, *e.g.,* the subject, a healthcare provider, or an entity that pays for the subject's treatment, *e.g.,* a government, insurance company, or other third party payer.

In an embodiment the method comprises confirming or determining, *e.g.,* by direct examination or evaluation of the subject, or sample *e.g.,* tissue or bodily fluid (*e.g.,* blood (*e.g.,* blood plasma), urine, lymph, or cerebrospinal fluid) therefrom, (*e.g.,* by DNA sequencing or immuno analysis or evaluation of the presence, distribution or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.,* IDH1 or IDH2, allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8), in specific embodiments, an IDH1 allele having His, Ser, Cys, Gly, Val, or Leu at residue 132 or an IDH1 allele having His or Cys at residue 132; or an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10).

In an embodiment, prior to or after treatment, the method includes evaluating the growth, size, weight, invasiveness, stage or other phenotype of the cell proliferation-related disorder.

In an embodiment the cell proliferation-related disorder is a tumor of the CNS, *e.g.,* a glioma, a leukemia, *e.g.,* AML or ALL, e.g., B-ALL or T-ALL, prostate cancer, or myelodysplasia or myelodysplastic syndrome and the evaluation is a or b. In an embodiment the method comprises evaluating a sample, e.g., a sample described

herein, e.g., a tissue, e.g., a cancer sample, or a bodily fluid, e.g., serum or blood, for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment, a subject is subjected to MRS and the evaluation comprises evaluating the presence or elevated amount of a peak correlated to or corresponding to 2HG, *e.g.,* R-2HG, as determined by magnetic resonance. For example, a subject can be analyzed for the presence and/or strength of a signal at about 2.5 ppm to determine the presence and/or amount of 2HG, *e.g.,* R-2HG in the subject.

In an embodiment the method comprises obtaining a sample from the subject and analyzing the sample, or analyzing the subject, *e.g.,* by imaging the subject and optionally forming a representationof the image on a computer.

In an embodiment the results of the analysis is compared to a reference.

In an embodiment a value for a parameter correlated to the presence, distribution, or level, *e.g.,* of 2HG, *e.g.,* R-2HG, is determined. It can be compared with a reference value, *e.g.,* the value for a reference subject not having abnormal presence, level, or distribution, *e.g.,* a reference subject cell not having a mutation in IDH, *e.g.,* IDH1 or IDH2, having a neoactivity described herein.

In an embodiment the method comprises determing if an IDH, e.g., IDH1 or IDH2, mutant allele that is associated with 2HG neoactivity is present. E.g., in the case of IDH1, the presence of a mutaton at residue 132 associated with 2HG neoactivity can be determined. In the case of IDH2, the presence of a mutaton at residue 172 associated with 2HG neoactivity can be determined. The determination can comprise sequencing a nucleic acid, e.g., genomic DNA or cDNA, from an affected cell, which encodes the relevant amino acid(s). The mutation can be a deletion, insertion, rearrangement, or substitution. The mutation can involve a single nucleotide, e.g., a single substitution, or more than one nucleotide, e.g., a deletion of more than one nucleotides.

In an embodiment the method comprises determining the sequence at position 394 or 395 of the IDH1 gene, or determining the identity of amino acid residue 132 (SEQ ID NO:8) in the IDH1 gene in a cell characterized by the cell proliferation related disorder.

In an embodiment the method comprises determining the amino acid sequence, *e.g.,* by DNA sequenceing, at position 172 of the IDH2 gene in a cell characterized by the cell proliferation related disorder.

In an embodiment a product of the neoactivity is 2-HG, *e.g.,* R-2HG, which acts as a metabolite. In another embodiment a product of the neoactivity is 2HG, *e.g.,* R-2HG, which acts as a toxin, *e.g.,* a carcinogen.

In an embodiment the disorder is other than a solid tumor. In an embodiment the disorder is a tumor that, at the time of diagnosis or treatment, does not have a necrotic portion. In an embodiment the disorder is a tumor in which at least 30, 40, 50, 60, 70, 80 or 90% of the tumor cells carry an IHD, e.g., IDH1 or IDH2, mutation having 2HG neoactivity, at the time of diagnosis or treatment.

In an embodiment the cell proliferation-related disorder is a cancer, e.g., a cancer described herein, characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the tumor is characterized by increased levels of an alpha hydroxy neoactivity product, 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a tumor of the CNS, *e.g.,* a glioma, *e.g.,* wherein the tumor is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. Gliomas include astrocytic tumors, oligodendroglial tumors, oligoastrocytic tumors, anaplastic astrocytomas, and glioblastomas. In an embodiment the tumor is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Yan *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **Fig. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132.

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation, specifically C394A or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having a glioma, wherein the cancer is characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8) (e.g., His, Ser, Cys, Gly, Val, or Leu; or His or Cys).

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8) (e.g., His, Ser, Cys, Gly, Val, or Leu; or His or Cys).

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment, the cell proliferation disorder is fibrosarcoma or paraganglioma wherein the cancer is characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment, the cell proliferation disorder is fibrosarcoma or paraganglioma wherein the cancer is characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment, the cell proliferation disorder is fibrosarcoma or paraganglioma wherein the cancer is characterized by increased levels of an alpha hydroxy neoactivity, product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is localized or metastatic prostate cancer, *e.g.,* prostate adenocarcinoma, *e.g.,* wherein the cancer is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type.

E.g., in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**) (e.g., His, Ser, Cys, Gly, Val, or Leu). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at

nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.,* prostate adenocarcinoma, wherein the cancer is characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having prostate cancer, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a hematological cancer, *e.g.,* a leukemia, *e.g.,* AML, or ALL, wherein the hematological cancer is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is acute lymphoblastic leukemia (*e.g.,* an adult or pediatric form), *e.g.,* wherein the acute lymphoblastic leukemia (sometimes referred to herein as ALL) is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. The ALL can be, e.g., B-ALL or T-ALL. In an embodiment the cancer is characterized by increased levels of 2 an alpha hydroxy neoactivity product, e.g., HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et a.l*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**) (e.g., His, Ser, Cys, Gly, Val, or Leu). In an embodiment the allele encodes an IDH1 having Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 according to the sequence of SEQ ID NO:8.

In an embodiment the method comprises selecting a subject ALL, e.g., B-ALL or T-ALL, on the basis of cancer being characterized by having an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is acute myelogenous leukemia (*e.g.,* an adult or pediatric form), *e.g.,* wherein the acute myelogenous leukemia (sometimes referred to herein as AML) is characterized by an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.,* a mutant described herein. In an embodiment the cancer is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.,* in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**) (e.g., His, Ser, Cys, Gly, Val or Leu). In an embodiment the allele encodes an IDH1 having Cys, His or Gly at residue 132, specifically, Cys.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML) characterized by an IDH1 allele described herein, *e.g.,* an IDH1 allele having Cys, His or Gly at residue 132 according to the sequence of SEQ ID NO:8, specifically, Cys.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML) on the basis of cancer being

characterized by having an IDH1 allele described herein, *e.g.*, an IDH1 allele having Cys, His or Gly at residue 132 (SEQ ID NO:8), specifically, Cys.

In an embodiment the method comprises selecting a subject having acute myelogenous lymphoplastic leukemia (AML), on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.*, R-2HG. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.*, R-2HG.

In an embodiment the method further comprises evaluating the subject for the presence of a mutation in the NRAS or NPMc gene.

In an embodiment the cell proliferation-related disorder is myelodysplasia or myelodysplastic syndrome, *e.g.*, wherein the myelodysplasia or myelodysplastic syndrome is characterized by having an IDH1 somatic mutant having alpha hydroxy neoactivity, e.g., 2HG neoactivity, *e.g.*, a mutant described herein. In an embodiment the disorder is characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, e.g., R-2HG, as compared to non-diseased cells of the same type. *E.g.*, in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.*, the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et a.l*, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**), specifically, His, Ser, Cys, Gly, Val, or Leu. In an embodiment the allele encodes an IDH1 having Cys at residue 132.

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome characterized by an IDH1 allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 according to the sequence of SEQ ID NO:8.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome on the basis of cancer being characterized by having an IDH1 allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG,

e.g., R-2HG. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a glioma, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically, Lys, Gly, Met, Trp or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having a glioma, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), specifically Lys, Gly, Met, Trp, or Ser; or Lys or Met.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), specifically Lys, Gly, Met, Trp, or Ser; or Lys or Met.

In an embodiment the method comprises selecting a subject having a glioma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is a prostate cancer, *e.g.,* prostate adenocarcinoma, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, wherein the cancer is characterized by having

an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having a prostate cancer, *e.g.,* prostate adenocarcinoma, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is ALL, e.g., B-ALL or T-ALL, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having ALL, e.g., B-ALL or T-ALL, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is AML, characterized by a mutation, or preselected allele, of IDH2 associated with an alpha hydroxy neoactivity, e.g., 2HG neoactivity. *E.g.,* in an embodiment, the IDH2 allele encodes

an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having AML, wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having AML, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having AML, on the basis of the cancer being characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In an embodiment the cell proliferation-related disorder is myelodysplasia or myelodysplastic syndrome, characterized by a mutation, or preselected allele, of IDH2. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome,wherein the cancer is characterized by having an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being characterized by an IDH2 allele described herein, *e.g.,* an IDH2 allele having Lys or Met at residue 172 (SEQ ID NO:10).

In an embodiment the method comprises selecting a subject having myelodysplasia or myelodysplastic syndrome, on the basis of the cancer being

characterized by increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. In an embodiment the method comprises evaluating a serum or blood sample for increased alpha neoactivity product, e.g., 2HG, *e.g.,* R-2HG.

In another aspect the invention features a pharmaceutical composition of an inhibitor (*e.g.,* a small molecule or a nucleic acid-based inhibitor) described herein.

In an embodiment a mutant protein specific reagent, e.g., an antibody that specifically binds an IDH mutant protein, e.g., an antibody that specifically binds an IDH1-R132H mutant protein, can be used to detect neoactive mutant enzyme see, for example, that described by Y.Kato et al., "A monoclonal antibody IMab-1 specifically recognizes $IDH1^{R132H}$, the most common glioma-derived mutation: (Kato, Biochem. Biophys. Res. Commun. (2009), which is hereby incorporated by reference in its entirety.

In another aspect, the invention features, a method of evaluating a candidate compound, *e.g.,* for the ability to inhibit a neoactivity of a mutant enzyme, *e.g.,* for use as an anti-proliferative or anti-cancer agent. In an embodiment the mutant enzyme is an IDH, *e.g.,* an IDH1 or IDH2 mutant, *e.g.,* a mutant described herein. In an embodiment the neaoctivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. The method comprises:

optionally supplying the candidate compound;

contacting the candidate compound with a mutant enzyme having a neoactivity, or with another enzyme, a referred to herein as a proxy enzyme, having an activity, referred to herein as a proxy activity, which is the same as the neoactivity (or with a cell or cell lysate comprising the same); and

evaluating the ability of the candidate compound to modulate, *e.g.,* inhibit or promote, the neoactivity or the proxy activity,

thereby evaluating the candidate compound.

In an embodiment the mutant enzyme is a mutant IDH1, *e.g.,* an IDH1 mutant described herein, and the neoactivity is an alpha hydroxy neoactivity, e.g., 2HG neoactivity. Mutations associated with 2HG neoactivity in IDH1 include mutations at residue 132, e.g., R132H, R132C, R132S, R132G, R132L,or R132V, more specifically, R132H or R132C.

In an embodiment the mutant enzyme is a mutant IDH2, *e.g.,* an IDH2 mutant described herein, and the neoactivity is an alpha hydroxy neoactivity, e.g., 2HG

neoactivity. Mutations associated with 2HG neoactivity in IDH2 inlcude mutations at residue 172, e.g., R172K, R172M, R172S, R172G, or R172W.

In an embodiment the method includes evaluating the ability of the candidate compound to inhibit the neoactivity or the proxy activity.

In an embodiment the method further comprises evaluating the ability of the candidate compound to inhibit the forward reaction of non-mutant or wild type enzyme activity, *e.g.,* in the case of IDH, *e.g.,* IDH1 or IDH2, the conversion of isocitrate to α-ketoglutarate (or an intermediate thereof, including the reduced hydroxyl intermediate).

In an embodiment, the contacting step comprises contacting the candidate compound with a cell, or a cell lysate thereof, wherein the cell comprises a mutant enzyme having the neoactivity or an enzyme having the activity.

In an embodiment, the cell comprises a mutation, or preselected allele, of a mutant IDH1 gene. *E.g.,* in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.,* the allele can encode His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**), specifically His, Ser, Cys, Gly, Val, or Leu.

In an embodiment the allele encodes an IDH1 having His at residue 132.

In an embodiment the allele encodes an IDH1 having Ser at residue 132.

In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment, the cell comprises a mutation, or preselected allele, of a mutant IDH2 gene. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically, Lys, Gly, Met, Trp, or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment, the cell includes a heterologous copy of a mutant IDH gene, *e.g.,* a mutant IDH1 or IDH2 gene. (Heterologous copy refers to a copy introduced or formed by a genetic engineering manipulation.)

In an embodiment, the cell is transfected (*e.g.,* transiently or stably transfected) or transduced (*e.g.,* transiently or stably transduced) with a nucleic acid sequence

encoding an IDH, *e.g.,* IDH1 or IDH2, described herein, *e.g.,* an IDH1 having other than an Arg at residue 132. In an embodiment, the IDH, *e.g.,* IDH1 or IDH2, is epitope-tagged, *e.g.,* myc-tagged.

In an embodiment, the cell, *e.g.,* a cancer cell, is non-mutant or wild type for the IDH, *e.g.,* IDH1 or IDH2, allele. The cell can include a heterologous IDH1 or IDH2 mutant.

In an embodiment, the cell is a cultured cell, *e.g.,* a primary cell, a secondary cell, or a cell line. In an embodiment, the cell is a cancer cell, *e.g.,* a glioma cell (*e.g.,* a glioblastoma cell), a prostate cancer cell, a leukemia cell (*e.g.,* an ALL, e.g., B-ALL or T-ALL, cell or AML cell) or a cell characterized by myelodysplasia or myelodysplastic syndrome. In embodiment, the cell is a 293T cell, a U87MG cell, or an LN-18 cell (*e.g.,* ATCC HTB-14 or CRL-2610).

In an embodiment, the cell is from a subject, *e.g.,* a subject having cancer, *e.g.,* a cancer characterized by an IDH, *e.g.,* IDH1 or IDH2, allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8); specifically His or Cys; or an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), specifically Lys, Gly, Met, Trp, or Ser.

In an embodiment, the evaluating step comprises evaluating the presence and/or amount of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, *e.g.,* in the cell lysate or culture medium, *e.g.,* by LC-MS.

In an embodiment, the evaluating step comprises evaluating the presence and/or amount of an alpha hydroxy neoactivity, e.g., 2HG neoactivity, in the cell lysate or culture medium.

In an embodiment, the method further comprises evaluating the presence/amount one or more of TCA metabolite(s), *e.g.,* citrate, α-KG, succinate, fumarate, and/or malate, *e.g.,* by LC-MS, *e.g.,* as a control.

In an embodiment, the method further comprises evaluating the oxidation state of NADPH, *e.g.,* the absorbance at 340 nm, *e.g.,* by spectrophotometer.

In an embodiment, the method further comprises evaluating the ability of the candidate compound to inhibit a second enzymatic activity, *e.g.,* the forward reaction of non-mutant or wild type enzyme activity, *e.g.,* in the case of IDH1 or IDH2 (e.g., IDH1), the conversion of isocitrate to α-ketoglutarate (or an intermediate thereof, including the reduced hydroxyl intermediate).

In an embodiment, the candidate compound is a small molecule, a polypeptide, peptide, a carbohydrate based molecule, or an aptamer (*e.g.,* a nucleic acid aptamer, or a peptide aptamer). The method can be used broadly and can, *e.g.,* be used as one or more of a primary screen, to confirm candidates produced by this or other methods or screens, or generally to guide drug discovery or drug candidate optimization.

In an embodiment, the method comprises evaluating, *e.g.,* confirming, the ability of a candidate compound (*e.g.,* a candidate compound which meets a predetermined level of inhibition in the evaluating step) to inhibit the neoactivity or proxy activity in a second assay.

In an embodiment, the second assay comprises repeating one or more of the contacting and/or evaluating step(s) of the basic method.

In another embodiment, the second assay is different from the first. *E.g.,* where the first assay can use a cell or cell lysate or other non-whole animal model the second assay can use an animal model, *e.g.,* a tumor transplant model, *e.g.,* a mouse having an IDH, *e.g.,* IDH1 or IDH2, mutant cell or tumor transplanted in it. E.g., a U87 cell, or glioma, e.g., glioblastoma, cell, harboring a transfected IDH, e.g., IDH1 or IDH2, neoactive mutant can be implanted as a xenograft and used in an assay. Primary human glioma or AML tumor cells can be grafted into mice to allow propogation of the tumor and used in an assay. A genetically engineered mouse model (GEMM) harboring an IDH1 or IDH2 mutation and/or other mutation, e.g., a p53 null mutation, can also be used in an assay.

In an embodiment the method comprises:

optionally supplying the candidate compound;

contacting the candidate compound with a cell comprising a nucleic acid sequence, *e.g.,* a heterologous sequence, encoding an IDH1 having other than an Arg at residue 132 (*e.g.,* IDH1R132H) or an IDH2 having other than an Arg at residue 172 (specifically an IDH1 having other than an Arg at residue 132); and

evaluating the presence and/or amount of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, in the cell lysate or culture medium, by LC-MS, thereby evaluating the compound.

In an embodiment the result of the evaluation is compared with a reference, *e.g.,* the level of product, *e.g.,* an alpha hydroxy neoactivity product, e.g., 2HG. *e.g.,* R-2HG, in a control cell, *e.g.,* a cell having inserted therein a wild type or non-mutant copy of IDH1 or IDH2 (e.g., IDH1).

In another aspect, the invention features, a method of evaluating a candidate compound, *e.g.,* for the ability to inhibit an RNA encoding a mutant enzyme having a neoactivity, *e.g.,* for use as an anti-proliferative or anti-cancer agent. In an embodiment the mutant enzyme is an IDH, *e.g.,* an IDH1 or IDH2 mutant, *e.g.,* a mutant described herein. In an embodiment the neaoctivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. The method comprises:

optionally supplying the candidate compound, *e.g.,* a nucleic acid based inhibitor (*e.g.,* a dsRNA (*e.g.,* siRNA or shRNA), an antisense, or a microRNA);

contacting the candidate compound with an RNA, *e.g.,* an mRNA, which encodes IDH, *e.g.,* an IDH1 or IDH2, *e.g.,* an RNA that encode mutant enzyme having a neoactivity (or with a cell or cell lysate comprising the same); and

evaluating the ability of the candidate compound to inhibit the RNA, thereby evaluating the candidate compound. By inhibit the RNA means, *e.g.,* to cleave or otherwise inactivate the RNA.

In an embodiment the RNA encodes a fusion of all or part of the IDH, *e.g.,* IDH1 or IDH2, wildtype or mutant protein to a second protein, *e.g.,* a reporter protein, *e.g.,* a fluorescent protein, *e.g.,* a green or red fluorescent protein.

In an embodiment the mutant enzyme is a mutant IDH1, *e.g.,* an IDH1 mutant described herein, and the neoactivity is an alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment the mutant enzyme is a mutant IDH2, *e.g.,* an IDH2 mutant described herein, and the neoactivity is an alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment, the contacting step comprises contacting the candidate compound with a cell, or a cell lysate thereof, wherein the cell comprises RNA encoding IDH, *e.g.,* IDH1 or IDH2, *e.g.,* a mutant IDH, *e.g.,* IDH1 or IDH2, enzyme having the neoactivity.

In an embodiment, the cell comprises a mutation, or preselected allele, of a mutant IDH1 gene. *E.g.,* in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.,* the allele can encode His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**), specifically His, Ser, Cys, Gly, Val, or Leu.

In an embodiment the allele encodes an IDH1 having His at residue 132.

In an embodiment the allele encodes an IDH1 having Ser at residue 132.

In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment, the cell comprises a mutation, or preselected allele, of a mutant IDH2 gene. *E.g.,* in an embodiment, the IDH2 allele encodes an IDH2 having other than an Arg at residue 172. *E.g.,* the allele encodes Lys, Gly, Met, Trp, Thr, Ser, or any residue described in described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10(see also **Fig. 22**), specifically Lys, Gly, Met, Trp or Ser. In an embodiment the allele encodes an IDH2 having Lys at residue 172. In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment, the cell includes a heterologous copy of a wildtype or mutant IDH gene, *e.g.,* a wildtype or mutant IDH1 or IDH2 gene. (Heterologous copy refers to a copy introduced or formed by a genetic engineering manipulation.) In an embodiment the heterologous gene comprises a fusion to a reporter protein, *e.g.,* a fluorescent protein, *e.g.,* a green or red fluorescent protein.

In an embodiment, the cell is transfected (*e.g.,* transiently or stably transfected) or transduced (*e.g.,* transiently or stably transduced) with a nucleic acid sequence encoding an IDH, *e.g.,* IDH1 or IDH2, described herein, *e.g.,* an IDH1 having other than an Arg at residue 132 or an IDH2 having other than an Arg at residue 172 (e.g., an IDH1 having other than an Arg at residue 132). In an embodiment, the IDH, *e.g.,* IDH1 or IDH2, is epitope-tagged, *e.g.,* myc-tagged.

In an embodiment, the cell, *e.g.,* a cancer cell, is non-mutant or wild type for the IDH, *e.g.,* IDH1 or IDH2, allele. The cell can include a heterologous IDH1 or IDH2 mutant.

In an embodiment, the cell is a cultured cell, *e.g.,* a primary cell, a secondary cell, or a cell line. In an embodiment, the cell is a cancer cell, *e.g.,* a glioma cell (*e.g.,* a glioblastoma cell), a prostate cancer cell, a leukemia cell (*e.g.,* an ALL, e.g., B-ALL or T-ALL cell or AML cell) or a cell characterized by myelodysplasia or myelodysplastic syndrome. In embodiment, the cell is a 293T cell, a U87MG cell, or an LN-18 cell (*e.g.,* ATCC HTB-14 or CRL-2610).

In an embodiment, the cell is from a subject, *e.g.,* a subject having cancer, *e.g.,* a cancer characterized by an IDH, *e.g.,* IDH1 or IDH2, allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8); specifically His or Cys. In an embodiment, the cancer is characterized by an IDH2

allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10), specifically Lys, Gly, Met, Trp, or Ser.

In an embodiment, the method comprises a second assay and the second assay comprises repeating one or more of the contacting and/or evaluating step(s) of the basic method.

In another embodiment, the second assay is different from the first. *E.g.,* where the first assay can use a cell or cell lysate or other non-whole animal model the second assay can use an animal model

In an embodiment the efficacy of the candidate is evaluated by its effect on reporter protein activity.

In another aspect, the invention features, a method of evaluating a candidate compound, *e.g.,* for the ability to inhibit transcription of an RNA encoding a mutant enzyme having a neoactivity, *e.g.,* for use as an anti-proliferative or anti-cancer agent. In an embodiment the mutant enzyme is an IDH, *e.g.,* an IDH1 or IDH2 mutant, *e.g.,* a mutant described herein. In an embodiment the neaoctivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity. The method comprises:

optionally supplying the candidate compound, *e.g.,* a small molecule, polypeptide, peptide, aptomer, a carbohydrate-based molecule or nucleic acid based molecule;

contacting the candidate compound with a system comprising a cell or cell lysate; and

evaluating the ability of the candidate compound to inhibit the translation of IDH, *e.g.,* IDH1 or IDH2, RNA, e.g,

thereby evaluating the candidate compound.

In an embodiment the the system comprises a fusion gene encoding of all or part of the IDH, *e.g.,* IDH1 or IDH2, wildtype or mutant protein to a second protein, *e.g.,* a reporter protein, *e.g.,* a fluorescent protein, *e.g.,* a green or red fluorescent protein.

In an embodiment the mutant enzyme is a mutant IDH1, *e.g.,* an IDH1 mutant described herein, and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment the mutant enzyme is a mutant IDH2, *e.g.,* an IDH2 mutant described herein, and the neoactivity is alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment, the system includes a heterologous copy of a wildtype or mutant IDH gene, *e.g.,* a wildtype or mutant IDH1 or IDH2 gene. (Heterologous copy refers to a copy introduced or formed by a genetic engineering manipulation.) In an embodiment the heterologous gene comprises a fusion to a reporter protein, *e.g.,* a fluorescent protein, *e.g.,* a green or red fluorescent protein.

In an embodiment the cell, *e.g.,* a cancer cell, is non-mutant or wild type for the IDH, *e.g.,* IDH1 or IDH2, allele. The cell can include a heterologous IDH1 or IDH2 mutant.

In an embodiment, the cell is a cultured cell, *e.g.,* a primary cell, a secondary cell, or a cell line. In an embodiment, the cell is a cancer cell, *e.g.,* a glioma cell (*e.g.,* a glioblastoma cell), a prostate cancer cell, a leukemia cell (*e.g.,* an ALL, e.g., B-ALL or T-ALL, cell or AML cell) or a cell characterized by myelodysplasia or myelodysplastic syndrome. In embodiment, the cell is a 293T cell, a U87MG cell, or an LN-18 cell (*e.g.,* ATCC HTB-14 or CRL-2610).

In an embodiment, the cell is from a subject, *e.g.,* a subject having cancer, *e.g.,* a cancer characterized by an IDH, *e.g.,* IDH1 or IDH2, allele described herein, *e.g.,* an IDH1 allele having His, Ser, Cys, Gly, Val, Pro or Leu at residue 132 (SEQ ID NO:8); specifically His, Ser, Cys, Gly, Val, or Leu. In an embodiment, the cancer is characterized an IDH2 allele having Lys, Gly, Met, Trp, Thr, or Ser at residue 172 (SEQ ID NO:10).

In an embodiment, the method comprises a second assay and the second assay comprises comprises repeating the method.

In another embodiment, the second assay is different from the first. *E.g.,* where the first assay can use a cell or cell lysate or other non-whole animal model the second assay can use an animal model.

In an embodiment the efficacy of the candidate is evaluated by its effect on reporter protein activity.

In another aspect, the invention features, a method of evaluating a candidate compound, e.g., a therapeutic agent, or inhibitor, described herein in an animal model. The candidate compound can be, *e.g.,* a small molecule, polypeptide, peptide, aptomer, a carbohydrate-based molecule or nucleic acid based molecule. The method comprises, contacting the candidate with the animal model and evaluating the animal model.

In an embodiment evaluating comprises;

determining an effect of the compound on the general health of the animal;

determining an effect of the compound on the weight of the animal;

determining an effect of the compound on liver function, e.g, on a liver enzyme;

determining an effect of the compound on the cardiovascular system of the animal;

determining an effect of the compound on neurofunction, *e.g.,* on neuromuscular control or response;

determining an effect tof the compound on eating or drinking;

determining the distribution of the compound in the animal;

determining the persistence of the compound in the animal or in a tissue or oragn of the animal, *e.g.,* determining plasma half-life; or

determining an effect of the compound on a selected cell in the animal;

determining an effect of the compound on the growth, size, weight, invasiveness or other phenotype of a tumor, *e.g.,* an endogenous tumor or a tumor arising from introduction of cells from the same or a different species.

In an embodiment the animal is a non-human primate, *e.g.,* a cynomolgus monkey or chimpanzee.

In an embodiment the animal is a rodent, *e.g.,* a rat or mouse.

In an embodiment the animal is a large animal, *e.g.,* a dog or pig, other than a non-human primate.

In an embodiment the evaluation is memorialized and optionally transmetted to another party.

In one aspect, the invention provides, a method of evaluating or processing a therapeutic agent, *e.g.,* a therapeutic agent referred to herein, *e.g.,* a therapeutic agent that results in a lowering of the level of a product of an IDH, *e.g.,* IDH1 or IDH2, mutant having a neoactivity. In an embodiment the neoactivity is an alpha hydroxy neoactivity, e.g., 2HG neoactivity, and the level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, is lowered.

The method includes:

providing, *e.g.,* by testing a sample, a value (*e.g.,* a test value) for a parameter related to a property of the therapeutic agent, *e.g.,* the ability to inhibit the conversion of alpha ketoglutarate to 2 hydroxyglutarate (i.e., 2HG), *e.g.,* R-2 hydroxyglutarate (i.e., R-2HG), and,

optionally, providing a determination of whether the value determined for the parameter meets a preselected criterion, *e.g.,* is present, or is present within a preselected range,

thereby evaluating or processing the therapeutic agent.

In an embodiment the therapeutic agent is approved for use in humans by a government agency, *e.g.,* the FDA.

In an embodiment the parameter is correlated to the ability to inhibit 2HG neoactivity, and, *e.g.,* the therapeutic agent is an inhibitor which binds to IDH1 or IDH2 protein and reduces an alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment the parameter is correlated to the level of mutant IDH, *e.g.,* IDH1 or IDH2, protein, and, *e.g.,* the therapeutic agent is an inhibitor which reduces the level of IDH1 or IDH2 mutant protein.

In an embodiment the parameter is correlated to the level of an RNA that encodes a mutant IDH, *e.g.,* IDH1 or IDH2, protein, and, *e.g.,* the therapeutic agent reduces the level of RNA, *e.g.,* mRNA, that encodes IDH1 or IDH2 mutant protein.

In an embodiment the method includes contacting the therapeutic agent with a mutant IDH, *e.g.,* IDH1 or IDH2, protein (or corresponding RNA).

In an embodiment, the method includes providing a comparison of the value determined for a parameter with a reference value or values, to thereby evaluate the therapeutic agent. In an embodiment, the comparison includes determining if a test value determined for the therapeutic agent has a preselected relationship with the reference value, *e.g.,* determining if it meets the reference value. The value need not be a numerical value but, *e.g.,* can be merely an indication of whether an activity is present.

In an embodiment the method includes determining if a test value is equal to or greater than a reference value, if it is less than or equal to a reference value, or if it falls within a range (either inclusive or exclusive of one or both endpoints). In an embodiment, the test value, or an indication of whether the preselected criterion is met, can be memorialized, *e.g.,* in a computer readable record.

In an embodiment, a decision or step is taken, *e.g.,* a sample containing the therapeutic agent, or a batch of the therapeutic agent, is classified, selected, accepted or discarded, released or withheld, processed into a drug product, shipped, moved to a different location, formulated, labeled, packaged, contacted with, or put into, a container, *e.g.,* a gas or liquid tight container, released into commerce, or sold or

offered for sale, or a record made or altered to reflect the determination, depending on whether the preselected criterion is met. *E.g.,* based on the result of the determination or whether an activity is present, or upon comparison to a reference standard, the batch from which the sample is taken can be processed, *e.g.,* as just described.

The evaluation of the presence or level of activity can show if the therapeutic agent meets a reference standard.

In an embodiment, methods and compositions disclosed herein are useful from a process standpoint, *e.g.,* to monitor or ensure batch-to-batch consistency or quality, or to evaluate a sample with regard to a reference, *e.g.,* a preselected value.

In an embodiment, the method can be used to determine if a test batch of a therapeutic agent can be expected to have one or more of the properties. Such properties can include a property listed on the product insert of a therapeutic agent, a property appearing in a compendium, *e.g.,* the US Pharmacopea, or a property required by a regulatory agency, *e.g.,* the FDA, for commercial use.

In an embodiment the method includes testing the therapeutic agent for its effect on the wildtype activity of an IDH, *e.g.,* IDH1 or IDH2, protein, and providing a determination of whether the value determined meets a preselected criterion, *e.g.,* is present, or is present within a preselected range.

In an embodiment the method includes:

contacting a therapeutic agent that is an inhibitor of IDH1 an alpha hydroxy neoactivity, e.g., 2HG neoactivity, with an IDH1 mutant having an alpha hydroxy neoactivity, e.g., 2HG neoactivity,

determining a value related to the inhibition of an alpha hydroxy neoactivity, e.g., 2HG neoactivity, and

comparing the value determined with a reference value, *e.g.,* a range of values, for the inhibition of an alpha hydroxy neoactivity, e.g., 2HG neoactivity. In an embodiment the reference value is an FDA required value, *e.g.,* a release criteria.

In an embodiment the method includes:

contacting a therapeutic agent that is an inhibitor of mRNA which encodes a mutant IDH1 having an alpha hydroxy neoactivity, e.g., 2HG neoactivity, with an mRNA that encodes an IDH1 mutant having an alpha hydroxy neoactivity, e.g., 2HG neoactivity,

determining a value related to the inhibition of the mRNA, and,

comparing the value determined with a reference value, *e.g.,* a range of values for inhibition of the mRNA. In an embodiment the reference value is an FDA required value, *e.g.,* a release criteria.

In one aspect, the invention features a method of evaluating a sample of a therapeutic agent, *e.g.,* a therapeutic agent referred to herein, that includes receiving data with regard to an activity of the therapeutic agent; providing a record which includes said data and optionally includes an identifier for a batch of therapeutic agent; submitting said record to a decision-maker, *e.g.,* a government agency, *e.g.,* the FDA; optionally, receiving a communication from said decision maker; optionally, deciding whether to release market the batch of therapeutic agent based on the communication from the decision maker. In one embodiment, the method further includes releasing, or other wise processing, *e.g.,* as described herein, the sample.

In another aspect, the invention features, a method of selecting a payment class for treatment with a therapeutic agent described herein, *e.g.,* an inhibitor of IDH, *e.g.,* IDH1 or IDH2, neoactivity, for a subject having a cell proliferation-related disorder. The method includes:

providing (*e.g.,* receiving) an evaluation of whether the subject is positive for increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., 2HG neoactivity, a mutant IDH1 or IDH2 having neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., 2HG neoactivity, (or a corresponding RNA), or a mutant IDH, *e.g.,* IDH1 or IDH2, somatic gene, *e.g.,* a mutant described herein, and

performing at least one of (1) if the subject is positive selecting a first payment class, and (2) if the subject is a not positive selecting a second payment class.

In an embodiment the selection is memorialized, *e.g.,* in a medical records system.

In an embodiment the method includes evaluation of whether the subject is positive for increased levels of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., 2HG neoactivity.

In an embodiment the method includes requesting the evaluation.

In an embodiment the evaluation is performed on the subject by a method described herein.

In an embodiment, the method comprises communicating the selection to another party, *e.g.,* by computer, compact disc, telephone, facsimile, email, or letter.

In an embodiment, the method comprises making or authorizing payment for said treatment.

In an embodiment, payment is by a first party to a second party. In some embodiments, the first party is other than the subject. In some embodiments, the first party is selected from a third party payor, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the drug. In some embodiments, the first party is an insurance company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the drug. In some embodiments, the first party is a governmental entity and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the drug.

As used herein, a cell proliferation-related disorder is a disorder characterized by unwanted cell proliferation or by a predisposition to lead to unwanted cell proliferation (sometimes referred to as a precancerous disorder). Examples of disorders characterized by unwanted cell proliferation include cancers, *e.g.,* tumors of the CNS, *e.g.,* a glioma. Gliomas include astrocytic tumors, oligodendroglial tumors, oligoastrocytic tumors, anaplastic astrocytomas, and glioblastomas. Other examples include hematological cancers, *e.g.,* a leukemia, *e.g.,* AML (*e.g.,* an adult or pediatric form) or ALL, e.g., B-ALL or T-ALL (*e.g.,* an adult or pediatric form), localized or metastatic prostate cancer, *e.g.,* prostate adenocarcinoma, fibrosarcoma, and paraganglioma; specificallya leukemia, *e.g.,* AML (*e.g.,* an adult or pediatric form) or ALL, e.g., B-ALL or T-ALL (*e.g.,* an adult or pediatric form), localized or metastatic prostate cancer, *e.g.,* prostate adenocarcinoma. Examples of disorders characterized by a predisposition to lead to unwanted cell proliferation include myelodysplasia or myelodysplastic syndrome, which are a diverse collection of hematological conditions marked by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

As used herein, specifically inhibits a neoactivity (and similar language), means the neoactivity of the mutant enzyme is inhibted to a significantly greater

degree than is the wildtype enzyme activity. By way of example, "specifically inhibits the 2HG neoactivity of mutant IDH1 (or IDH2)" means the 2HG neoactivity is inhibited to a significantly greater degree than is the forward reaction (the conversion of isocitrate to alpha ketoglutarate) of wildtype IDH1 (or IDH2) activity. In embodiments the neactivity is inhibited at least 2, 5, 10, or 100 fold more than the wildtype activity. In embodiments an inhibitor that is specfic for the 2HG neaoctivity of IDH, e.g., IDH1 or IDH2, will also inhibit another dehydrogenase, e.g., malate dehydrogenase. In other embodiments the specific inhibitor does inhibit other dehydrogenases, e.g., malate dehydrogenase.

As used herein, a cell proliferation-related disorder, *e.g.,* a cancer, characterized by a mutation or allele, means a cell proliferation-related disorder having a substantial number of cells which carry that mutation or allele. In an embodiment at least 10, 25, 50, 75, 90, 95 or 99% of the cell proliferation-related disorder cells, *e.g.,* the cells of a cancer, or a representative, average or typical sample of cancer cells, *e.g.,* from a tumor or from affected blood cells, carry at least one copy of the mutation or allele. A cell proliferation-related disorder, characterized by a mutant IDH, *e.g.,* a mutant IDH1 or mutant IDH2, having 2HG neoactivity is exemplary. In an embodiment the mutation or allele is present as a heterozygote at the indicated frequencies.

As used herein, a "SNP" is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual).

As used herein, a subject can be a human or non-human subject. Non-human subjects include non-human primates, rodents, *e.g.,* mice or rats, or other non-human animals.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts DNA sequence verification of pET41a-IDH1 and alignment against published IDH1 CDS. The sequence of IDH1 (CDS) corresponds to SEQ ID NO:5. The sequence of pET41a-IDH1 corresponds to SEQ ID NO:6, and the "consensus" sequence corresponds to SEQ ID NO:7.

FIG. 2 depicts DNA sequence verification of R132S and R132H mutants according to the SEQ ID NO:8. The amino acid sequence of IDH1 (SEQ ID NO:8) is provided in FIG. 21.

FIG. 3 depicts separation of wild type IDH1 protein on Ni-Sepharose column.

FIG. 4 depicts protein analysis of wild type IDH1 on SDS gel pre and post Ni column fractionation. T: total protein; I: insoluble fractions; S: soluble fraction; L: sample for loading on Ni-column. The numbers in the figure indicates the fraction numbers. Fractions #17 ~ #27 were collected for further purification.

FIG. 5A depicts separation of wild type IDH1 protein through SEC column S-200.

FIG. 5B depicts protein analysis of wild type IDH1 on SDS gel pre and post S-200 column fractionation. M: molecular weight marker; Ni: nickel column fraction prior to S-200; S200: fraction from SEC column.

FIG. 6 depicts separation of mutant R132S protein on Ni-Sepharose column.

FIG. 7 depicts protein analysis of mutant R132S on SDS gel pre and post Ni column fractionation. M: protein marker (KDa): 116, 66.2, 45, 35, 25, 18.4, 14.4; T: total cell protein; So: soluble fraction; In: insoluble fraction; Ft: flow through. #3-#7 indicate the corresponding eluted fraction numbers.

FIG. 8A depicts separation of mutant R132S protein through SEC column S-200.

FIG. 8B depicts protein analysis of mutant R132S on SDS gel post S-200 column fractionation. M: molecular weight marker; R132S: fraction from SEC column.

FIG. 9 depicts separation of mutant R132H protein on Ni-Sepharose column.

FIG. 10 depicts protein analysis of mutant R132H on SDS gel pre and post Ni column fractionation. M: protein marker (KDa): 116, 66.2, 45, 35, 25, 18.4, 14.4; T: total cell protein; So: soluble fraction; In: insoluble fraction; Ft: flow through; #5-#10 indicate the corresponding eluted fraction numbers; Ni: sample from Ni-Sepharose column, pool #5-#10 together.

FIG. 11A depicts separation of mutant R132H protein through SEC column S-200.

FIG. 11B depicts protein analysis of mutant R132H on SDS gel post S-200 column fractionation. M: molecular weight marker; R132H: fraction from SEC column.

FIG. 12A depicts Michaelis-Menten plot of IDH1 wild-type in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 12B depicts Michaelis-Menten plot of R132H mutant enzyme in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 12C depicts Michaelis-Menten plot of R132S mutant enzyme in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 13A depicts α-KG inhibition of IDH1 wild-type.

FIG. 13B depicts α-KG inhibition of R132H mutant enzyme.

FIG. 13C depicts α-KG inhibition of R132S mutant enzyme.

FIG. 14 depicts IDH1 wt, R132H, and R132S in the conversion α-ketoglutarate to 2-hydroxyglutarate.

FIG. 15A depicts Substrate-Concentration velocity plot for R132H mutant enzyme.

FIG. 15B depicts Substrate-Concentration velocity plot for R132S mutant enzyme.

FIG. 16 depicts IDH1 wt, R132H, and R132S in the conversion α-ketoglutarate to 2-hydroxyglutarate with NADH.

FIG. 17A depicts oxalomalate inhibition to IDH1 wt.

FIG. 17B depicts oxalomalate inhibition to R132H.

FIG. 17C depicts oxalomalate inhibition to R132S.

FIG. 18A depicts LC-MS/MS analysis of the control reaction.

FIG. 18B depicts LC-MS/MS analysis of the reaction containing enzyme.

FIG. 18C depicts LC-MS/MS analysis of the spiked control reaction.

FIG. 19 depicts LC-MS/MS analysis of alpha-hydroxyglutarate.

FIG. 20 depicts LC-MS/MS analysis showing that R132H consumes α-KG to produce 2-hydroxyglutaric acid.

FIG. 21 depicts the amino acid sequence of IDH1 (SEQ ID NO:13) as described in GenBank Accession No. NP_005887.2 (GI No. 28178825) (record dated May 10, 2009).

FIG. 21A is the cDNA sequence of IDH1 as presented at GenBank Accession No. NM_005896.2 (Record dated May 10, 2009; GI No. 28178824) (SEQ ID NO:8).

FIG. 21B depicts the mRNA sequence of IDH1 as described in GenBank Accession No. NM_005896.2 (Record dated May 10, 2009; GI No. 28178824) (SEQ ID NO:9).

FIG. 22 is the amino acid sequence of IDH2 as presented at GenBank Accession No. NM_002168.2 (Record dated August 16, 2009; GI28178831) (SEQ ID NO:10).

FIG. 22A is the cDNA sequence of IDH2 as presented at GenBank Accession No. NM_002168 (Record dated August 16, 2009; GI28178831) (SEQ ID NO:11).

FIG. 22B is the mRNA sequence of IDH2 as presented at GenBank Accession No. NM_002168.2 (Record dated August 16, 2009; GI28178831) (SEQ ID NO:12).

FIG. 23 depicts the progress of forward reactions (isocitrate to α-KG) for the mutant enzyme R132H and R132S.

FIG. 24A depicts LC-MS/MS analysis of derivitized 2-HG racemic mixture.

FIG. 24B depicts LC-MS/MS analysis of derivitized R-2HG standard.

FIG. 24C depicts LC-MS/MS analysis of a coinjection of derivitized 2-HG racemate and R-2-HG standard.

FIG. 24D depicts LC-MS/MS analysis of the deriviatized neoactivity reaction product.

FIG. 24E depicts LC-MS/MS analysis of a coinjection of the neoactivty enzyme reaction product and the R-2-HG standard.

FIG. 24F depicts LC-MS/MS analysis of a coinjection of the neoactivity enzyme reaction product and the 2-HG racemic mixture.

FIG. 25 depicts the inhibitory effect of 2-HG derived from the reduction of α-KG by ICDH1 R132H on the wild-type ICDH1 catalytic oxidative decarboxylation of isocitrate to α-KG.

FIG. 26A depicts levels of 2-HG in CRL-2610 cell lines expressing wildtype or IDH-1 R132H mutant protein.

FIG. 26B depicts levels of 2-HG in HTB-14 cell lines expressing wildtype or IDH-1 R132H mutant protein.

FIG. 27 depicts human IDH1 genomic DNA: intron/2nd exon sequence.

FIG. 28 depicts concentrations of 2HG in human malignant gliomas containing R132 mutations in IDH1. Human glioma samples obtained by surgical resection were snap frozen, genotyped to stratify as wild-type (WT) (N=10) or carrying an R132 mutant allele (Mutant) (n=12) and metabolites extracted for LC-MS analysis. Among the 12 mutant tumors, 10 carried a R132H mutation, one an R132S mutation, and one an R132G mutation. Each symbol represents the amount of the listed metabolite found in each tumor sample. Red lines indicate the group sample means. The difference in 2HG observed between WT and R132 mutant IDH1 mutant tumors was statistically significant by Student's t-test ($p<0.0001$). There were no statistically significant differences in αKG, malate, fumarate, succinate, or isocitrate levels between the WT and R132 mutant IDH1 tumors.

FIG. 29A depicts the structural analysis of R132H mutant IDH1. On left is shown an overlay structure of R132H mutant IDH1 and WT IDH1 in the 'closed' conformation.

On the right is shown an overlay structure of WT IDH1 in the 'open' conformation with mutant IDH1 for comparison.

FIG. 29B depicts the close-up structural comparison of the R132H IDH1 (left) and wild-type (WT) IDH1 (right) active-site containing both αKG and NADPH. In addition to changes at residue 132, the position of the catalytic residues Tyr 139 and Lys 212 are different and αKG is oriented differently relative to NADPH for catalytic hydride transfer in the WT versus R132H mutant enzymes.

FIG. 30A depicts the enzymatic properties of IDH1 R132H mutants when ecombinant human wild-type (WT) and R132H mutant (R132H) IDH1 enzymes were assessed for oxidative decarboxylation of isocitrate to αKG with $NADP^+$ as cofactor. Different concentrations of enzyme were used to generate the curves.

FIG. 30B depicts the enxymatic properties of IDH R132 mutants when WT and R132H mutant IDH1 enzymes were assessed for reduction of αKG with NADPH as cofactor. Different concentrations of enzyme were used to generate the curves.

FIG. 30C depicts kinetic parameters of oxidative and reductive reactions as measured for WT and R132H IDH1 enzymes are shown. K_m and k_{cat} values for the reductive activity of the WT enzyme were unable to be determined as no measurable enzyme activity was detectable at any substrate concentration.

FIG. 31A depicts the LC-MS/MS analysis identifying 2HG as the reductive reaction product of recombinant human R132H mutant IDH1.

FIG. 31B depicts the diacetyl-L-tartaric anhydride derivatization and LC-MS/MS analysis of the chirality of 2HG produced by R132H mutant IDH1. Normalized LC-MS/MS signal for the reductive reaction (rxn) product alone, an R(-)-2HG standard alone, and the two together (Rxn + R(-)-2HG) are shown as is the signal for a racemic mixture of R(-) and S(+) forms (2HG Racemate) alone or with the reaction products (Rxn + Racemate).

FIG. 32A depicts SDS-PAGE and Western blot analyses of C-terminal affinity-purification tagged IDH1 R132S protein used for crystallization.

FIG. 32B depicts the chromatogram of FPLC analysis of the IDH1 R132S protein sample.

FIG. 33 depicts crystals obtained from a protein solution contained 5 mM NADP, 5 mM isocitrate, 10 mM Ca2+. Precipitant solution contained 100 mM MES (pH 6.0) and 20% PEG 6000 using a hanging drop method of crystallization.

FIG. 34 depicts crystal obtained from a protein solution contained 5 mM NADP, 5 mM α-ketoglutarate, 10 mM Ca2+. Precipitant contained 100 mM MES (pH 6.5) and 12% PEG 20000.

FIG. 35 is a bar graph depicting elevated NADPH reductive catalysis activity in IDH2-R172K mutant enzyme as compared to wildtype IDH2.

FIGs. 36A-C are graphs depicting the following: **(A)** Extracts from IDH1/2 wt (n=10), and IDH1/2 mutant (n=16) patient leukemia cells obtained at presentation and relapse, and IDH1 R132 mutant leukemia cells grown in culture for 14 days (n=14) analyzed by LC-MS to measure levels of 2-HG; and **(B)** 2-HG measured in serum of patients with IDH1 wt or IDH1 R132 mutant leukemia. In **(A)** and **(B)**, each point represents an individual patient sample. Diamonds represent wildtype, circles represent IDH1 mutants, and triangles represent IDH2 mutants. Horizontal bars indicate the mean. (*) indicates a statistically significant difference relative to wild-type patient cells ($p<0.05$). **(C)** depicts *In vitro* growth curves of IDH1 R132 mutant and IDH1 wild-type AML cells.

FIG. 37 is a graph depicting the results of extracts from leukemia cells of AML patients carrying an IDH1/2 mutant (n=16) or wild-type (n=10) allele obtained at initial presentation and relapse assayed by LC-MS for levels of a-KG, succinate, malate, and fumarate. Each point represents an individual patient sample. Open circles represent wild-types, closed circles represent IDH1 mutants, and triangles represent IDH2 mutants. Horizontal bars represent the mean. There were no statistically significant differences between the wild-type and IDH1/2 mutant AML samples.

FIG. 38 depicts graphical representations of LC-MS analysis of *in vitro* reactions using recombinant IDH1 R132C and IDH2 R172K confirming that 2-HG and not isocitrate is the end product of the mutant enzyme reactions.

FIGs. 39A and B depict **(A)** the wild-type IDH1 enzyme catalysis of the oxidative decarboxylation of isocitrate to alpha-ketoglutarate with the concomitant reduction of NADP to NADPH; and **(B)** the IDH1 R132C mutant reduction of alpha-ketoglutarate to 2-hydroxyglutarate while oxidizing NADPH to NADP. These are referred to as the "forward" and "partial reverse" reactions, respectively.

DETAILED DESCRIPTION

The inventors have discovered that certain mutated forms of an enzyme (*e.g.,* IDH1 or IDH2) have a gain of function, referred to herein as a neoactivity, which can be targeted in the treatment of a cell proliferation-related disorder, *e.g.,* a proliferative disorder such as cancer. For example, in the case of a metabolic pathway enzyme, a gain of function or neoactivity can serve as a target for treatment of cancer. Described herein are methods and compositions for the treatment of a cell proliferation-related disorder, *e.g.,* a proliferative disorder such as cancer. The methods include, *e.g.,* treating a subject having a glioma or brain tumor characterized by a preselected IDH1 allele, *e.g.,* an allele having A at position 394, such as a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation, (*e.g.,* a C394A mutant) or an A at position 395 (*e.g.,* a G395A mutant) according to the sequence of SEQ ID NO:5, that encodes an IDH1 having His, Ser, Cys, Gly, Val, Pro or Leu at position 132 (e.g., His); or a preselected IDH2 allelle that encodes an IDH2 having Lys, Gly, Met, Trp, Thr, or Ser at position 172 and having a neoactivity disclosed herein, by administering to the subject a therapeutically effective amount of an inhibitor of IDH1 or IDH2 (e.g., IDH1), *e.g.,* a small molecule or nucleic acid. The nucleic acid based inhibitor is, for example, a dsRNA, *e.g.,* a dsRNA that comprises the primary sequences of the sense strand and antisense strands of **Tables 7-14**. The dsRNA is composed of two separate strands, or a single strand folded to form a hairpin structure (*e.g.,* a short hairpin RNA (shRNA)). In some embodiments, the nucleic acid based inhibitor is an antisense nucleic acid, such as an antisense having a sequence that overlaps, or includes, an antisense sequence provided in **Tables 7-14**.

Neoactivity of an enzyme

Neoactivity, as used herein, means an activity that arises as a result of a mutation, *e.g.,* a point mutation, *e.g.,* a substitution, *e.g.,* in the active site of an enzyme. In an embodiment the neoactivity is substantially absent from wild type or non-mutant enzyme. This is sometimes referred to herein as a first degree neoactivity. An example of a first degree neoactivity is a "gain of function" wherein the mutant enzyme gains a new catalytic activity. In an embodiment the neoactivity is present in wild type or non-mutant enzyme but at a level which is less than 10, 5, 1, 0.1, 0.01 or 0.001 % of what is seen in the mutant enzyme. This is sometimes referred to herein as a second degree neoactivity. An example of a second degree neoactivity is a "gain of function" wherein the mutant enzyme has an increase, for example, a 5 fold

increase in the rate of a catalytic activity possessed by the enzyme when lacking the mutation.

In some embodiments, a non-mutant form the enzyme, *e.g.,* a wild type form, converts substance A (*e.g.,* isocitrate) to substance B (*e.g.,* α-ketoglutarate), and the neoactivity converts substance B (*e.g.,* α-ketoglutarate) to substance C, sometimes referred to as the neoactivity product (*e.g.,* 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate). In some embodiments, the enzyme is in a metabolic pathway, *e.g.,* a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.,* IDH1 or IDH2.

In some embodiments, a non-mutant form the enzyme, *e.g.,* a wild type form, converts substance A to substance B, and the neoactivity converts substance B to substance A. In some embodiments, the enzyme is in a metabolic pathway, *e.g.,* a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway.

Isocitrate Dehydrogenases

Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate (*i.e.,* α-ketoglutarate). These enzymes belong to two distinct subclasses, one of which utilizes NAD(+) as the electron acceptor and the other NADP(+). Five isocitrate dehydrogenases have been reported: three NAD(+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP(+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP(+)-dependent isozyme is a homodimer.

IDH1 (isocitrate dehydrogenase 1 (NADP+), cytosolic) is also known as IDH; IDP; IDCD; IDPC or PICD. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. It contains the PTS-1 peroxisomal targeting signal sequence. The presence of this enzyme in peroxisomes suggests roles in the regeneration of NADPH for intraperoxisomal reductions, such as the conversion of 2, 4-dienoyl-CoAs to 3-enoyl-CoAs, as well as in peroxisomal reactions that consume 2-oxoglutarate, namely the

alpha-hydroxylation of phytanic acid. The cytoplasmic enzyme serves a significant role in cytoplasmic NADPH production.

The human IDH1 gene encodes a protein of 414 amino acids. The nucleotide and amino acid sequences for human IDH1 can be found as GenBank entries NM_005896.2 and NP_005887.2 respectively. The nucleotide and amino acid sequences for IDH1 are also described in, *e.g.,* Nekrutenko *et al.*, Mol. Biol. Evol. 15:1674-1684(1998); Geisbrecht *et al.*, J. Biol. Chem. 274:30527-30533(1999); Wiemann *et al.*, Genome Res. 11:422-435(2001); The MGC Project Team, Genome Res. 14:2121-2127(2004); Lubec *et al.*, Submitted (DEC-2008) to UniProtKB; Kullmann *et al.*, Submitted (JUN-1996) to the EMBL/GenBank/DDBJ databases; and Sjoeblom *et al.*, Science 314:268-274(2006).

IDH2 (isocitrate dehydrogenase 2 (NADP+), mitochondrial) is also known as IDH; IDP; IDHM; IDPM; ICD-M; or mNADP-IDH. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the mitochondria. It plays a role in intermediary metabolism and energy production. This protein may tightly associate or interact with the pyruvate dehydrogenase complex. Human IDH2 gene encodes a protein of 452 amino acids. The nucleotide and amino acid sequences for IDH2 can be found as GenBank entries NM_002168.2 and NP_002159.2 respectively. The nucleotide and amino acid sequence for human IDH2 are also described in, *e.g.,* Huh *et al.*, Submitted (NOV-1992) to the EMBL/GenBank/DDBJ databases; and The MGC Project Team, Genome Res. 14:2121-2127(2004).

Non-mutant, *e.g.,* wild type, IDH1 catalyzes the oxidative decarboxylation of ioscitrate to α-ketoglutarate thereby reducing NAD^+ ($NADP^+$) to NADP (NADPH), *e.g.,* in the forward reaction:

Isocitrate + NAD^+ ($NADP^+$) $\rightarrow$ α-KG + CO_2 + NADH (NADPH) + H^+

In some embodiments, the neoactivity of a mutant IDH1 can have the ability to convert α-ketoglutarate to 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate:

α-KG + NADH (NADPH) + H^+ $\rightarrow$ 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate + NAD^+ ($NADP^+$).

In some embodiments, the neoactivity can be the reduction of pyruvate or malate to the corresponding α-hydroxyl compounds.

In some embodiments, the neoactivity of a mutant IDH1 can arise from a mutant IDH1 having a His, Ser, Cys, Gly, Val, Pro or Leu, or any other mutations described in Yan *et al.*, at residue 132 (e.g., His, Ser, Cys, Gly, Val or Leu; or His,

Ser, Cys or Lys). In some embodiments, the neoactivity of a mutant IDH2 can arise from a mutant IDH2 having a Lys, Gly, Met, Trp, Thr, or Ser (e.g., Lys, Gly, Met, Trp, or Ser; or Gly, Met or Lys), or any other mutations described in Yan H *et al.*, at residue 172. Exemplary mutations include the following: R132H, R132C, R132S, R132G, R132L, and R132V.

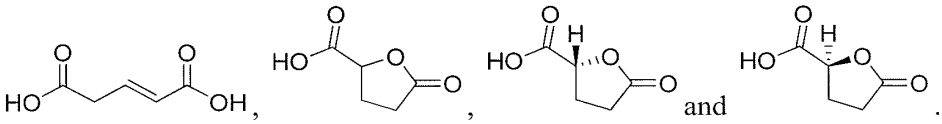
In some embodiments, the mutant IDH1 and/or IDH2 (*e.g.,* a mutant IDH1 and/or IDH2 having a neoactivity described herein) could lead to an increased level of 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate in a subject. The accumulation of 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate in a subject, *e.g.,* in the brain of a subject, can be harmful. For example, in some embodiments, elevated levels of 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate can lead to and/or be predictive of cancer in a subject such as a cancer of the central nervous system, *e.g.,* brain tumor, *e.g.,* glioma, *e.g.,* glioblastoma multiforme (GBM). Accordingly, in some embodiments, a method described herein includes administering to a subject an inhibitor of the neoactivity.

Detection of 2-hydroxyglutarate

2-hydroxyglutarate can be detected, *e.g.,* by LC/MS. To detect secreted 2-hydroxyglutarate in culture media, 500 μL aliquots of conditioned media can be collected, mixed 80:20 with methanol, and centrifuged at 3,000 rpm for 20 minutes at 4 degrees Celsius. The resulting supernatant can be collected and stored at -80 degrees Celsius prior to LC-MS/MS to assess 2-hydroxyglutarate levels. To measure whole-cell associated metabolites, media can be aspirated and cells can be harvested, *e.g.,* at a non-confluent density. A variety of different liquid chromatography (LC) separation methods can be used. Each method can be coupled by negative electrospray ionization (ESI, -3.0 kV) to triple-quadrupole mass spectrometers operating in multiple reaction monitoring (MRM) mode, with MS parameters optimized on infused metabolite standard solutions. Metabolites can be separated by reversed phase chromatography using 10 mM tributyl-amine as an ion pairing agent in the aqueous mobile phase, according to a variant of a previously reported method (Luo *et al. J Chromatogr A* 1147, 153-64, 2007). One method allows resolution of TCA metabolites: t = 0, 50% B; t = 5, 95% B; t= 7, 95% B; t= 8, 0% B, where B refers to an organic mobile phase of 100% methanol. Another method is specific for 2-hydroxyglutarate, running a fast linear gradient from 50% -95% B (buffers as

defined above) over 5 minutes. A Synergi Hydro-RP, 100mm × 2 mm, 2.1 μm particle size (Phenomonex) can be used as the column, as described above. Metabolites can be quantified by comparison of peak areas with pure metabolite standards at known concentration. Metabolite flux studies from ^{13}C-glutamine can be performed as described, *e.g.,* in Munger *et al.* Nat Biotechnol 26, 1179-86, 2008.

In an embodiment 2HG, e.g., R-2HG, is evaluated and the analyte on which the determination is based is 2HG, e.g., R-2HG. In an embodiment the analyte on which the determination is based is a derivative of 2HG, *e.g.,* R-2HG, formed in process of performing the analytic method. By way of example such a derivative can be a derivative formed in MS analysis. Derivatives can include a salt adduct, *e.g.,* a Na adduct, a hydration variant, or a hydration variant which is also a salt adduct, e.g., a Na adduct, e.g., as formed in MS analysis. Exemplary 2HG derivatives include dehydrated derivatives such as the compounds provided below or a salt adduct thereof:



Methods of evaluating samples and/or subjects

This section provides methods of obtaining and analyzing samples and of analyzing subjects.

Embodiments of the method comprise evaluation of one or more parameters related to IDH, e.g., IDH1 or IDH2, an alpha hydroxy neoactivity, e.g., 2HG neoactivity, e.g., to evaluate the IDH1 or IDH2 2HG neoactivity genotype or phenotype. The evaluation can be performed, e.g., to select, diagnose or prognose the subject, to select a therapeutic agent, e.g., an inhibitor, or to evaluate response to the treatment or progression of disease. In an embodiment the evaluation, which can be performed before and/or after treatment has begun, is based, at least in part, on analysis of a tumor sample, cancer cell sample, or precancerous cell sample, from the subject. *E.g.,* a sample from the patient can be analyzed for the presence or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, by evaluating a parameter correlated to the presence or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. An alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, in the sample can be determined by a chromatographic method, *e.g.,* by LC-MS analysis. It can also be determined by contact with a specific binding agent, *e.g.,* an

antibody, which binds the alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, and allows detection. In an embodiment the sample is analyzed for the level of neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., 2HG neoactivity. In an embodment the sample is analysed for the presence of a mutant IDH, *e.g.,* IDH1 or IDH2, protein having an alpha hydroxy neoactivity, e.g., 2HG neoactivity (or a corresponding RNA). E.g., a mutant protein specific reagent, e.g., an antibody that specifically binds an IDH mutant protein, e.g., an antibody that specifically binds an IDH1-R132H mutant protein or an IDH2-R172 mutant protein (e.g., an IDH1-R132H mutant protein), can be used to detect neoactive mutant enzymeIn an embodiment a nucleic acid from the sample is sequenced to determine if a selected allele or mutation of IDH1 or IDH2 disclosed herein is present. In an embodiment the analysis is other than directly determining the presence of a mutant IDH, *e.g.,* IDH1 or IDH2, protein (or corresponding RNA) or sequencing of an IDH, *e.g.,* IDH1 or IDH2 gene. In an embodiment the analysis is other than directly determining, e.g., it is other than sequencing genomic DNA or cDNA, the presence of a mutation at residue 132 of IDH1 and/or a mutation at residue 172 of IDH2. *E.g.,* the analysis can be the detection of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or the measurement of the mutation's an alpha hydroxy neoactivity, e.g., 2HG neoactivity. In an embodiment the sample is removed from the patient and analyzed. In an embodiment the evaluation can include one or more of performing the analysis of the sample, requesting analysis of the sample, requesting results from analysis of the sample, or receiving the results from analysis of the sample. (Generally herein, analysis can include one or both of performing the underlying method or receiving data from another who has performed the underlying method.)

In an embodiment the evaluation, which can be performed before and/or after treatment has begun, is based, at least in part, on analysis of a tissue (e.g., a tissue other than a tumor sample), or bodily fluid, or bodily product. Exemplary tissues include lymph node, skin, hair follicles and nails. Exemplary bodily fluids include blood, plasma, urine, lymph, tears, sweat, saliva, semen, and cerebrospinal fluid. Exemplary bodily products include exhaled breath. *E.g.,* the tissue, fluid or product can be analyzed for the presence or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, by evaluating a parameter correlated to the presence or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG. An alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, in the sample can be determined by a

chromatographic method, *e.g.,* by LC-MS analysis. It can also be determend by contact with a specific binding agent, *e.g.,* an antibody, which binds the alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, and allows detection. In embodiments where sufficient levels are present, the tissue, fluid or product can be analyzed for the level of neoactivity, *e.g.,* an alpha hydroxy neoactivity, e.g., the 2HG neoactivity. In an embodment the sample is analysed for the presence of a mutant IDH, *e.g.,* IDH1 or IDH2, protein having an alpha hydroxy neoactivity, e.g., 2HG neoactivity (or a corresponding RNA). E.g., a mutant protein specific reagent, e.g., an antibody that specifically binds an IDH mutant protein, e.g., an antibody that specifically binds an IDH1-R132H mutant protein or an IDH2-R172 mutant protein (e.g., an IDH1-R132H mutant protein), can be used to detect neoactive mutant enzyme. In an embodiment a nucleic acid from the sample is sequenced to determine if a selected allele or mutation of IDH1 or IDH2 disclosed herein is present. In an embodiment the analysis is other than directly determining the presence of a mutant IDH, *e.g.,* IDH1 or IDH2, protein (or corresponding RNA) or sequencing of an IDH, *e.g.,* IDH1 or IDH2 gene. *E.g.,* the analysis can be the detection of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or the measurement of 2HG neoactivity. In an embodiment the tissue, fluid or product is removed from the patient and analyzed. In an embodiment the evaluation can include one or more of performing the analysis of the tissue, fluid or product, requesting analysis of the tissue, fluid or product, requesting results from analysis of the tissue, fluid or product, or receiving the results from analysis of the tissue, fluid or product.

In an embodiment the evaluation, which can be performed before and/or after treatment has begun, is based, at least in part, on alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, imaging of the subject. In embodiments magnetic resonance methods are is used to evaluate the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, in the subject. In an embodiment the subject is subjected to imaging and/or spectroscopic analysis, *e.g.,* magnetic resonance-based analysis, *e.g.,* MRI and/or MRS *e.g.,*analysis, and optionally an image corresponding to the presence, distribution, or level of an alpha hydroxy neoactivity product, e.g., 2HG, *e.g.,* R-2HG, or of the tumor, is formed. Optionally the image or a value related to the image is stored in a tangible medium and/or transmitted to a second site. In an embodiment the evaluation can include one or

more of performing imaging analysis, requesting imaging analysis, requesting results from imaging analysis, or receiving the results from imaging analysis.

Methods of treating a proliferative disorder

Described herein are methods of treating a cell proliferation-related disorder, *e.g.,* a cancer, *e.g.,* a glioma, *e.g.,* by inhibiting a neoactivity of a mutant enzyme, *e.g.,* an enzyme in a metabolic pathway, *e.g.,* a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.,* IDH1 or IDH2. The cancer can be characterized by the presence of a neoactivity, such as a gain of function in one or more mutant enzymes (*e.g.,* an enzyme in the metabolic pathway, *e.g.,* a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway *e.g.,* IDH1 or IDH2). In some embodiments, the gain of function is the conversion of α-ketoglurarate to 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate.

Compounds for the treatment of cancer

A candidate compound can be evaluated for modulation (*e.g.,* inhibition) of neoactivity, for example, using an assay described herein. A candidate compound can also be evaluated for modulation (*e.g.,* inhibition) of wild type or non-mutant activity. For example, the formation of a product or by-product of any activity (*e.g.,* enzymatic activity) can be assayed, thus evaluating a candidate compound. In some embodiments, the activity (*e.g.,* wild type/non-mutant or neoactivity) can be evaluated by measuring one or more readouts from an enzymatic assay. For example, the change in nature and/or amount of substrate and/or product can be measured, *e.g.,* using methods such as fluorescent or radiolabeled substrates. Exemplary substrates and/or products include α-ketoglutarate, CO_2, NADP, NADPH, NAD, NADH, and 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate. In some embodiments, the rate of reaction of the enzyme can also be evaluated as can the nature and/or amount of a product of the enzymatic reaction. In addition to the measurement of potential enzymatic activities, activity (*e.g.,* wild type/non-mutant or neoactivity) can be detected by the quenching of protein fluorescence upon binding of a potential substrate, cofactor, or enzymatic activity modulator to the enzyme.

In one embodiment, assay progress can be monitored by changes in the OD340 or fluorescence of the NAD or NADP cofactor. In another embodiment, the reaction progress can be coupled to a secondary enzyme assay system in continuous mode or endpoint mode for increasing the dynamic range of the assay. For example, an endpoint assay can be performed by adding to the reaction an excess of diaphorase and rezasarin. Diaphorase consumes the remaining NADPH or NADH while producing resorufin from rezasarin. Resorufin is a highly fluorescent product which can be measured by fluorescence at Ex544 Em590. This not only terminates the reaction but also generates an easily detectable signal with greater quantum yield than the fluorescence of the cofactor.

A continuous assay can be implemented through coupling a product of the primary reaction to a secondary enzyme reaction that yields detectable results of greater dynamic range or more convenient detection mode. For example, inclusion in the reaction mix of aldehyde dehydrogenase (ALDH), which is an NADP+ dependent enzyme, and 6-methoxy-2-napthaldehye, a chromogenic substrate for ALDH, will result in the production of the fluorescent product 6-methoxy-2-napthoate (Ex310 Em 360) at a rate dependent on the production of NADP+ by isocitrate dehydrogenase. The inclusion of a coupling enzyme such as aldehyde dehydrogenase has the additional benefit of allowing screening of neoactivity irrespective of whether NADP+ or NAD+ is produced, since this enzyme is capable of utilizing both. Additionally, since the NADPH or NADH cofactor required for the "reverse" assay is regenerated, a coupled enzyme system which cycles the cofactor back to the IDH enzyme has the further advantage of permitting continuous assays to be conducted at cofactor concentrations much below Km for the purpose of enhancing the detection of competitive inhibitors of cofactor binding.

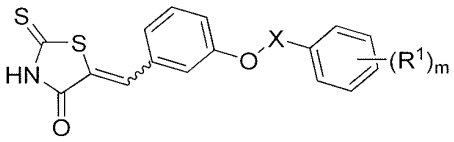
In yet a third embodiment of an activity (*e.g.,* wild type/non-mutant or neoactivity) screen, one or a number of IDH substrates, cofactors, or products can be isotopically labeled with radioactive or "heavy" elements at defined atoms for the purpose of following specific substrates or atoms of substrates through the chemical reaction. For example, the alpha carbon of a-KG, isocitrate, or 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate may be ^{14}C or ^{13}C. Amount, rate, identity and structure of products formed can be analyzed by means known to those of skill in the art, for example mass spectroscopy or radiometric HPLC.

Compounds that inhibit a neoactivity, *e.g.,* a neoactivity described herein, can include, *e.g.,* small molecule, nucleic acid, protein and antibody.

Exemplary small molecules include, *e.g*, small molecules that bind to enzymes and decrease their activity, *e.g.,* a neoactivity described herein. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically. These inhibitors can modify key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both.

In some embodiments, the small molecule is oxalomalate, oxalofumarate, or oxalosuccinate.

In some embodiments, the small molecule is a compound of formula (X), or a compound as listed in **Table 24a**. The compound of formula (X) is provided below:



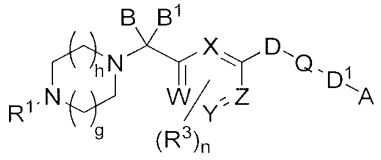
Formula (X)

wherein X is C_1-C_6 alkylene (*e.g.,* methylene), C(O), or C(O)C_1-C_6 alkylene; wherein X is optionally substituted;

R^1 is halo (*e.g.,* fluoro), C_1-C_6 alkyl, C_1-C_6 haloalkyl, hydroxyl, C_1-C_6 alkoxy, cyano, nitro, amino, alkylamino, dialkylamino, amido, -C(O)OH, or C(O)OC_1-C_6alkyl; and

m is 0, 1, 2, or 3.

In some embodiments, the compound is a compound of formula (XI) or a pharmaceutically acceptable salt thereof or a compound listed in Table 24b



(XI)

wherein:

W, X, Y and Z are each independently selected from CH or N;

B and B^1 are independently selected from hydrogen, alkyl or when taken together with the carbon to which they are attached form a carbonyl group;
Q is C=O or SO_2;
D and D^1 are independently selected from a bond, oxygen or NR^c;
A is optionally substituted aryl or optionally substituted heteroaryl;
R^1 is independently selected from alkyl, acyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, cycloalkylalkyl, aralkyl, and heteroaralkyl; each of which may be optionally substituted with 0-3 occurrences of R^d;
each R^3 is independently selected from halo, haloalkyl, alkyl and $-OR^a$;
each R^a is independently selected from alkyl, and haloalkyl;
each R^b is independently alkyl;
each R^c is independently selected from hydrogen, alkyl and alkenyl;
each R^d is independently selected from halo, haloalkyl, alkyl, nitro, cyano, and $-OR^a$, or two R^d taken together with the carbon atoms to which they are attached form an optionally substituted heterocyclyl;
n is 0, 1, or 2;
h is 0, 1, 2; and
g is 0, 1 or 2.

In some embodiments, the small molecule is a selective inhibitor of the neoactivity (*e.g.,* relative to the wild type activity).

Nucleic acids can be used to inhibit a neoactivity, *e.g.,* a neoactivity described herein, *e.g.,* by decreasing the expression of the enzyme. Exemplary nucleic acids include, *e.g.,* siRNA, shRNA, antisense RNA, aptamer and ribozyme. Art-known methods can be used to select inhibitory molecules, *e.g.,* siRNA molecules, for a particular gene sequence.

Proteins can also be used to inhibit a neoactivity, *e.g.,* a neoactivity described herein, by directly or indirectly binding to the enzyme and/or substrate, or competing binding to the enzyme and/or substrate. Exemplary proteins include, *e.g.,* soluble receptors, peptides and antibodies. Exemplary antibodies include, *e.g.,* whole antibody or a fragment thereof that retains its ability to bind to the enzyme or substrate.

Exemplary candidate compounds, which can be tested for inhibitin of a neoactivity described herein (*e.g.,* a neoactivity associated with mutant IDH1), are described in the following references, each of which are incorporated herein by

reference: Bioorganic & Medicinal Chemistry (2008), 16(7), 3580-3586; Free Radical Biology & Medicine (2007), 42(1), 44-51; KR 2005036293 A; Applied and Environmental Microbiology (2005), 71(9), 5465-5475; KR 2002095553 A; U.S. Pat. Appl. US 2004067234 A1; PCT Int. Appl. (2002), WO 2002033063 A1; Journal of Organic Chemistry (1996), 61(14), 4527-4531; Biochimica et Biophysica Acta, Enzymology (1976), 452(2), 302-9; Journal of Biological Chemistry (1975), 250(16), 6351-4; Bollettino - Societa Italiana di Biologia Sperimentale (1972), 48(23), 1031-5; Journal of Biological Chemistry (1969), 244(20), 5709-12.

Isomers

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, atropic, stereoisomer, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

In one embodiment, a compound described herein, *e.g.,* an inhibitor of a neoactivity or 2-HG is an enantiomerically enriched isomer of a stereoisomer described herein. For example, the compound has an enantiomeric excess of at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. Enantiomer, when used herein, refers to either of a pair of chemical compounds whose molecular structures have a mirror-image relationship to each other.

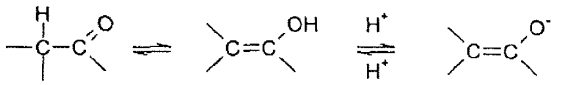
In one embodiment, a preparation of a compound disclosed herein is enriched for an isomer of the compound having a selected stereochemistry, *e.g.,* R or S, corresponding to a selected stereocenter, *e.g.,* the 2-position of 2-hydroxyglutaric acid. 2HG can be purchased from commercial sources or can be prepared using methods known in the art, for example, as described in Org. Syn. Coll vol., 7, P-99, 1990. For example, the compound has a purity corresponding to a compound having a selected stereochemistry of a selected stereocenter of at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%.

In one embodiment, a composition described herein includes a preparation of a compound disclosed herein that is enriched for a structure or structures having a selected stereochemistry, *e.g.,* R or S, at a selected stereocenter, *e.g.,* the 2-position of 2-hydroxyglutaric acid. Exemplary R/S configurations can be those provided in an example described herein.

An "enriched preparation," as used herein, is enriched for a selected stereoconfiguration of one, two, three or more selected stereocenters within the subject compound. Exemplary selected stereocenters and exemplary stereoconfigurations thereof can be selected from those provided herein, *e.g.,* in an example described herein. By enriched is meant at least 60%, *e.g.,* of the molecules of compound in the preparation have a selected stereochemistry of a selected stereocenter. In an embodiment it is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. Enriched refers to the level of a subject molecule(s) and does not connote a process limitation unless specified.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers," as used herein, are structural (or constitutional) isomers (*i.e.*, isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH3, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH2OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (*e.g.,* C1-7alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hydroxyazo, and nitro/aci-nitro.



keto enol enolate

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including 1H, 2H (D), and 3H (T); C may be in any isotopic form, including 12C, 13C, and 14C; O may be in any isotopic form, including 16O and 18O; and the like. Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (*e.g.,* asymmetric synthesis) and separation (*e.g.,* fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Salts

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge *et al.*, 1977, "Pharmaceutically Acceptable Salts." J. Pharm. ScL. Vol. 66, pp. 1-19.

For example, if the compound is anionic, or has a functional group which may be anionic (*e.g.,* -COOH may be -COO"), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na+ and K+, alkaline earth cations such as Ca2+ and Mg2+, and other cations such as Al+3. Examples of suitable organic cations include, but are not limited to, ammonium ion (*i.e.*, NH4+) and substituted ammonium ions (*e.g.,* NH3R+, NH2R2+, NHR3+, NR4+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is N(CH3)4+.

If the compound is cationic, or has a functional group that may be cationic (*e.g.,* -NH2 may • be -NH3+), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

Unless otherwise specified, a reference to a particular compound also includes salt forms thereof.

Chemically Protected Forms

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form" is used herein in the conventional chemical sense and pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions under specified conditions (*e.g.,* pH, temperature, radiation, solvent, and the like). In practice, well known chemical methods are employed to reversibly render unreactive a functional group, which otherwise would be reactive, under specified conditions. In a chemically protected form, one or more reactive functional groups are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999). Unless otherwise specified, a reference to a particular compound also includes chemically protected forms thereof.

A wide variety of such "protecting," "blocking," or "masking" methods are widely used and well known in organic synthesis. For example, a compound which has two nonequivalent reactive functional groups, both of which would be reactive under specified conditions, may be derivatized to render one of the functional groups

"protected," and therefore unreactive, under the specified conditions; so protected, the compound may be used as a reactant which has effectively only one reactive functional group. After the desired reaction (involving the other functional group) is complete, the protected group may be "deprotected" to return it to its original functionality.

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal (R-CH(OR)2) or ketal (R2C(OR)2), respectively, in which the carbonyl group (>C=O) is converted to a diether (>C(OR)2), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide (-NRCO-R) or a urethane (-NRCO-OR), for example, as: a methyl amide (-NHCO-CH3); a benzyloxy amide (-NHCO-OCH2C6H5, -NH-Cbz); as a t-butoxy amide (-NHCO-OC(CH3)3, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-OC(CH3)2C6H4C6H5, -NH-Bpoc), as a 9- fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases (*e.g.,* cyclic amines), as a nitroxide radical (>N-O«).

For example, a carboxylic acid group may be protected as an ester for example, as: an C^alkyl ester (*e.g.,* a methyl ester; a t-butyl ester); a Cvrhaloalkyl ester (*e.g.,* a C1-7trihaloalkyl ester); a triC1-7alkylsilyl-Ci.7alkyl ester; or a C5.2oaryl-C1-7alkyl ester (*e.g.,* a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-CH2NHC(=O)CH3).

Nucleic acid based inhibitors

Nucleic acid-based inhibitors for inhibition IDH, *e.g.,* IDH1, can be, *e.g.,* double stranded RNA (dsRNA) that function, *e.g.,* by an RNA interference (RNAi

mechanism), an antisense RNA, or a microRNA (miRNA). In an embodiment the nucleic-acid based inhibitor binds to the target mRNA and inhibits the production of protein therefrom, *e.g.*,. by cleavage of the targent mRNA.

Double stranded RNA (dsRNA)

A nucleic acid based inhibitor useful for decreasing IDH1 or IDH2 mutant function is, *e.g.*, a dsRNA, such as a dsRNA that acts by an RNAi mechanism. RNAi refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). dsRNAs as used herein are understood to include siRNAs. Typically, inhibition of IDH, *e.g.*, IDH1,by dsRNAs does not trigger the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

dsRNAs targeting an IDH,*e.g.*, IDH1,enzyme, *e.g.*, a wildtype or mutant IDH1, can be unmodified or chemically modified. The dsRNA can be chemically synthesized, expressed from a vector or enzymatically synthesized. The invention also features various chemically modified synthetic dsRNA molecules capable of modulating IDH1 gene expression or activity in cells by RNA interference (RNAi). The use of chemically modified dsRNA improves various properties of native dsRNA molecules, such as through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake.

The dsRNAs targeting nucleic acid can be composed of two separate RNAs, or of one RNA strand, which is folded to form a hairpin structure. Hairpin dsRNAs are typically referred to as shRNAs.

An shRNA that targets IDH, *e.g.*, a mutant or wildtype IDH1 gene can be expressed from a vector, *e.g.*, viral vector, such as a lentiviral or adenoviral vector. In certain embodiments, a suitable dsRNA for inhibiting expression of an IDH1 gene will be identified by screening an siRNA library, such as an adenoviral or lentiviral siRNA library.

In an embodiment, a dsRNA that targets IDH, *e.g.*, IDH1, is about 15 to about 30 base pairs in length (*e.g.*, about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) basepairs in length. In another embodiment, the dsRNA includes overhanging ends of about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotides. By "overhang" is meant that 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand,

or vice versa. The dsRNA can have an overhang on one or both ends of the dsRNA molecule. In some embodiments, the single-stranded overhang is located at the 3'-terminal end of the antisense strand, or, alternatively, at the 3'-terminal end of the sense strand. In some embodiments, the overhang is a TT or UU dinucleotide overhang, *e.g.,* a TT or UU dinucleotide overhang. For example, in an embodiment, the dsRNA includes a 21-nucleotide antisense strand, a 19 base pair duplex region, and a 3'-terminal dinucleotide. In yet another embodiment, a dsRNA includes a duplex nucleic acid where both ends are blunt, or alternatively, where one of the ends is blunt.

In an embodiment, the dsRNA includes a first and a second strand, each strand is about 18 to about 28 nucleotides in length, *e.g.,* about 19 to about 23 nucleotides in length, the first strand of the dsRNA includes a nucleotide sequence having sufficient complementarity to the IDH, *e.g.,* IDH1, RNA for the dsRNA to direct cleavage of the IDH, *e.g.,* IDH1, mRNA via RNA interference, and the second strand of the dsRNA includes a nucleotide sequence that is complementary to the first strand.

In an embodiment, a dsRNA targeting an IDH, *e.g.,* IDH1, gene can target wildtype and mutant forms of the gene, or can target different allelic isoforms of the same gene. For example, the dsRNA will target a sequence that is identical in two or more of the different isoforms. In an embodiment, the dsRNA targets an IDH1 having G at position 395 or C at position 394 (*e.g.,* a wildtype IDH1 RNA) and an IDH1 having A at position 395 or A at position 394, such as a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation, (*e.g.,* an IDH1 RNA carrying a G395A and/or a C394A mutation) (**FIG. 2**).

In an embodiment, a dsRNA will preferentially or specifically target a mutant IDH RNA, or a particular IDH polymorphism. In some embodiments, the IDH has a mutation at position 394 or 395 such as a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation. For example, in an embodiment, the dsRNA targets an IDH1 RNA carrying an A at position 395, *e.g.,* G395A, and in another embodiment, the dsRNA targets an IDH1 RNA carrying an A at position 394, *e.g.,* C394A mutation.

In an embodiment, a dsRNA targeting an IDH RNA includes one or more chemical modifications. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and

terminal glyceryl and/or inverted deoxy abasic residue incorporation. Such chemical modifications have been shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, one or more phosphorothioate substitutions are well-tolerated and have been shown to confer substantial increases in serum stability for modified dsRNA constructs.

In an embodiment, a dsRNA targeting an IDH, *e.g.*, IDH1, RNA includes modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, the dsRNA can include modified nucleotides as a percentage of the total number of nucleotides present in the molecule. As such, the dsRNA can generally include about 5% to about 100% modified nucleotides (*e.g.*, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides).

In some embodiments, the dsRNA targeting IDH, *e.g.*, IDH1, is about 21 nucleotides long. In another embodiment, the dsRNA does not contain any ribonucleotides, and in another embodiment, the dsRNA includes one or more ribonucleotides. In an embodiment, each strand of the dsRNA molecule independently includes about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein each strand includes about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In an embodiment, one of the strands of the dsRNA includes a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the IDH1 or IDH2 gene, and the second strand of the dsRNA includes a nucleotide sequence substantially similar to the nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof.

In an embodiment, the dsRNA targeting IDH1 or IDH2 includes an antisense region having a nucleotide sequence that is complementary to a nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof, and a sense region having a nucleotide sequence substantially similar to the nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof. In an embodiment, the antisense region and the sense region independently include about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, where the antisense region includes

about 15 to about 30 (*e.g.,* about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

As used herein, the term "dsRNA" is meant to include nucleic acid molecules that are capable of mediating sequence specific RNAi, such as short interfering RNA (siRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term "RNAi" is meant to include sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

Nucleic acid-based IDH inhibitors

In an embodiment the inhibitor is a nucleic acid-based inhibitor, such as a double stranded RNA (dsRNA) or antisense RNA that targets a mutant IDH, *e.g.,* mutant IDH1or IDH2.

In one embodiment, the nucleic acid based inhibitor, e.g., a dsRNA or antisense molecule, decreases or inhibits expression of an IDH1 having other than an Arg, e.g., having a His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 enzyme having His at residue 132

In an embodiment the nucleic acid-based inhibitor is a dsRNA that targets an mRNA that encodes an IDH1 allele described herein, *e.g.,* an IDH1 allele having other than an Arg at residue 132. *E.g.,* the allele encodes His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Yan *et al.*, at residue 132, according to the sequence of SEQ ID NO:8 (see also **Fig. 21**).

In an embodiment the allele encodes an IDH1 having His at residue 132.

In an embodiment the allele encodes an IDH1 having Ser at residue 132.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.,* an IDH1 having an A or a T (or a nucleotide other than C) at nucleotide position 394 or an A (or a nucleotide other than G) at nucleotide position 395, *e.g.,* a mutant allele carrying a C394T mutation or a G395A mutation according to the IDH1 sequence of SEQ ID NO:8 (see also Fig 21A).

In an embodiment, the dsRNA targets an IDH1 having other than C, *e.g.,* a T or an A, at nucleotide position 394 or and other than G, *e.g.,* an A, at 395 (*e.g.,* a mutant) and an IDH1 having a C at nucleotide position 394 or a G at nucleotide position 395 (*e.g.,* a wildtype), *e.g.,* by targeting a region of the IDH1 mRNA that is identical between the wildtype and mutant transcripts. In yet another embodiment, the dsRNA targets a particular mutant or polymorphism (such as a single nucleotide polymorphism (SNP)), but not a wildtype allele. In this case, the nucleic acid based inhibitor, *e.g.,* a dsRNA, targets the region of the IDH1 containing the mutation.

In some embodiments, the nucleic acid based inhibitor, *e.g.,* a dsRNA preferentially or specifically inhibits the product of a mutant IDH1 as compared to the product of a wildtype IDH1. In some embodiments, the IDH has a mutation at position 394 or 395 such as a C394A, a C394G, a C394T, a G395C, a G395T or a G395A mutation. For example, in one embodiment, a dsRNA targets a region of an IDH1 mRNA that carries the mutation (*e.g.,* a C394A of C394T or a G395A mutation according to SEQ ID NO:5).

In one embodiment, the nucleic acid-based inhibitor is a dsRNA including a sense strand and an antisense strand having a primary sequence presented in **Tables 7- 14**. In another embodiment, the nucleic acid based inhibitor is an antisense oligonucleotide that includes all or a part of an antisense primary sequence presented in **Tables 7- 14** or which targets the same or substantially the same region as does a dsRNA from **Tables 7- 14**.

In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH2 having Lys, Gly, Met, Trp, Thr, Ser, or any residue described in Yan *et al.*, at residue 172, according to the amino acid sequence of SEQ ID NO:10 (see also **FIG. 22**). In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH2 enzyme having Lys at residue 172.

In an embodiment the nucleic acid-based inhibitor is a dsRNA that targets an mRNA that encodes an IDH2 allele described herein, *e.g.,* an IDH2 allele having other than an Arg at residue 172. *E.g.,* the allele can have Lys, Gly, Met, Trp, Thr, Ser, or any residue described in Yan *et al.*, at residue 172, according to the sequence of SEQ ID NO:10 (see also **Fig. 22**).

In an embodiment the allele encodes an IDH2 having Lys at residue 172.

In an embodiment the allele encodes an IDH2 having Met at residue 172.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH2, *e.g.,* an IDH2 having a G or a T (or a nucleotide other than A or C) at nucleotide position 514 or an A or T or C (or a nucleotide other than G) at nucleotide position 515, *e.g.,* a mutant allele carrying a A514G mutation or a G515T or a G515A mutation according to the IDH2 sequence of SEQ ID NO:10 (**Fig. 22A**). In one embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH2, *e.g.,* an IDH2 having a C or a T (or a nucleotide other than G or A) at nucleotide position 516 according to the IDH2 sequence of SEQ ID NO:10.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH2, *e.g.,* an IDH2 having a G at nucleotide position 514 or a T at nucleotide position 515 or an A at position 515, according to the IDH2 sequence of SEQ ID NO:10.

In an embodiment, the dsRNA targets an IDH2 having other than A, *e.g.,* a G or a T, at nucleotide position 514, or other than G, *e.g.,* an A or C or T at position 515 (*e.g.,* a mutant), or other than G, *e.g.,* C or T, and an IDH2 having an A at nucleotide position 514 or a G at nucleotide position 515 or a G at position 516 (*e.g.,* a wildtype), *e.g.,* by targeting a region of the IDH2 mRNA that is identical between the wildtype and mutant transcripts. In yet another embodiment, the dsRNA targets a particular mutant or polymorphism (such as a single nucleotide polymorphism (SNP)), but not a wildtype allele. In this case, the nucleic acid based inhibitor, *e.g.,* a dsRNA, targets the region of the IDH2 containing the mutation.

In some embodiments, the nucleic acid based inhibitor, *e.g.,* a dsRNA, preferentially or specifically inhibits the product of a mutant IDH2 as compared to the product of a wildtype IDH2. For example, in one embodiment, a dsRNA targets a region of an IDH2 mRNA that carries the mutation (*e.g.,* an A514G or G515T or a G515U mutation according to SEQ ID NO:10).

In one embodiment, the nucleic acid-based inhibitor is a dsRNA including a sense strand and an antisense strand having a primary sequence presented in **Tables 15-23**. In another embodiment, the nucleic acid based inhibitor is an antisense oligonucleotide that includes all or a part of an antisense primary sequence presented in **Tables 15-23** or which targets the same or substantially the same region as does a dsRNA from **Tables 15-23**.

In an embodiment, the nucleic acid based inhibitor is delivered to the brain, *e.g.,* directly to the brain, *e.g.,* by intrathecal or intraventricular delivery. The nucleic

acid based inhibitor can also be delivered from an inplantable device. In an embodiment, the nucleic acid-based inhibitor is delivered by infusion using, *e.g.*, a catheter, and optionally, a pump.

Antisense

Suitable nucleic acid based inhibitors include antisense nucleic acids. While not being bound by theory it is believed that antisense inhibition is typically based upon hydrogen bonding-based hybridization of oligonucleotide strands or segments such that at least one strand or segment is cleaved, degraded, or otherwise rendered inoperable.

An antisense agent can bind IDH1 or IDH2 DNA. In embodiments it inhibits replication and transcription. While not being bound by theory it is believed that an antisense agent can also function to inhibit target RNA translocation, *e.g.*, to a site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA.

An antisense agents can have a chemical modification described above as being suitable for dsRNA.

Antisense agents can include, for example, from about 8 to about 80 nucleobases (*i.e.*, from about 8 to about 80 nucleotides), *e.g.*, about 8 to about 50 nucleobases, or about 12 to about 30 nucleobases. Antisense compounds include ribozymes, external guide sequence (EGS) oligonucleotides (oligozymes), and other short catalytic RNAs or catalytic oligonucleotides which hybridize to the target nucleic acid and modulate its expression. Anti-sense compounds can include a stretch of at least eight consecutive nucleobases that are complementary to a sequence in the target gene. An oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment or, in the case of *in vitro* assays, under conditions in which the assays are conducted.

Hybridization of antisense oligonucleotides with mRNA (*e.g.,* an mRNA encoding IDH1 or IDH2) can interfere with one or more of the normal functions of mRNA. While not being bound by theory it is believed that athe functions of mRNA to be interfered with include all key functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in by the RNA. Binding of specific protein(s) to the RNA may also be interfered with by antisense oligonucleotide hybridization to the RNA.

Exemplary antisense compounds include DNA or RNA sequences that specifically hybridize to the target nucleic acid, *e.g.,* the mRNA encoding IDH1 or IDH2. The complementary region can extend for between about 8 to about 80 nucleobases. The compounds can include one or more modified nucleobases. Modified nucleobases may include, *e.g.,* 5-substituted pyrimidines such as 5-iodouracil, 5-iodocytosine, and C5-propynyl pyrimidines such as C5-propynylcytosine and C5-propynyluracil. Other suitable modified nucleobases include N^4-(C_1-C_{12}) alkylaminocytosines and N^4,N^4-(C_1-C_{12}) dialkylaminocytosines. Modified nucleobases may also include 7-substituted-5-aza-7-deazapurines and 7-substituted-7-deazapurines such as, for example, 7-iodo-7-deazapurines, 7-cyano-7-deazapurines, 7-aminocarbonyl-7-deazapurines. Examples of these include 6-amino-7-iodo-7-deazapurines, 6-amino-7-cyano-7-deazapurines, 6-amino-7-aminocarbonyl-7-deazapurines, 2-amino-6-hydroxy-7-iodo-7-deazapurines, 2-amino-6-hydroxy-7-cyano-7-deazapurines, and 2-amino-6-hydroxy-7-aminocarbonyl-7-deazapurines. Furthermore, N^6-(C_1-C_{12}) alkylaminopurines and N^6,N^6-(C_1-C_{12}) dialkylaminopurines, including N^6-methylaminoadenine and N^6,N^6-dimethylaminoadenine, are also suitable modified nucleobases. Similarly, other 6-substituted purines including, for example, 6-thioguanine may constitute appropriate modified nucleobases. Other suitable nucleobases include 2-thiouracil, 8-bromoadenine, 8-bromoguanine, 2-fluoroadenine, and 2-fluoroguanine. Derivatives of any of the aforementioned modified nucleobases are also appropriate. Substituents of any of the preceding compounds may include C_1-C_{30} alkyl, C_2-C_{30} alkenyl, C_2-C_{30} alkynyl, aryl, aralkyl, heteroaryl, halo, amino, amido, nitro, thio, sulfonyl, carboxyl, alkoxy, alkylcarbonyl, alkoxycarbonyl, and the like.

MicroRNA

In some embodiments, the nucleic acid-based inhibitor suitable for targeting IDH, *e.g.,* IDH1, is a microRNA (miRNA). A miRNA is a single stranded RNA that regulates the expression of target mRNAs either by mRNA cleavage, translational repression/inhibition or heterochromatic silencing. The miRNA is 18 to 25 nucleotides, typically 21 to 23 nucleotides in length. In some embodiments, the miRNA includes chemical modifications, such as one or more modifications described herein.

In some embodiments, a nucleic acid based inhibitor targeting IDH has partial complementarity (*i.e.*, less than 100% complementarity) with the target IDH, *e.g.,* IDH1 or IDH2, mRNA. For example, partial complementarity can include various mismatches or non-base paired nucleotides (*e.g.,* 1, 2, 3, 4, 5 or more mismatches or non-based paired nucleotides, such as nucleotide bulges), which can result in bulges, loops, or overhangs that result between the antisense strand or antisense region of the nucleic acid-based inhibitor and the corresponding target nucleic acid molecule.

The nucleic acid-based inhibitors described herein, *e.g.,* antisense nucleic acid described herein, can be incorporated into a gene construct to be used as a part of a gene therapy protocol to deliver nucleic acids that can be used to express and produce agents within cells. Expression constructs of such components may be administered in any biologically-effective carrier, *e.g.,* any formulation or composition capable of effectively delivering the component gene to cells *in vivo*. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (*e.g.,* antibody conjugated) polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular earners, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out *in vivo*.

In an embodiment, *in vivo* introduction of nucleic acid into a cell includes use of a viral vector containing nucleic acid, *e.g.,* a cDNA. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.,* by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retroviral vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo* particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. *et al.* (eds.) Greene Publishing Associates (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE, and pEM which are known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include Crip, Cre, 2, and Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, for example, Eglitis *et al.* (1985) Science 230:1395-1398; Danos and Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.* (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury *et al.* (1991) Science 254:1802-1805; van Beusechem *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay *et al.* (1992) Human Gene Therapy 3:641-647; Dai *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu *et al.* (1993) J. Immunol. 150:4104-4115; U.S. Pat. Nos. 4,868,116 and 4,980,286; PCT Pub. Nos. WO 89/07136, WO 89/02468, WO 89/05345, and WO 92/07573).

Another viral gene delivery system utilizes adenovirus-derived vectors. See, for example, Berkner *et al.* (1988) BioTechniques 6:616; Rosenfeld *et al.* (1991) Science 252:431-434; and Rosenfeld *et al.* (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (*e.g.,* Ad2, Ad3, Ad7 etc.) are known to those skilled in the art.

Yet another viral vector system useful for delivery of the subject gene is the adeno-associated virus (AAV). See, for example, Flotte *et al.* (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski *et al.* (1989) J. Virol. 63:3822-3828; and McLaughlin *et al.* (1989) J. Virol. 62:1963-1973.

Pharmaceutical compositions

The compositions delineated herein include the compounds delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of disease or disease symptoms, including those described herein.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-β-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions containing inhibitors of IDH, *e.g.,* IDH1, may be administered directly to the central nervous system, such as into the cerebrospinal fluid or into the brain. Delivery can be, for example, in a bolus or by continuous pump infusion. In certain embodiments, delivery is by intrathecal delivery or by intraventricular injection directly into the brain. A catheter and, optionally, a pump can be used for delivery. The inhibitors can be delivered in and released from an implantable device, *e.g.,* a device that is implanted in association with surgical

removal of tumor tissue. *E.g.,* for delivery to the brain, the delivery can be analogous to that with Gliadel, a biopolymer wafer designed to deliver carmustine directly into the surgical cavity created when a brain tumor is resected. The Gliadel wafer slowly dissolves and delivers carmustine.

The therapeutics disclosed herein, *e.g.,* nucleic acid based inhibitors, *e.g.* siRNAs can be administered directly to the CNS, *e.g.,* the brain, *e.g.,* using a pump and/or catheter system. In one embodiment, the pump is implanted under the skin. In an embodiment and a catheter attached to a pump is inserted into the CNS, *e.g.,* into the brain or spine. In one embodiment, the pump (such as the IsoMed Drug Pump from Medtronic) delivers dosing, *e.g*, constant dosing, of a nucleic acid based inhibitor. In an embodiment, the pump is programmable to administer variable or constant doses at predetermined time intervals. For example, the IsoMed Drug pump from Medtronic (or a similar device) can be used to administer a constant supply of the inhibitor, or the SynchroMedII Drug Pump (or a similar device) can be used to administer a variable dosing regime.

Methods and devices described in US patents 7,044,932, 6,620,151, 6,283949, and 6,685,452 can be used in methods described herein.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents

that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

Topical administration of the pharmaceutical compositions of this invention is useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active

components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

The compounds described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a dosage ranging from about 0.02 to about 100 mg/kg of body weight, alternatively dosages between 1 mg and 1000 mg/dose, every 4 to 120 hours, or according to the requirements of the particular drug. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated

effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

Kits

A compound described herein can be provided in a kit.

In an embodiment the kit includes (a) a compound described herein, *e.g.,* a composition that includes a compound described herein (wherein, *e.g.,* the compound can be an inhibitor described herein), and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of a compound described herein for the methods described herein.

In an embodiment the kit provides materials for evaluating a subject. The evaluation can be, *e.g.,*for: identifying a subject having unwanted levels (e.g., higher than present in normal or wildtype cells) of any of 2HG, 2HG neoactivity, or mutant

IDH1 or IDH2 protien having 2HG neoactivity (or corresponding RNA), or having a somatic mutation in IDH1 or IDH2 characterized by 2HG neoactivity; diagnosing, prognosing, or staging, a subject, *e.g.,* on the basis of having increased levels of 2HG, 2HG neoactivity, or mutant IDH1 or IDH2 protien having 2HG neoactivity (or corresponding RNA), or having a somatic mutation in IDH1 or IDH2 characterized by 2HG neoactivity; selecting a treatment for, or evaluating the efficacy of, a treatment, *e.g.,* on the basis of the subject having increased levels of 2HG, 2HG neoactivity, or mutant IDH1 or IDH2 protien having 2HG neoactivity (or corresponding RNA), or having a somatic mutation in IDH1 or IDH2 characterized by 2HG neoactivity. The kit can include one or more reagent useful in the evaluation, *e.g.,* reagents mentioned elsewhere herein. A detection reagent, *e.g.,* an antibody or other specific bindng reagent can be included. Standards or reference samples, *e.g.,* a positive or negative control standard can be included. *E.g.,* if the evaluation is based on the presence of 2HG the kit can include a reagent, e.g, a positive or negative control standards for an assay, *e.g.,* a LC-MS assay.

If the evaluation is based on the presence of 2HG neoactivity, the kit can include a reagent, *e.g.,* one or more of those mentioned elsewhere herein, for assaying 2HG neoactivity. If the evaluation is based on sequencing, the kit can include primers or other matierials useful for sequencing the relevant nucleic acids for identifying an IHD, e.g., IDH1 or IDH2, neoactive mutant. E.g., the kit can contain a reagent that provides for interrogation of the indentity, i.e., sequencing of, residue 132 of IDH1 to determine if a neoactive mutant is present. The kit can include nucleic acids, e.g., an oligomer, e.g., primers, which allow sequencing of of the nucleotides that encode residue 132 of IDH1. In an embodiment the kit includes a nucleic acid whose hybridization, or ability to be amplified, is dependent on the indentity of residue 132 of IDH1. In other embodiments the kit includes a reagent, e.g., an antibody or other specific binding molecule that can identify the presence of a neoactive mutant, *e.g.*, a protein encoded by a neoactive mutant at 132 of IDH1. As described below, a kit can also include buffers, solvents, and information related to the evaluation.

In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to methods for administering the compound.

In one embodiment, the informational material can include instructions to administer a compound described herein in a suitable manner to perform the methods described herein, *e.g.,* in a suitable dose, dosage form, or mode of administration (*e.g.,* a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer a compound described herein to a suitable subject, *e.g.,* a human, *e.g.,* a human having or at risk for a disorder described herein.

The informational material of the kits is not limited in its form. In many cases, the informational material, *e.g.,* instructions, is provided in printed matter, *e.g.,* a printed text, drawing, and/or photograph, *e.g.,* a label or printed sheet. However, the informational material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. In another embodiment, the informational material of the kit is contact information, *e.g.,* a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about a compound described herein and/or its use in the methods described herein. Of course, the informational material can also be provided in any combination of formats.

In addition to a compound described herein, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer, a preservative, a flavoring agent (*e.g.,* a bitter antagonist or a sweetener), a fragrance or other cosmetic ingredient, and/or a second agent for treating a condition or disorder described herein. Alternatively, the other ingredients can be included in the kit, but in different compositions or containers than a compound described herein. In such embodiments, the kit can include instructions for admixing a compound described herein and the other ingredients, or for using a compound described herein together with the other ingredients.

A compound described herein can be provided in any form, *e.g.,* liquid, dried or lyophilized form. It is preferred that a compound described herein be substantially pure and/or sterile. When a compound described herein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When a compound described herein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, *e.g.,* sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing a compound described herein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (*e.g.,* a pack) of individual containers, each containing one or more unit dosage forms (*e.g.,* a dosage form described herein) of a compound described herein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of a compound described herein. The containers of the kits can be air tight, waterproof (*e.g.,* impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, *e.g.,* a syringe, inhalant, pipette, forceps, measured spoon, dropper (*e.g.,* eye dropper), swab (*e.g.,* a cotton swab or wooden swab), or any such delivery device. In an embodiment, the device is a medical implant device, *e.g.,* packaged for surgical insertion.

Combination therapies

In some embodiments, a compound or composition described herein, is administered together with an additional cancer treatment. Exemplary cancer treatments include, for example: surgery, chemotherapy, targeted therapies such as antibody therapies, immunotherapy, and hormonal therapy. Examples of each of these treatments are provided below.

Chemotherapy

In some embodiments, a compound or composition described herein, is administered with a chemotherapy. Chemotherapy is the treatment of cancer with drugs that can destroy cancer cells. "Chemotherapy" usually refers to cytotoxic drugs which affect rapidly dividing cells in general, in contrast with targeted therapy. Chemotherapy drugs interfere with cell division in various possible ways, *e.g.,* with the duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy tar*get al*l rapidly dividing cells and are not specific for cancer cells,

although some degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can.

Examples of chemotherapeutic agents used in cancer therapy include, for example, antimetabolites (*e.g.,* folic acid, purine, and pyrimidine derivatives) and alkylating agents (*e.g.,* nitrogen mustards, nitrosoureas, platinum, alkyl sulfonates, hydrazines, triazenes, aziridines, spindle poison, cytotoxic agents, toposimerase inhibitors and others). Exemplary agents include Aclarubicin, Actinomycin, Alitretinon, Altretamine, Aminopterin, Aminolevulinic acid, Amrubicin, Amsacrine, Anagrelide, Arsenic trioxide, Asparaginase, Atrasentan, Belotecan, Bexarotene, endamustine, Bleomycin, Bortezomib, Busulfan, Camptothecin, Capecitabine, Carboplatin, Carboquone, Carmofur, Carmustine, Celecoxib, Chlorambucil, Chlormethine, Cisplatin, Cladribine, Clofarabine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Decitabine, Demecolcine, Docetaxel, Doxorubicin, Efaproxiral, Elesclomol, Elsamitrucin, Enocitabine, Epirubicin, Estramustine, Etoglucid, Etoposide, Floxuridine, Fludarabine, Fluorouracil (5FU), Fotemustine, Gemcitabine, Gliadel implants, Hydroxycarbamide, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Irofulven, Ixabepilone, Larotaxel, Leucovorin, Liposomal doxorubicin, Liposomal daunorubicin, Lonidamine, Lomustine, Lucanthone, Mannosulfan, Masoprocol, Melphalan, Mercaptopurine, Mesna, Methotrexate, Methyl aminolevulinate, Mitobronitol, Mitoguazone, Mitotane, Mitomycin, Mitoxantrone, Nedaplatin, Nimustine, Oblimersen, Omacetaxine, Ortataxel, Oxaliplatin, Paclitaxel, Pegaspargase, Pemetrexed, Pentostatin, Pirarubicin, Pixantrone, Plicamycin, Porfimer sodium, Prednimustine, Procarbazine, Raltitrexed, Ranimustine, Rubitecan, Sapacitabine, Semustine, Sitimagene ceradenovec, Strataplatin, Streptozocin, Talaporfin, Tegafur-uracil, Temoporfin, Temozolomide, Teniposide, Tesetaxel, Testolactone, Tetranitrate, Thiotepa, Tiazofurine, Tioguanine, Tipifarnib, Topotecan, Trabectedin, Triaziquone, Triethylenemelamine, Triplatin, Tretinoin, Treosulfan, Trofosfamide, Uramustine, Valrubicin, Verteporfin, Vinblastine, Vincristine, Vindesine, Vinflunine, Vinorelbine, Vorinostat, Zorubicin, and other cytostatic or cytotoxic agents described herein.

Because some drugs work better together than alone, two or more drugs are often given at the same time. Often, two or more chemotherapy agents are used as combination chemotherapy. In some embodiments, the chemotherapy agents

(including combination chemotherapy) can be used in combination with a compound described herein, *e.g.,* phenformin.

Targeted therapy

In some embodiments, a compound or composition described herein, is administered with a targeted therapy. Targeted therapy constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, overexpressed, or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors such as Axitinib, Bosutinib, Cediranib, desatinib, erlotinib, imatinib, gefitinib, lapatinib, Lestaurtinib, Nilotinib, Semaxanib, Sorafenib, Sunitinib, and Vandetanib, and also cyclin-depdendent kinase inhibitors such as Alvocidib and Seliciclib. Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells. Examples include the anti-HER2/neu antibody trastuzumab (HERCEPTIN®) typically used in breast cancer, and the anti-CD20 antibody rituximab and Tositumomab typically used in a variety of B-cell malignancies. Other exemplary antibodies include Cetuximab, Panitumumab, Trastuzumab, Alemtuzumab, Bevacizumab, Edrecolomab, and Gemtuzumab. Exemplary fusion proteins include Aflibercept and Denileukin diftitox. In some embodiments, the targeted therapy can be used in combination with a compound described herein, *e.g.,* a biguanide such as metformin or phenformin, preferably phenformin.

Targeted therapy can also involve small peptides as "homing devices" which can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (*e.g.,* RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. An example of such therapy includes BEXXAR®.

Immunotherapy

In some embodiments, a compound or composition described herein, is administered with an immunotherapy. Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the tumor. Contemporary methods for generating an immune response against tumors include intravesicular BCG immunotherapy for superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients.

Allogeneic hematopoietic stem cell transplantation can be considered a form of immunotherapy, since the donor's immune cells will often attack the tumor in a graft-versus-tumor effect. In some embodiments, the immunotherapy agents can be used in combination with a compound or composition described herein.

Hormonal therapy

In some embodiments, a compound or composition described herein, is administered with a hormonal therapy. The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial. In some embodiments, the hormonal therapy agents can be used in combination with a compound or a composition described herein.

In some embodiments, a compound or composition described herein, is administered together with an additional cancer treatment (*e.g.,* surgical removal), in treating cancer in nervous system, *e.g.,* cancer in central nervous system, *e.g.,* brain tumor, *e.g.,* glioma, *e.g.,* glioblastoma multiforme (GBM).

Several studies have suggested that more than 25% of glioblastoma patients obtain a significant survival benefit from adjuvant chemotherapy. Meta-analyses have suggested that adjuvant chemotherapy results in a 6-10% increase in 1-year survival rate.

Temozolomide is an orally active alkylating agent that is used for persons newly diagnosed with glioblastoma multiforme. It was approved by the United States Food and Drug Administration (FDA) in March 2005. Studies have shown that the drug was well tolerated and provided a survival benefit. Adjuvant and concomitant temozolomide with radiation was associated with significant improvements in median progression-free survival over radiation alone (6.9 vs 5 mo), overall survival (14.6 vs 12.1 mo), and the likelihood of being alive in 2 years (26% vs 10%).

Nitrosoureas: BCNU (carmustine)-polymer wafers (Gliadel) were approved by the FDA in 2002. Though Gliadel wafers are used by some for initial treatment, they have shown only a modest increase in median survival over placebo (13.8 vs. 11.6 months) in the largest such phase III trial, and are associated with increased rates of CSF leak and increased intracranial pressure secondary to edema and mass effect.

MGMT is a DNA repair enzyme that contributes to temozolomide resistance. Methylation of the MGMT promoter, found in approximately 45% of glioblastoma multiformes, results in an epigenetic silencing of the gene, decreasing the tumor cell's capacity for DNA repair and increasing susceptibility to temozolomide.

When patients with and without MGMT promoter methylation were treated with temozolomide, the groups had median survivals of 21.7 versus 12.7 months, and 2-year survival rates of 46% versus 13.8%, respectively.

Though temozolomide is currently a first-line agent in the treatment of glioblastoma multiforme, unfavorable MGMT methylation status could help select patients appropriate for future therapeutic investigations.

O6-benzylguanine and other inhibitors of MGMT as well as RNA interference-mediated silencing of MGMT offer promising avenues to increase the effectiveness of temozolomide and other alkylating antineoplastics, and such agents are under active study.

Carmustine (BCNU) and cis -platinum (cisplatin) have been the primary chemotherapeutic agents used against malignant gliomas. All agents in use have no greater than a 30-40% response rate, and most fall into the range of 10-20%.

Data from the University of California at San Francisco indicate that, for the treatment of glioblastomas, surgery followed by radiation therapy leads to 1-, 3-, and 5-year survival rates of 44%, 6%, and 0%, respectively. By comparison, surgery followed by radiation and chemotherapy using nitrosourea-based regimens resulted in 1-, 3-, and 5-year survival rates of 46%, 18%, and 18%, respectively.

A major hindrance to the use of chemotherapeutic agents for brain tumors is the fact that the blood-brain barrier (BBB) effectively excludes many agents from the CNS. For this reason, novel methods of intracranial drug delivery are being developed to deliver higher concentrations of chemotherapeutic agents to the tumor cells while avoiding the adverse systemic effects of these medications.

Pressure-driven infusion of chemotherapeutic agents through an intracranial catheter, also known as convection-enhanced delivery (CED), has the advantage of delivering drugs along a pressure gradient rather than by simple diffusion. CED has shown promising results in animal models with agents including BCNU and topotecan.

Initial attempts investigated the delivery of chemotherapeutic agents via an intraarterial route rather than intravenously. Unfortunately, no survival advantage was observed.

Chemotherapy for recurrent glioblastoma multiforme provides modest, if any, benefit, and several classes of agents are used. Carmustine wafers increased 6-month survival from 36% to 56% over placebo in one randomized study of 222 patients, though there was a significant association between the treatment group and serious intracranial infections.

Genotyping of brain tumors may have applications in stratifying patients for clinical trials of various novel therapies.

The anti-angiogenic agent bevacizumab, when used with irinotecan improved 6-month survival in recurrent glioma patients to 46% compared with 21% in patients treated with temozolomide. This bevacizumab and irinotecan combination for recurrent glioblastoma multiforme has been shown to improve survival over bevacizumab alone. Anti-angiogenic agents also decrease peritumoral edema, potentially reducing the necessary corticosteroid dose.

Some glioblastomas responds to gefitinib or erlotinib (tyrosine kinase inhibitors). The simultaneous presence in glioblastoma cells of mutant EGFR (EGFRviii) and PTEN was associated with responsiveness to tyrosine kinase inhibitors, whereas increased p-akt predicts a decreased effect. Other targets include PDGFR, VEGFR, mTOR, farnesyltransferase, and PI3K.

Other possible therapy modalities include imatinib, gene therapy, peptide and dendritic cell vaccines, synthetic chlorotoxins, and radiolabeled drugs and antibodies.

Patient selection/monitoring

Described herein are methods of treating a cell proliferation-related disorder, *e.g.*, cancer, in a subject and methods of identifying a subject for a treatment described herein. Also described herein are methods of predicting a subject who is at risk of developing cancer (*e.g.*, a cancer associate with a mutation in an enzyme (*e.g.*, an enzyme in the metabolic pathway such as IDH1 and/or IDH2)). The cancer is generally characterized by the presence of a neoactivity, such as a gain of function in one or more mutant enzymes (*e.g.*, an enzyme in the metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or

IDH2). The subject can be selected on the basis of the subject having a mutant gene having a neoactivity, *e.g.*, a neoactivity described herein. As used herein, "select" means selecting in whole or part on said basis.

In some embodiments, a subject is selected for treatment with a compound described herein based on a determination that the subject has a mutant enzyme described herein (*e.g.*, an enzyme in the metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2). In some embodiments, the mutant enzyme has a neoactivity and the patient is selected on that basis. The neoactivity of the enzyme can be identified, for example, by evaluating the subject or sample (*e.g.*, tissue or bodily fluid) therefrom, for the presence or amount of a substrate, cofactor and/or product of the enzyme. The presence and/or amount of substrate, cofactor and/or product can correspond to the wild-type/non-mutant activity or can correspond to the neoactivity of the enzyme. Exemplary bodily fluid that can be used to identifty (*e.g.*, evaluate) the neoactivity of the enzyme include amniotic fluid surrounding a fetus, aqueous humour, blood (*e.g.*, blood plasma), Cerebrospinal fluid, cerumen, chyme, Cowper's fluid, female ejaculate, interstitial fluid, lymph, breast milk, mucus (*e.g.*, nasal drainage or phlegm), pleural fluid, pus, saliva, sebum, semen, serum, sweat, tears, urine, vaginal secretion, or vomit.

In some embodiments, a subject can be evaluated for neoactivity of an enzyme using magnetic resonance. For example, where the mutant enzyme is IDH1 or IDH2 and the neoactivity is conversion of α-ketoglutarate to 2-hydroxyglutarate, the subject can be evaluated for the presence of and/or an elevated amount of 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate relative to the amount of 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate present in a subject who does not have a mutation in IDH1 or IDH2 having the above neoactivity. In some embodiments, neoactivity of IDH1 or IDH2 can be determined by the presence or elevated amount of a peak corresponding to 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate as determined by magnetic resonance. For example, a subject can be evaluated for the presence and/or strength of a signal at about 2.5 ppm to determine the presence and/or amount of 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate in the subject. This can be correlated to and/or predictive of a neoactivity described herein for the mutant enzyme IDH. Similarly, the presence,

strength and/or absence of a signal at about 2.5 ppm could be predictive of a response to treatment and thereby used as a noninvasive biomarker for clinical response.

Neoactivity of a mutant enzyme such as IDH can also be evaluated using other techniques known to one skilled in the art. For example, the presence or amount of a labeled substrate, cofactor, and/or reaction product can be measured such as a ^{13}C or ^{14}C labeled substrate, cofactor, and/or reaction product. The neoactivity can be evaluated by evaluating the forward reaction of the wild-type/non mutant enzyme (such as the oxidative decarboxylation of ioscitrate to α-ketoglutarate in a mutant IDH1 or IDH2 enzyme, specifically a mutant IDH1 enzyme) and/or the reaction corresponding to the neoactivity (*e.g.,* the conversion of α-ketoglutarate to 2-hydroxyglutarate, *e.g.,* R-2-hydroxyglutarate in a mutant IDH1 or IDH2 enzyme, specifically a mutant IDH1 enzyme).

Disorders

The IDH-related methods disclosed herein, *e.g.,* methods of evaluating or treating subjects, are directed to subjects having a cell proliferation-related disorder characterized by an IDH mutant, *e.g.,* an IDH1 or IDH2, mutant having neoactivity, *e.g.,* 2HG neoactivity. Examples of some of the disorders below have been shown to be characterized by an IDH1 or IDH2 mutation. Others can be analyzed, *e.g.,* by sequencing cell samples to determine the presence of a somatic mutation at amino acid 132 of IDH1 or at amino acid 172 of IDH2. Without being bound by theory it is expected that a portion of the tumors of given type of cancer will have an IDH, e.g., IDH1 or IDH2, mutant having 2HG neoactivity.

The disclosed methods are useful in evaluating or treating proliferative disorders, *e.g.* evaluating or treating solid tumors, soft tissue tumors, and metastases thereof wherein the solid tumor, soft tissue tumor or metastases thereof is a cancer described herein. Exemplary solid tumors include malignancies (*e.g.,* sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those of brain, lung, breast, lymphoid, gastrointestinal (*e.g.,* colon), and genitourinary (*e.g.,* renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine. The disclosed methods are also useful in evaluating or treating non-solid cancers.

The methods described herein can be used with any cancer, for example those described by the National Cancer Institute. A cancer can be evaluated to determine whether it is using a method described herein. Exemplary cancers described by the National Cancer Institute include: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood; Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primaiy; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma, Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's

Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS- Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's, Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non- Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood; Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's

Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T- Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor. Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein.

The methods described herein are useful in treating cancer in nervous system, *e.g.,* brain tumor, *e.g.,* glioma, *e.g.,* glioblastoma multiforme (GBM), *e.g.,* by inhibiting a neoactivity of a mutant enzyme, *e.g.,* an enzyme in a metabolic pathway, *e.g.,* a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.,* IDH1 or IDH2.

Gliomas, a type of brain tumors, can be classified as grade I to grade IV on the basis of histopathological and clinical criteria established by the World Health Organization (WHO). WHO grade I gliomas are often considered benign. Gliomas of WHO grade II or III are invasive, progress to higher-grade lesions. WHO grade IV tumors (glioblastomas) are the most invasive form. Exemplary brain tumors include, *e.g.,* astrocytic tumor (*e.g.,* pilocytic astrocytoma, subependymal giant-cell

astrocytoma, diffuse astrocytoma, pleomorphic xanthoastrocytoma, anaplastic astrocytoma, astrocytoma, giant cell glioblastoma, glioblastoma, secondary glioblastoma, primary adult glioblastoma, and primary pediatric glioblastoma); oligodendroglial tumor (*e.g.,* oligodendroglioma, and anaplastic oligodendroglioma); oligoastrocytic tumor (*e.g.,* oligoastrocytoma, and anaplastic oligoastrocytoma); ependymoma (*e.g.,* myxopapillary ependymoma, and anaplastic ependymoma); medulloblastoma; primitive neuroectodermal tumor, schwannoma, meningioma, meatypical meningioma, anaplastic meningioma; and pituitary adenoma. Exemplary cancers are described in Acta Neuropathol (2008) 116:597–602 and N Engl J Med. 2009 Feb 19;360(8):765-73, the contents of which are each incorporated herein by reference.

In embodiments the disorder is glioblastoma.

In an embodiment the disorder is prostate cancer, e.g., stage T1 (*e.g.,* T1a, T1b and T1c), T2 (*e.g.,* T2a, T2b and T2c), T3 (*e.g.,* T3a and T3b) and T4, on the TNM staging system. In embodiments the prostate cancer is grade G1, G2, G3 or G4 (where a higher number indicates greater difference from normal tissue).. Types of prostate cancer include, *e.g.,* prostate adenocarcinoma, small cell carcinoma, squamous carcinoma, sarcomas, and transitional cell carcinoma.

Methods and compositions of the inventin can be combined with art-known treatment. Art-known treatment for prostate cancer can include, *e.g.,* active surveillance, surgery (*e.g.,* radical prostatectomy, transurethral resection of the prostate, orchiectomy, and cryosurgegry), radiation therapy including brachytherapy (prostate brachytherapy) and external beam radiation therapy, High-Intensity Focused Ultrasound (HIFU), chemotherapy, cryosurgery, hormonal therapy (*e.g.,* antiandrogens (*e.g.,* flutamide, bicalutamide, nilutamide and cyproterone acetate, ketoconazole, aminoglutethimide), GnRH antagonists (*e.g.,* Abarelix)), or a combination thereof.

All references described herein are expressly incorporated herein by reference.

EXAMPLES

Example 1 IDH1 cloning, mutagenesis, expression and purification

1. Wild type IDH1 was cloned into pET41a, creating His8 tag at C-terminus.

The IDH1 gene coding region (cDNA) was purchased from Invitrogen in pENTR221 vector (www.invitrogen.com, Cat#B-068487_Ultimate_ORF). Oligo

nucleotides were designed to PCR out the coding region of IDH1 with NdeI at the 5' end and XhoI at the 3'. (IDH1-f: TAATCATATGTCCAAAAAAATCAGT (SEQ ID NO:1), IDH1-r: TAATCTCGAGTGAAAGTTTGGCCTGAGCTAGTT (SEQ ID NO:2)). The PCR product is cloned into the NdeI/XhoI cleaved pET41a vector. NdeI/XhoI cleavage of the vector pET41a releases the GST portion of the plasmid, and creating a C-terminal His8 tag (SEQ ID NO:3) without the N-terminal GST fusion. The original stop codon of IDH1 is change to serine, so the junction sequence in final IDH1 protein is: Ser-Leu-Glu-His-His-His-His-His-His-His-His-Stop (SEQ ID NO:4).

The C-terminal His tag strategy instead of N-terminal His tag strategy was chosen, because C-terminal tag might not negatively impact IDH1 protein folding or activity. See, *e.g.,* Xu X *et al*, J Biol Chem. 2004 Aug 6; 279(32):33946-57.

The sequence for pET41a-IDH1 plasmid is confirmed by DNA sequencing. **FIG. 1** shows detailed sequence verification of pET41a-IDH1and alignment against published IDH1 CDS below.

2. IDH1 site directed mutagenesis to create the IDHr132s and IDHr132h mutants.

Site directed mutagenesis was performed to convert R132 to S or H, DNA sequencing confirmed that G395 is mutated to A (creating Arg→His mutation in the IDH1 protein), and C394 is mutated to A (creating Arg→Ser in the IDH1 protein). Detailed method for site directed mutagenesis is described in the user manual for QuikChange® MultiSite-Directed Mutagenesis Kit (Stratagene, cat# 200531). **FIG. 2** shows DNA sequence verification of such mutations. Highlighted nucleotides were successfully changed in the mutagenesis: G395→A mutation allows amino acid Arg132→His; C394→A mutation allows amino acid Arg132→Ser.

3. IDH1 protein expression and purification.

IDHwt, IDHR132S, and IDHR132H proteins were expressed in the *E. coli* strain Rosetta and purified according to the detailed procedure below. Active IDH1 proteins are in dimer form, and SEC column fraction/peak that correspond to the dimer form were collected for enzymology analysis and cross comparison of catalytic activities of these proteins.

A. Cell culturing:

Cells were grown in LB (20 μg/ml Kanamycin) at 37°C with shaking until OD600 reaches 0.6. The temperature was changed to 18°C and protein was induced by adding IPTG to final concentration of 1 mM. Cells were collected 12-16 hours after IPTG induction.

B. Buffer system:

Lysis buffer: 20mM Tris, pH7.4, 0.1% Triton X-100, 500 mM NaCl, 1 mM PMSF, 5 mM β-mercaptoethanol, 10 % glycerol.

Ni-Column Buffer A: 20 mM Tris, pH7.4, 500mM NaCl, 5 mM β-mercaptoethanol, 10% glycerol.

Ni-column Buffer B: 20 mM Tris, pH7.4, 500 mM NaCl, 5 mM β-mercaptoethanol , 500 mM Imidazole, 10% glycerol

Gel filtration Buffer C: 200 mM NaCl, 50 mM Tris 7.5, 5 mM β-mercaptoethanol, 2 mM $MnSO_4$, 10% glycerol.

C. Protein purification procedure

1. Cell pellet were resuspended in the lysis buffer (1gram cell/5-10 ml buffer).
2. Cells were broken by passing the cell through Microfludizer with at a pressure of 15,000 psi for 3 times.
3. Soluble protein was collected from supernatant after centrifugation at 20,000g (Beckman Avanti J-26XP) for 30 min at 4°C.
4. 5-10 ml of Ni-column was equilibrated by Buffer A until the A280 value reached baseline. The supernatant was loaded onto a 5-ml Ni-Sepharose column (2 ml/min). The column was washed by 10-20 CV of washing buffer (90 % buffer A+10 % buffer B) until A280 reach the baseline (2 ml/min).
5. The protein was eluted by liner gradient of 10-100% buffer B (20 CV) with the flow rate of 2 ml/min and the sample fractions were collected as 2 ml/tube.
6. The samples were analyzed on SDS-PAGE gel.
7. The samples were collected and dialyzed against 200x Gel filtration buffer for 2 times (1 hour and > 4 hours).
8. The samples were concentrated to 10 ml.
9. 200 ml of S-200 Gel-filtration column was equilibrated by buffer C until the A280 value reached baseline. The samples were loaded onto Gel filtration column (0.5 ml/min).

10. The column was washed by 10 CV of buffer C, collect fractions as 2-4 ml/tube.
11. The samples were analyzed on SDS-PAGE gel and protein concentration was determined.

D. Protein purification results

The results for purification of wild type IDH1 are shown in **FIGs. 3, 4, 5A** and **5B**.

The results for purification of mutant IDH1R132S are shown in **FIGs. 6, 7, 8A** and **8B**.

The results for purification of wild type IDH1R132H are shown in **FIGs. 9, 10, 11A** and **11B**.

EXAMPLE 2 ENZYMOLOGY ANALYSIS OF IDH1 WILD TYPE AND MUTANTS

1. Analysis of IDH1 wild-type and mutants R132H and R132S in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

A. Methods

To determine the catalytic efficiency of enzymes in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG) direction, reactions were performed to determine Vmax and Km for isocitrate. In these reactions, the substrate was varied while the cofactor was held constant at 500 uM. All reactions were performed in 150 mM NaCl, 20 mM Tris-Cl, pH 7.5, 10% glycerol, and 0.03% (w/v) BSA). Reaction progress was followed by spectroscopy at 340 nM monitoring the change in oxidation state of the cofactor. Sufficient enzyme was added to give a linear change in absorbance for 10 minutes.

B. ICDH1 R132H and ICDH1 R132S are impaired for conversion of isocitrate to α-KG.

Michaelis-Menten plots for the relationship of isocitrate concentration to reaction velocity are presented in **FIGs. 12A-12C**. Kinetic parameters are summarized in the **Table 1**. All data was fit to the Hill equation by least-squares regression analysis.

Table 1

Enzyme	Vmax (umol/min/mg)	Km (uM)	Hill Constant	Vmax/Km	Relative Catalytic Efficiency
Wt	30.5	56.8	1.8	0.537	100%
R132H	0.605	171.7	0.6	0.0035	0.35%
R132S	95	>1e6	0.479	<9.5e7	<.001%

Both mutant enzymes display a reduced Hill coefficient and an increase in Km for isocitrate, suggesting a loss of co-operativity in substrate binding and/or reduced affinity for substrate. R132H enzyme also displays a reduced Vmax, suggestive of a lower kcat. R132S displays an increase in Vmax, suggesting an increase in kcat, although this comes at the expense of a 20,000 fold increase in Km so that the overall effect on catalytic efficiency is a great decrease as compared to the wild-type enzyme. The relative catalytic efficiency, described as Vmax/Km, is dramatically lower for the mutants as compared to wild-type. The *in vivo* effect of these mutations would be to decrease the flux conversion of isocitrate to α-KG.

C. The ICDH1 R132H and R132S mutants display reduced product inhibition in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

A well-known regulatory mechanism for control of metabolic enzymes is feedback inhibition, in which the product of the reaction acts as a negative regulator for the generating enzyme. To examine whether the R132S or R132H mutants maintain this regulatory mechanism, the Ki for α-KG in the oxidative decarboxylation of ioscitrate to α-ketoglutarate was determined. Data is presented in **FIGs. 13A-13C** and summarized in **Table 2**. In all cases, α-KG acts as a competitive inhibitor of the isocitrate substrate. However, R132H and R132S display a 20-fold and 13-fold increase in sensitivity to feedback inhibition as compared to the wild-type enzyme.

Table 2

Enzyme	Ki (uM)
Wt	612.2
R132H	28.6
R132S	45.3

D. The effect of $MnCl_2$ in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

$MnCl_2$ can be substituted with $MgCl_2$ to examine if there is any difference in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

E. The effect of R132 mutations on the inhibitory effect of oxalomalate on IDH1

The purpose of this example is to examine the susceptibility of IDH1R132S and IDH1R132H in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG) to the known IDH1 inhibitor oxalomalate. Experiments were performed to examine if R132 mutations circumvent the inhibition by oxalomalate.

Final concentrations: Tris 7.5 20 mM, NaCl 150 mM, $MnCl_2$ 2 mM, Glycerol 10%, BSA 0.03%, NADP 0.5 mM, IDH1 wt 1.5 ug/ml, IDH1R132S 30 ug/ml, IDH1R132H 60 ug/ml, DL-isocitrate (5 – 650 uM). The results are summarized in **FIG. 17** and Table 3. The R132S mutation displays approximately a two-fold increase in susceptibility to inhibition by oxalomalate, while the R132H mutation is essentially unaffected. In all three cases, the same fully competitive mode of inhibition with regards to isocitrate was observed.

Table 3

Enzyme	Oxalomalate Ki (uM)
wt	955.4
R132S	510
R132H	950.8

F. Forward reactions (isocitrate to α-KG) of mutant enzyme do not go to completion.

Forward reactions containing ICDH1 R132S or ICDH1 R132H were assembled and reaction progress monitored by an increase in the OD340 of the reduced NADPH cofactor. It was observed (**FIG. 23**), that these reactions proceed in the forward direction for a period of time and then reverse direction and oxidize the cofactor reduced in the early stages of the reaction, essentially to the starting concentration present at the initiation of the experiment. Addition of further isocitrate re-initiated the forward reaction for a period of time, but again did not induce the reaction to proceed to completion. Rather, the system returned to initial concentrations of NADPH. This experiment suggested that the mutant enzymes were performing a reverse reaction other than the conversion of α-KG to isocitrate.

2. Analysis of IDH1 wild-type and mutants R132H and R132S in the reduction of α-Ketoglutarate (α-KG).

A. Methods

To determine the catalytic efficiency of enzymes in the reduction of α-Ketoglutarate (α-KG), reactions were performed to determine Vmax and Km for α-KG. In these reactions, substrate was varied while the cofactor was held constant at 500 uM. All reactions were performed in 50 mM potassium phosphate buffer, pH 6.5, 10% glycerol, 0.03% (w/v) BSA, 5 mM $MgCl_2$, and 40 mM sodium hydrocarbonate. Reaction progress was followed by spectroscopy at 340 nM monitoring the change in oxidation state of the cofactor. Sufficient enzyme was added to give a linear change in absorbance for 10 minutes.

B. The R132H and R132S mutant enzymes, but not the wild-type enzyme, support the reduction of α-KG.

To test the ability of the mutant and wild-type enzymes to perform the reduction of α-KG, 40 ug/ml of enzyme was incubated under the conditions for the reduction of α-Ketoglutarate (α-KG) as described above. Results are presented in **FIG. 14**. The wild-type enzyme was unable to consume NADPH, while R132S and R132H reduced α-KG and consumed NADPH.

C. The reduction of α-KG by the R132H and R132S mutants occurs *in vitro* at physiologically relevant concentrations of α-KG.

To determine the kinetic parameters of the reduction of α-KG performed by the mutant enzymes, a substrate titration experiment was performed, as presented in **FIGs. 15A-15B**. R132H maintained the Hill-type substrate interaction as seen in the oxidative decarboxylation of isocitrate, but displayed positive substrate co-operative binding. R132S showed a conversion to Michaelis-Menten kinetics with the addition of uncompetitive substrate inhibition, as compared to wild-type enzyme in the oxidative decarboxylation of isocitrate. The enzymatic parameters of the mutant enzyme are presented in **Table 4**. Since the wild-type enzyme did not consume measurable NADPH in the experiment described above, a full kinetic workup was not performed.

Table 4

Enzyme	Vmax (umol/min/mg)	Km (mM)	Hill Constant	Ki (mM)	Vmax/Km
R132H	1.3	0.965	1.8		1.35
R132S	2.7	0.181	0.479	24.6	14.92

The relative catalytic efficiency of reduction of α-KG is approximately ten-fold higher in the R132S mutant than in the R132H mutant. The biological consequence is that the rate of metabolic flux should be greater in cells expressing R132S as compared to R132H.

D. Analysis of IDH1 wild-type and mutants R132H and R132S in the reduction of alpha-ketoglutarate with NADH.

In order to evaluate the ability of the mutant enzymes to utilize NADH in the reduction of alpha-ketoglutarate, the following experiment was conducted. Final concentrations: NaHCO3 40mM, MgCl2 5mM, Glycerol 10%, K2HPO4 50mM, BSA 0.03%, NADH 0.5mM, IDH1wt 5ug/ml, R132S 30ug/ml, R132H 60ug/ml, alpha-Ketoglutarate 5mM.

The results are shown in **FIG. 16** and **Table 5**. The R132S mutant demonstrated the ability to utilize NADH while the wild type and R132H show no measurable consumption of NADH in the presence of alpha-ketoglutarate.

Table 5: Consumption of NADH by R132S in the presence of alpha-ketoglutarate

	R132S		Mean	SD
Rate (ΔA/sec)	0.001117	0.001088	0.001103	2.05E-05
Umol/min/mg	0.718328	0.699678	**0.709003**	0.013187

Summary

To understand how R132 mutations alter the enzymatic properties of IDH1, wild-type and R132H mutant IDH1 proteins were produced and purified from *E. coli*. When $NADP^+$-dependent oxidative decarboxylation of isocitrate was measured using purified wild-type or R132H mutant IDH1 protein, it was confirmed that R132H mutation impairs the ability of IDH1 to catalyze this reaction (Yan, H. et al. N Engl J Med 360, 765-73 (2009); Zhao, S. et al. Science 324, 261-5 (2009)), as evident by the loss in binding affinity for both isocitrate and $MgCl_2$ along with a 1000-fold decrease

in catalytic turnover (**FIGs. 30A** and **30C**). In contrast, when NADPH-dependent reduction of αKG was assessed using either wild-type or R132H mutant IDH1 protein, only R132H mutant could catalyze this reaction at a measurable rate (**FIGs. 30** and **30C**). Part of this increased rate of αKG reduction results from an increase in binding affinity for both the cofactor NADPH and substrate αKG in the R132H mutant IDH1 (**FIG. 30C**). Taken together, these data demonstrate that while the R132H mutation leads to a loss of enzymatic function for oxidative decarboxylation of isocitrate, this mutation also results in a gain of enzyme function for the NADPH-dependent reduction of αKG.

2: Analysis of mutant IDH1

The R132H mutant does not result in the conversion of α-KG to isocitrate.

Using standard experimental methods, an API2000 mass spectrometer was configured for optimal detection of α-KG and isocitrate (Table 6). MRM transitions were selected and tuned such that each analyte was monitored by a unique transition. Then, an enzymatic reaction containing 1 mM α-KG, 1 mM NADPH, and ICDH1 R132H were assembled and run to completion as judged by the decrease to baseline of the optical absorbance at 340 nM. A control reaction was performed in parallel from which the enzyme was omitted. Reactions were quenched 1:1 with methanol, extracted, and subjected to analysis by LC-MS/MS.

FIG. 18A presents the control reaction indicating that aKG was not consumed in the absence of enzyme, and no detectable isocitrate was present. **FIG. 18B** presents the reaction containing R132H enzyme, in which the α-KG has been consumed, but no isocitrate was detected. **FIG. 18C** presents a second analysis of the reaction containing enzyme in which isocitrate has been spiked to a final concentration of 1 mM, demonstrating that had α-KG been converted to isocitrate at any appreciable concentration greater than 0.01%, the configured analytical system would have been capable of detecting its presence in the reaction containing enzyme. The conclusion from this experiment is that while α-KG was consumed by R132H, isocitrate was not produced. This experiment indicates that one neoactivity of the R132H mutant is the reduction of α-KG to a compound other than isocitrate.

Table 6. Instrument settings for MRM detection of compounds

Compound	Q1	Q3	DP	FP	EP	CEP	CE	CXP
α-KG	144.975	100.6	-6	-220	-10	-16	-10	-22
isocitrate	191.235	110.9	-11	-230	-4.5	-14	-16	-24
a-hydroxyglutarate	147.085	128.7	-11	-280	-10	-22	-12	-24

The R132H mutant reduces α-KG to 2-hydroxyglutaric acid.

Using standard experimental methods, an API2000 mass spectrometer was configured for optimal detection 2-hydroxyglutarate (**Table 6** and **FIG. 19**). The reaction products of the control and enzyme-containing reactions from above were investigated for the presence of 2-hydroxyglutaric acid, **FIG. 20**. In the control reaction, no 2-hydroxyglutaric acid was detected, while in reaction containing R132H, 2-hydroxyglutaric acid was detected. This data confirms that one neoactivity of the R132H mutant is the reduction of α-KG to 2-hydroxyglutaric acid.

To determine whether R132H mutant protein directly produced 2HG from αKG, the product of the mutant IDH1 reaction was examined using negative ion mode triple quadrupole electrospray LC-MS. These experiments confirmed that 2HG was the direct product of NADPH-dependent αKG reduction by the purified R132H mutant protein through comparison with a known metabolite standards (**FIG. 31A**). Conversion of αKG to isocitrate was not observed.

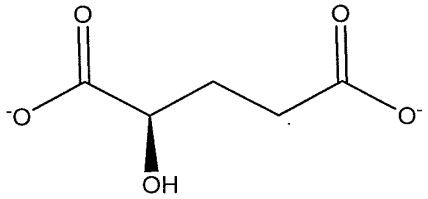
One can determine the enantiomeric specificity of the reaction product through derivitazation with DATAN (diacetyl-L-tartaric acid) and comparing the retention time to that of known R and S standards. This method is described in Struys *et al.* Clin Chem 50:1391-1395(2004). The stereo-specific production of either the R or S enantomer of alpha-hydroxyglutaric acid by ICDH1 R132H may modify the biological activity of other enzymes present in the cell. The racemic production may also occur.

For example, one can measure the inhibitory effect of alpha-hydroxyglutaric acid on the enzymatic activity of enzymes which utilize α-KG as a substrate. In one embodiment, alpha-hydroxyglutaric acid may be a substrate- or product- analogue inhibitor of wild-type ICDH1. In another embodiment alpha-hydroxyglutaric acid may be a substrate- or product- analogue inhibitor of HIF1 prolyl hydroxylase. In the former case, inhibition of wild type ICDH1 by the enzymatic product of R132H will reduce the circulating levels of aKG in the cell. In the latter case, inhibition of HIF1

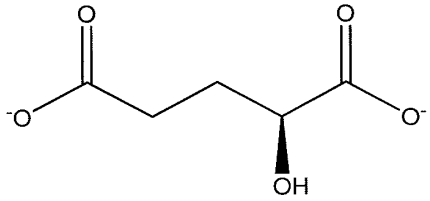
prolyl hydroxylase will result in the stabilization of HIF1 and an induction of the hypoxic response cohort of cellular responses.

ICDH R132H reduces aKG to the R-enantiomer of 2-hydroxyglutarate.

There are two possible enantiomers of the ICDHR132H reductive reaction product, converting alpha-ketoglutarate to 2-hydroxyglutarate, with the chiral center being located at the alpha-carbon position. Exemplary products are depicted below.



R-2-hydroxyglutarate

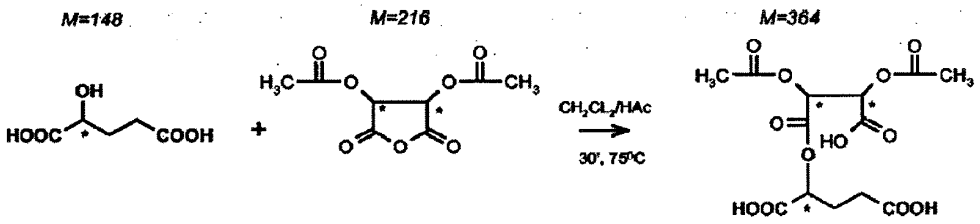


S-2-hydroxyglutarate

These are referred to by those with knowledge in the art as the R (or pro-R) and S (or pro-S) enantiomers, respectively. In order to determine which form or both is produced as a result of the ICDH1 neoactivity described above, the relative amount of each chiral form in the reaction product was determined in the procedure described below.

Reduction of α-KG to 2-HG was performed by ICDHR132H in the presence of NADPH as described above, and the reaction progress was monitored by a change in extinction coefficient of the nucleotide cofactor at 340 nM; once the reaction was judged to be complete, the reaction was extracted with methanol and dried down completely in a stream of nitrogen gas. In parallel, samples of chirally pure R-2-HG and a racemic mixture of R- and S-2-HG (produced by a purely chemical reduction of α-KG to 2-HG) were resuspended in ddH_2O, similarly extracted with methanol, and dried.

The reaction products or chiral standards were then resuspended in a solution of dichloromethane:acetic acid (4:1) containing 50 g/L DATAN and heated to 75°C for 30 minutes to promote the derivitization of 2-HG in the scheme described below:



After cooling to room temperature, the derivitization reactions were dried to completion and resuspended in ddH_2O for analysis on an LC-MS/MS system. Analysis of reaction products and chiral standards was performed on an API2000 LC-MS/MS system using a 2 x 150 mM C18 column with an isocratic flow of 200 µl/min of 90:10 (ammonium formate, pH 3.6:methanol) and monitoring the retention times of the 2-HG-DATAN complex using XIC and the diagnostic MRM transition of 363/147 in the negative ion mode.

It should be noted that retention times in the experiments described below are approximate and accurate to within +/- 1 minute; the highly reproducible peak seen at 4 minutes is an artefact of a column switching valve whose presence has no result on the conclusions drawn from the experiment.

Injection of the racemic mixture gave two peaks of equal area at retention times of 8 and 10 minutes (**FIG. 24A**), while injection of the R-2-HG standard resulted in a major peak of >95% area at 10 minutes and a minor peak <5% area at 8 minutes (**FIG. 24B**); indicating that the R-2-HG standard is approximately 95% R and 5% S. Thus, this method allows us to separate the R and S-2-HG chiral forms and to determine the relative amounts of each in a given sample. Coinjection of the racemic mixture and the R-2-HG standard resulted in two peaks at 8 and 10 minutes, with a larger peak at 10 minutes resulting from the addition of surplus pro-R-form (the standard) to a previously equal mixture of R- and S-2-HG (**FIG. 24C**). These experiments allow us to assign the 8 minute peak to the S-2-HG form and the 10 minute peak to the R-2-HG form.

Injection of the derivatized neoactivity enzyme reaction product alone yields a single peak at 10 minutes, suggesting that the neoactivity reaction product is chirally pure R-2-HG (**FIG. 24D**). Coinjection of the neoactivity reaction product with the R-

2-HG standard results in a major peak of >95% area at 10 minutes (**FIG. 24E**) and a single minor peak of <5% area at 8 minutes (previously observed in injection of the R-2-HG standard alone) confirming the chirality of the neoactivity product as R. Coinjection of a racemic mixture and the neoactivity reaction product (**FIG. 24F**) results in a 60% area peak at 10 minutes and a 40% area peak at 8 minutes; this deviation from the previously symmetrical peak areas observed in the racemate sample being due to the excess presence of R-2-HG form contributed by the addition of the neoactivity reaction product.

These experiments allow us to conclude that the ICDH1 neoactivity is a highly specific chiral reduction of α-KG to R-2-HG.

Enzyme properties of other IDH1 mutations

To determine whether the altered enzyme properties resulting from R132H mutation were shared by other R132 mutations found in human gliomas, recombinant R132C, R132L and R132S mutant IDH1 proteins were generated and the enzymatic properties assessed. Similar to R132H mutant protein, R132C, R132L, and R132S mutations all result in a gain-of-function for NADPH-dependent reduction of αKG (data not shown). Thus, in addition to impaired oxidative decarboxylation of isocitrate, one common feature shared among the IDH1 mutations found in human gliomas is the ability to catalyze direct NADPH-dependent reduction of αKG.

Identification of 2-HG production in glioblastoma cell lines containing the IDH-1 R132H mutant protein.

Generation of genetic engineered glioblastoma cell lines expressing wildtype or mutant IDH-1 protein. A carboxy-terminal Myc-DDK-tagged open reading frame (ORF) clone of human isocitrate dehydrogenase 1 (IDH1; Ref. ID: NM_005896) cloned in vector pCMV6 was obtained from commercial vendor Origen Inc. Vector pCMV6 contains both kanamycin and neomycin resistance cassettes for selection in both bacterial and mammalian cell systems. Standard molecular biology mutagenesis techniques were utilized to alter the DNA sequence at base pair 364 of the ORF to introduce base pair change from guanine to adenine resulting in a change in the amino acid code at position 132 from argentine (wt) to histidine (mutant; or R132H). Specific DNA sequence alteration was confirmed by standard methods for

DNA sequence analysis. Parental vector pCMV6 (no insert), pCMV6-wt IDH1 or pCMV6-R132H were transfected into immortalized human glioblastoma cell lines ATCC® CRL-2610 (LN-18) or HTB-14 (U-87) in standard growth medium (DMEM; Dulbecco's modified Eagles Medium containing 10 % fetal bovine serum). Approximately 24 hrs after transfection, the cell cultures were transitioned to DMEM containing G418 sodium salt at concentrations of either 750 ug/ml (CRL-2610) or 500 ug/ml (HTB-14) to select those cells in culture that expressed the integrated DNA cassette expressing both the neomycin selectable marker and the ORF for human wild type or R132H. Pooled populations of G418 resistant cells were generated and expression of either wild type IDH1 or R132 IDH1 was confirmed by standard Western blot analysis of cell lysates using commercial antibodies recognizing either human IDH1 antigen or the engineered carboxy-terminal MYC-DDK expression tag. These stable clonal pools were then utilized for metaobolite preparation and analysis.

Procedure for metabolite preparation and analysis. Glioblastoma cell lines (CRL-2610 and HTB-14) expressing wildtype or mutant IDH-1 protein were grown using standard mammalian tissue culture techniques on DMEM media containing 10% FCS, 25 mM glucose, 4 mM glutamine, and G418 antibiotic (CRL-2610 at 750 ug/mL; HTB-14 at 500 ug/mL) to insure ongoing selection to preserve the transfected mutant expression sequences. In preparation for metabolite extraction experiments, cells were passaged into 10 cm round culture dishes at a density of $1x10^6$ cells. Approximately 12 hours prior to metabolite extraction, the culture media was changed (8 mL per plate) to DMEM containing 10% dialyzed FCS (10,000 mwco), 5 mM glucose, 4 mM glutamine, and G-418 antibiotic as before; the dialyzed FCS removes multiple small molecules form the culture media and enables cell culture-specific assessment of metabolite levels. The media was again changed 2 horrs prior to metabolite extraction. Metabolite extraction was accomplished by quickly aspirating the media from the culture dishes in a sterile hood, immediately placing the dishes in a tray containing dry ice to cool them to -80°C, and as quickly as possible, adding 2.6 mL of 80% MeOH/20% water, pre-chilled to -80°C in a dry-ice/acetone bath. These chilled, methanol extracted cells were then physically separated from the culture dish by scraping with a sterile polyethylene cell lifter (Corning #3008), brought into suspension and transferred to a 15 mL conical vial, then chilled to -20°C. An additional 1.0 mL of 80% MeOH/20% water was applied to the chilled culture dish

and the cell lifting procedure repeated, to give a final extraction volume of 3.6 mL. The extracts were centrifuged at 20,000 x g for 30 minutes to sediment the cell debris, and 3.0 mL of the supernatants was transferred to a screw-cap freezer vial and stored at -80°C until ready for analysis.

In preparation for analysis, the extracts were removed from the freezer and dried on a nitrogen blower to remove methanol. The 100% aqueous samples were analyzed by LCMS as follows. The extract (10 µL) was injected onto a reverse-phase HPLC column (Synergi 150mm x 2 mm, Phenomenex Inc.) and eluted using a linear gradient of LCMS-grade methanol (Buffer B) in Aq. 10 mM tributylamine , 15 mM Acetic acid (Buffer A), running from 3% Buffer B to 95% Buffer B over 45 minutes at 200 µL/min. Eluted metabolite ions were detected using a triple-quadrapole mass spectrometer, tuned to detect in negative mode with multiple-reaction-monitoring mode transition set (MRM's) according to the molecular weights and fragmentation patterns for 38 known central metabolites, including 2-hydroxyglutarate (MRM parameters were optimized by prior infusion of known compound standards). Data was processed using Analyst Software (Applied Biosystems, Inc.) and metabolite signal intensities were converted into absolute concentrations using signal build-up curves from injected mixtures of metabolite standards at known concentrations. Final metabolite concentrations were reported as mean of at least three replicates, +/- standard deviation.

Results. Analyses reveal significantly higher levels of 2-HG in cells that express the IDH-1 R132H mutant protein. As shown in **FIG. 26A**, levels of 2-HG in CRL-2610 cell lines expressing the IDH-1 R132H mutant protein are approximately 28-fold higher than identical lines expressing the wild-type protein. Similarly, levels of 2-HG in HTB-14 cell lines expressing the IDH-1 R132H mutant protein are approximately 38-fold higher than identical lines expressing the wild-type protein, as shown in **FIG. 26B**.

Evaluation of 2-hydroxyglutarate (2-HG) production in human glioblastoma tumors containing mutations in isocitrate dehydrogenase 1 (IDH1) at amino acid 132.

Heterozygous somatic mutations at nucleotide position 395 (amino acid codon 132) in the transcript encoding isocitrate dehydrogenase 1(IDH1) can occur in brain tumors.

***Tissue source*:** Human brain tumors were obtained during surgical resection, flash frozen in liquid nitrogen and stored at -80°C. Clinical classification of the tissue as gliomas was performed using standard clinical pathology categorization and grading.

Genomic sequence analysis to identify brain tumor samples containing either wild type isocitrate dehydrogenase (IDH1) or mutations altering amino acid 132. Genomic DNA was isolated from 50-100 mgs of brain tumor tissue using standard methods. A polymerase chain reaction (PCR) procedure was then performed on the isolated genomic DNA to amplify a 295 base pair fragment of the genomic DNA that contains both intron and 2nd exon sequences of human IDH1 (**FIG. 27**). In **FIG. 27**, intron sequence is shown in lower case font; 2nd exon IDH1 DNA sequence is shown in upper case font; forward (5') and reverse (3') primer sequences are shown in underlined font; guanine nucleotide mutated in a subset of human glioma tumors is shown in bold underlined font.

The amplified DNA fragment was then sequenced using standard protocols and sequence alignments were performed to classify the sequences as either wild type or mutant at the guanine nucleotide at base pair 170 of the amplified PCR fragment. Tumors were identified that contained genomic DNA having either two copies of guanine (wild type) or a mixed or monoalellic combination of one IDH1 allele containing guanine and the other an adenine (mutant) sequence at base pair 170 of the amplified product (**Table 15**). The nucleotide change results in a change at amino acid position 132 of human IDH1 protein from arginine (wild type) to histidine (mutant) as has been previously reported.

Table 15. Sequence variance at base pair 170 of the amplified genomic DNA from human glioma samples.

Sample ID	Base 170	IDH1 Amino Acid 132	Genotype
1102	G	arginine	wild type
1822	A	histidine	mutant
496	G	arginine	wild type
1874	A	histidine	mutant
816	A	histidine	mutant
534	G	arginine	wild type
AP-1	A	histidine	mutant
AP-2	A	histidine	mutant

Procedure for metabolite preparation and analysis. Metabolite extraction was accomplished by adding a 10 X volume (m/v ratio) of -80 C methanol:water mix (80%:20%) to the brain tissue (approximately 100mgs) followed by 30 s homogenization at 4 C. These chilled, methanol extracted homogenized tissues were then centrifuged at 14,000 rpm for 30 minutes to sediment the cellular and tissue debris and the cleared tissue supernatants were transferred to a screw-cap freezer vial and stored at -80℃. For analysis, a 2X volume of tributylamine (10 mM) acetic acid (10 mM) pH 5.5 was added to the samples and analyzed by LCMS as follows. Sample extracts were filtered using a Millex-FG 0.20 micron disk and 10 μL were injected onto a reverse-phase HPLC column (Synergi 150mm x 2 mm, Phenomenex Inc.) and eluted using a linear gradient LCMS-grade methanol (50%) with 10 mM tributylamine and 10 mM acetic acid) ramping to 80 % methanol:10 mM tributylamine: 10 mM acetic acid over 6 minutes at 200 μL/min. Eluted metabolite ions were detected using a triple-quadrapole mass spectrometer, tuned to detect in negative mode with multiple-reaction-monitoring mode transition set (MRM's) according to the molecular weights and fragmentation patterns for 8 known central metabolites, including 2-hydroxyglutarate (MRM parameters were optimized by prior infusion of known compound standards). Data was processed using Analyst Software (Applied Biosystems, Inc.) and metabolite signal intensities were obtained by standard peak integration methods.

Results. Analyses revealed dramatically higher levels of 2-HG in cells tumor samples that express the IDH-1 R132H mutant protein. Data is summarized in **Table 16** and **FIG. 28**.

Table 16

mple ID	Primary Specimen Diagnosis	Grade	Tumor Cells in Tumor Foci (%)	Geno-type	Nucleo-tide change	Codon	2HG (□mole/g)	□KG (□mole/g)	Malate (□mole/g)	Fumarate (□mole/g)	Succinate (□mole/g)	Isocitrate (□mole/g)
1	Glioblastoma, residual/recurrent	WHO grade IV	n/a	wild type	wild type	R132	0.18	0.161	1.182	0.923	1.075	0.041
2	Glioblastoma	WHO grade IV	n/a	wild type	wild type	R132	0.16	0.079	1.708	1.186	3.156	0.100
3	Glioblastoma	WHO grade IV	n/a	wild type	wild type	R132	0.13	0.028	0.140	0.170	0.891	0.017
4	Oligoastrocytoma	WHO grade II	n/a	wild type	wild type	R132	0.21	0.016	0.553	1.061	1.731	0.089
5	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	16.97	0.085	1.091	0.807	1.357	0.058
6	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	19.42	0.023	0.462	0.590	1.966	0.073
7	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	31.56	0.068	0.758	0.503	2.019	0.093
8	Oligodendroglioma, anaplastic	WHO grade III	75	mutant	G364A	R132H	12.49	0.033	0.556	0.439	0.507	0.091
9	Oligodendroglioma, anaplastic	WHO grade III	90	mutant	G364A	R132H	4.59	0.029	1.377	1.060	1.077	0.574
10	Oligoastrocytoma	WHO grade II	n/a	mutant	G364A	R132H	6.80	0.038	0.403	0.503	1.561	0.065
11	Glioblastoma	WHO grade IV	n/a	wild type	wild type	R132	0.686	0.686	0.686	0.686	0.686	0.007
12	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	18.791	18.791	18.791	18.791	18.791	0.031
13	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	4.59	0.029	1.377	1.060	1.077	0.043
14	Glioblastoma	WHO grade IV	n/a	wild type	wild type	R132	0.199	0.046	0.180	0.170	0.221	0.014
15	Glioblastoma	WHO grade IV	n/a	mutant	C363G	R132G	13.827	0.030	0.905	0.599	1.335	0.046
16	Glioblastoma	WHO grade IV	n/a	mutant	G364A	R132H	28.364	0.068	0.535	0.488	2.105	0.054
17	Glioblastoma	WHO grade IV	n/a	mutant	C363A	R132S	9.364	0.029	1.038	0.693	2.151	0.121
18	Glioblastoma	WHO grade IV	n/a	wild type	wild type	R132	0.540	0.031	0.468	0.608	1.490	0.102
19	Glioma, malignant, astrocytoma	WHO grade IV	80	mutant	G364A	R132H	19.000	0.050	0.654	0.391	2.197	0.171
20	Oligodendroglioma	WHO grade III	80	wild type	wild type	R132	0.045	0.037	1.576	0.998	1.420	0.018
21	Glioma, malignant, astrocytoma	WHO grade	95	wild type	wild type	R132	0.064	0.034	0.711	0.710	2.105	0.165

		IV										
22	Glioblastoma	WHO grade IV	70	wild type	wild type	R132	0.171	0.041	2.066	1.323	0.027	0.072

To determine if 2HG production is characteristic of tumors harboring mutations in IDH1, metabolites were extracted from human malignant gliomas that were either wild-type or mutant for IDH1. It has been suggested that αKG levels are decreased in cells transfected with mutant IDH1 (Zhao, S. et al. Science 324, 261-5 (2009)). The average αKG level from 12 tumor samples harboring various R132 mutations was slightly less than the average αKG level observed in 10 tumors which are wild-type for IDH1. This difference in αKG was not statistically significant, and a range of αKG levels was observed in both wild-type and mutant tumors. In contrast, increased 2HG levels were found in all tumors that contained an R132 IDH1 mutation. All R132 mutant IDH1 tumors examined had between 5 and 35 μmol of 2HG per gram of tumor, while tumors with wild-type IDH1 had over 100 fold less 2HG. This increase in 2HG in R132 mutant tumors was statistically significant ($p<0.0001$). It was confirmed that (R)-2HG was the isomer present in tumor samples (data not shown). Together these data establish that the novel enzymatic activity associated with R132 mutations in IDH1 results in the production of 2HG in human brain tumors that harbor these mutations.

2HG is known to accumulate in the inherited metabolic disorder 2-hydroxyglutaric aciduria. This disease is caused by deficiency in the enzyme 2-hydroxyglutarate dehydrogenase, which converts 2HG to αKG (Struys, E. A. et al. Am J Hum Genet 76, 358-60 (2005)). Patients with 2-hydroxyglutarate dehydrogenase deficiencies accumulate 2HG in the brain as assessed by MRI and CSF analysis, develop leukoencephalopathy, and have an increased risk of developing brain tumors (Aghili, M., Zahedi, F. & Rafiee, J Neurooncol 91, 233-6 (2009); Kolker, S., Mayatepek, E. & Hoffmann, G. F. Neuropediatrics 33, 225-31 (2002); Wajner, M., Latini, A., Wyse, A. T. & Dutra-Filho, C. S. J Inherit Metab Dis 27, 427-48 (2004)). Furthermore, elevated brain levels of 2HG result in increased ROS levels (Kolker, S. et al. Eur J Neurosci 16, 21-8 (2002); Latini, A. et al. Eur J Neurosci 17, 2017-22 (2003)), potentially contributing to an increased risk of cancer. The ability of 2HG to act as an NMDA receptor agonist may contribute to this effect (Kolker, S. et al. Eur J

Neurosci 16, 21-8 (2002)). 2HG may also be toxic to cells by competitively inhibiting glutamate and/or αKG utilizing enzymes. These include transaminases which allow utilization of glutamate nitrogen for amino and nucleic acid biosynthesis, and αKG-dependent prolyl hydroxylases such as those which regulate Hif1α levels. Alterations in Hif1α have been reported to result from mutant IDH1 protein expression (Zhao, S. et al. Science 324, 261-5 (2009)). Regardless of mechanism, it appears likely that the gain-of-function ability of cells to produce 2HG as a result of R132 mutations in IDH1 contributes to tumorigenesis. Patients with 2-hydroxyglutarate dehydrogenase deficiency have a high risk of CNS malignancy (Aghili, M., Zahedi, F. & Rafiee, E. J Neurooncol 91, 233-6 (2009)). The ability of mutant IDH1 to directly act on αKG may explain the prevalence of IDH1 mutations in tumors from CNS tissue, which are unique in their high level of glutamate uptake and its ready conversion to αKG in the cytosol (Tsacopoulos, M. J Physiol Paris 96, 283-8 (2002)), thereby providing high levels of substrate for 2HG production. The apparent co-dominance of the activity of mutant IDH1 with that of the wild-type enzyme is consistent with the genetics of the disease, in which only a single copy of the gene is mutated. As discussed above, the wild-type IDH1 could directly provide NADPH and αKG to the mutant enzyme. These data also demonstrate that mutation of R132 to histidine, serine, cysteine, glycine or leucine share a common ability to catalyze the NADPH-dependent conversion of αKG to 2HG. These findings help clarify why mutations at other amino acid residues of IDH1, including other residues essential for catalytic activity, are not found. Finally, these findings have clinical implications in that they suggest that 2HG production will identify patients with IDH1 mutant brain tumors. This will be important for prognosis as patients with IDH1 mutations live longer than patients with gliomas characterized by other mutations (Parsons, D. W. et al. Science 321, 1807-12 (2008)). In addition, patients with lower grade gliomas may benefit by the therapeutic inhibition of 2HG production. Inhibition of 2HG production by mutant IDH1 might slow or halt conversion of lower grade glioma into lethal secondary glioblastoma, changing the course of the disease.

The reaction product of ICDH1 R132H reduction of α-KG inhibits the oxidative decarboxylation of isocitrate by wild-type ICDH1.

A reaction containing the wild-type ICDH1, NADP, and α-KG was assembled (under conditions as described above) to which was added in a titration series either (R)-2-hydroxyglutarate or the reaction product of the ICDH1 R1321H mutant reduction of α-KG to 2-hydroxyglutarate. The reaction product 2-HG was shown to inhibit the oxidative decarboxylation of isocitrate by the wild-type ICDH1, while the (R)-2-hydroxyglutarate did not show any effect on the rate of the reaction. Since there are only two possible chiral products of the ICDH1 R132H mutant reduction of α-KG to 2-HG, and the (R)-2-HG did not show inhibition in this assay, it follows that the product of the mutant reaction is the (S)-2-HG form. This experiment is presented in **FIG. 25**.

To determine the chirality of the 2HG produced, the products of the R132H reaction was derivatized with diacetyl-L-tartaric anhydride, which allowed separating the (S) and (R) enantiomers of 2HG by simple reverse-phase LC and detecting the products by tandem mass spectrometry (Struys, E. A., Jansen, E. E., Verhoeven, N. M. & Jakobs, C. Clin Chem 50, 1391-5 (2004)) (**FIG. 31B**). The peaks corresponding to the (S) and (R) isomers of 2HG were confirmed using racemic and R(-)-2HG standards. The reaction product from R132H co-eluted with R(-)-2HG peak, demonstrating that the R(-) stereoisomer is the product produced from αKG by R132H mutant IDH1.

The observation that the reaction product of the mutant enzyme is capable of inhibiting a metabolic reaction known to occur in cells suggests that this reaction product might also inhibit other reactions which utilize α-KG, isocitrate, or citrate as substrates or produce them as products in vivo or in vitro.

EXAMPLE 3 METABOLOMICS ANALYSIS OF IDH1 WILD TYPE AND MUTANTS

Metabolomics research can provide mechanistic basis for why R132 mutations confer survival advantage for GBM patients carrying such mutations.

1. Metabolomics of GBM tumor cell lines: wild type vs R132 mutants

Cell lines with R132 mutations can be identified and profiled. Experiments can be performed in proximal metabolite pool with a broad scope of metabolites.

2. Oxalomalate treatment of GBM cell lines

Oxalomalate is a competitive inhibitor of IDH1. Change of NADPH (metabolomics) when IDH1 is inhibited by a small molecule can be examined.

3. Metabolomics of primary GBM tumors: wild type vs R132 mutations

Primary tumors with R132 mutations can be identified. Experiments can be performed in proximal metabolite pool with a broad scope of metabolites.

4. Detection of 2-hydroxyglutarate in cells that overexpress IDH1 132 mutants

Overexpression of an IDH1 132 mutant in cells may cause an elevated level of 2-hydroxyglutarate and/or a reduced level of alpha-ketoglutarate. One can perform a metabolomic experiment to demonstrate the consequence of this mutation on the cellular metabolite pool.

EXAMPLE 4 EVALUATION OF IDH1 AS A CANCER TARGET

shRNAmir inducible knockdown can be performed to examine the cellular phenotype and metabolomics profiles. HTS grade IDH1 enzymes are available. The IDH mutations described herein can be used for patient selection.

EXAMPLE 5 siRNAs

IDH1

Exemplary siRNAs are presented in the following tables. Art-known methods can be used to select other siRNAs. siRNAs can be evaluated, *e.g.,* by determining the ability of an siRNA to silence an IDH, *e.g.,* IDH1, *e.g.,* in an *in vitro* system, *e.g.,* in cultured cells, *e.g.,* HeLa cells or cultured glioma cells. siRNAs known in the art for silencing the target can also be used, see, *e.g., Silencing of cytosolic NADP+ dependent isoccitrate dehydrogenase by small interfering RNA enhances the sensitivity of HeLa cells toward stauropine*, Lee *et al.*, 2009, Free Radical Research, 43: 165-173.

The siRNAs in **Table 7** (with the exception of entry 1356) were generated using the siRNA selection tool available on the worldwide web at jura.wi.mit.edu/bioc/siRNAext/. (Yuan *et al.* Nucl. Acids. Res. 2004 32:W130-W134.) Other selection tools can be used as well. Entry 1356 was adapted from *Silencing of cytosolic NADP+ dependent isoccitrate dehydrogenase by small interfering RNA enhances the sensitivity of HeLa cells toward stauropine*, Lee *et al.*, 2009, Free Radical Research, 43: 165-173.

The siRNAs in Tables 7, 8, 9, 10, 11, 12, 13 and 14 represent candidates spanning the IDH1 mRNA at nucleotide positions 628 and 629 according to the sequence at GenBank Accession No. NM_005896.2 (SEQ ID NO:9, FIG. 22).

The RNAs in the tables can be modified, *e.g.,* as described herein. Modifications include chemical modifications to enhance properties, *e.g.,* resistance to degradation, or the use of overhangs. For example, either one or both of the sense and antisense strands in the tables can include an additional dinucleotide at the 3' end, *e.g.,* TT, UU, dTdT.

Table 7. siRNAs targeting wildtype IDH1

Position on mRNA (FIG. 21B)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
13	GGUUUCUGCAGAGUCUACU	14	AGUAGACUCUGCAGAAACC	15
118	CUCUUCGCCAGCAUAUCAU	16	AUGAUAUGCUGGCGAAGAG	17
140	GGCAGGCGAUAAACUACAU	18	AUGUAGUUUAUCGCCUGCC	19
145	GCGAUAAACUACAUUCAGU	20	ACUGAAUGUAGUUUAUCGC	21
199	GAAAUCUAUUCACUGUCAA	22	UUGACAGUGAAUAGAUUUC	23
257	GUUCUGUGGUAGAGAUGCA	24	UGCAUCUCUACCACAGAAC	25
272	GCAAGGAGAUGAAAUGACA	26	UGUCAUUUCAUCUCCUUGC	27
277	GGAGAUGAAAUGACACGAA	28	UUCGUGUCAUUUCAUCUCC	29
278	GAGAUGAAAUGACACGAAU	30	AUUCGUGUCAUUUCAUCUC	31
280	GAUGAAAUGACACGAAUCA	32	UGAUUCGUGUCAUUUCAUC	33
292	CGAAUCAUUUGGGAAUUGA	34	UCAAUUCCCAAAUGAUUCG	35
302	GGGAAUUGAUUAAAGAGAA	36	UUCUCUUUAAUCAAUUCCC	37
332	CCUACGUGGAAUUGGAUCU	38	AGAUCCAAUUCCACGUAGG	39
333	CUACGUGGAAUUGGAUCUA	40	UAGAUCCAAUUCCACGUAG	41
345	GGAUCUACAUAGCUAUGAU	42	AUCAUAGCUAUGUAGAUCC	43
356	GCUAUGAUUUAGGCAUAGA	44	UCUAUGCCUAAAUCAUAGC	45
408	GGAUGCUGCAGAAGCUAUA	46	UAUAGCUUCUGCAGCAUCC	47
416	CAGAAGCUAUAAAGAAGCA	48	UGCUUCUUUAUAGCUUCUG	49
418	GAAGCUAUAAAGAAGCAUA	50	UAUGCUUCUUUAUAGCUUC	51
432	GCAUAAUGUUGGCGUCAAA	52	UUUGACGCCAACAUUAUGC	53
467	CUGAUGAGAAGAGGGUUGA	54	UCAACCCUCUUCUCAUCAG	55
481	GUUGAGGAGUUCAAGUUGA	56	UCAACUUGAACUCCUCAAC	57
487	GAGUUCAAGUUGAAACAAA	58	UUUGUUUCAACUUGAACUC	59
495	GUUGAAACAAAUGUGGAAA	60	UUUCCACAUUUGUUUCAAC	61
502	CAAAUGUGGAAAUCACCAA	62	UUGGUGAUUUCCACAUUUG	63
517	CCAAAUGGCACCAUACGAA	64	UUCGUAUGGUGCCAUUUGG	65
528	CAUACGAAAUAUUCUGGG	66	ACCCAGAAUAUUUCGUAU	67