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INHALED ILOPROST FOR SEVERE PULMONARY HYPERTENSION

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ABSTRACT

Background Uncontrolled studies suggested that aerosolized iloprost, a stable analogue of prostacyclin, causes selective pulmonary vasodilatation and improves hemodynamics and exercise capacity in patients with pulmonary hypertension.

Methods We compared repeated daily inhalations of 2.5 or 5.0 μg of iloprost (six or nine times per day; median inhaled dose, 30 μg per day) with inhalation of placebo. A total of 203 patients with selected forms of severe pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension (New York Heart Association [NYHA] functional class III or IV) were included. The primary end point was met if, after week 12, the NYHA class and distance walked in six minutes were improved by at least one class and at least 10 percent, respectively, in the absence of clinical deterioration according to predefined criteria and death.

Results The combined clinical end point was met by 16.8 percent of the patients receiving iloprost, as compared with 4.9 percent of the patients receiving placebo (P=0.007). There were increases in the distance walked in six minutes of 36.4 m in the iloprost group as a whole (P=0.004) and of 58.8 m in the subgroup of patients with primary pulmonary hypertension. Overall, 4.0 percent of patients in the iloprost group (including one who died) and 13.7 percent of those in the placebo group (including four who died) did not complete the study (P=0.024); the most common reason for withdrawal was clinical deterioration. As compared with base-line values, hemodynamic values were significantly improved at 12 weeks when measured after iloprost inhalation (P<0.001), were largely unchanged when measured before iloprost inhalation, and were significantly worse in the placebo group. Further significant beneficial effects of iloprost treatment included an improvement in the NYHA class (P=0.03), dyspnea (P=0.015), and quality of life (P=0.026). Syncope occurred with similar frequency in the two groups but was more frequently rated as serious in the iloprost group, although this adverse effect was not associated with clinical deterioration.

Conclusions Inhaled iloprost is an effective therapy for patients with severe pulmonary hypertension. (N Engl J Med 2002;347:322-9.)

A CONTINUOUS infusion of prostacyclin was the first therapy shown to reduce mortality in a controlled study of patients with severe pulmonary hypertension.[1] However, its use is associated with a number of serious drawbacks. The lack of pulmonary selectivity results in systemic side effects, tolerance leads to progressive increases in the dose, and there may be recurrent infections of the intravenous catheter.[2] As an alternative, inhaled nitric oxide possesses pulmonary selectivity, but it is less potent than prostacyclin in the pulmonary vasculature.[3,4] Moreover, an interruption in the inhalation of continuous nitric oxide may cause rebound pulmonary hypertension.[5,6] Designed to combine the beneficial effects of prostacyclin with those of an inhalational application, aerosolized prostacyclin was found to be a potent pulmonary vasodilator in patients with acute respiratory failure, exerting preferential vasodilatation in well-ventilated lung regions.[7-10] Similar results were obtained in spontaneously breathing patients who had lung fibrosis and severe pulmonary hypertension.[11]

Iloprost is a stable analogue of prostacyclin that is associated with a longer duration of vasodilatation.[12] When administered during a short aerosolization ma-

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*The other members of the Aerosolized Iloprost Randomized (AIR) study group are listed in the Appendix.

neuver to patients with pulmonary hypertension, its pulmonary vasodilative potency was similar to that of prostacyclin, but its effects lasted for 30 to 90 minutes, as compared with 15 minutes.[4,11,13-15] Several open-label, uncontrolled studies of patients with severe pulmonary hypertension suggested that long-term use of aerosolized iloprost results in substantial clinical improvement.[11,13,16-20] Our objective in this trial was to evaluate the effects of inhaled iloprost using a rigorous end point of clinical efficacy.

METHODS

Selection of Patients

Patients with primary pulmonary hypertension and selected forms of nonprimary pulmonary hypertension were candidates for the study. The forms of nonprimary pulmonary hypertension included appetite-suppressant–associated and scleroderma-associated pulmonary hypertension as well as inoperable chronic thromboembolic pulmonary hypertension. The inclusion criteria were a mean pulmonary-artery pressure greater than 30 mm Hg, the ability to cover between 50 and 500 m without encouragement on a six-minute walk test,[21] and a New York Heart Association (NYHA) functional class of III or IV[22] despite the use of standard conventional therapy (anticoagulants, diuretics, digitalis, calcium-channel blockers, and supplemental oxygen). Patients who were taking investigational drugs, prostanoids, or beta-blockers were excluded. The doses of calcium-channel blockers had to be constant for more than six weeks before study entry. Exclusion criteria were a pulmonary-artery wedge pressure at rest of more than 15 mm Hg, a cardiac index at rest of less than 1.5 or more than 4 liters per minute per square meter of body-surface area, bleeding disorders, a bilirubin level of more than 3 mg per deciliter (51 μmol per liter), creatinine clearance below 30 ml per minute, a forced vital capacity below 50 percent, a forced expiratory volume in one second that was less than the mean normal value minus twice the standard deviation, and clinical instability.

Study Design

A total of 203 patients participated after giving written informed consent and after the study had been approved by the local ethics committees at 37 European specialist centers. Patients were randomly assigned to receive iloprost (Ilomedin, Schering) or placebo after stratification according to NYHA functional class (III or IV) and type of pulmonary hypertension (primary or nonprimary) by an independent committee whose members were unaware of patients' identities. A total of 101 patients were randomly assigned to the iloprost group, and 102 were assigned to the placebo group.

For inhalation, iloprost or placebo was diluted with saline to a concentration of 10 μg per milliliter, and 2 ml was added to a nebulizer (HaloLite, MedicAid). This device delivered short pulses of aerosolized particles (geometric median [±SD] aerodynamic diameter of particles, 4.3±0.05 μm)[23] during the first part of each inspiration until a predefined total inhaled dose of 2.5 μg had been dispensed. The inhalation was then stopped or repeated once, to achieve a total dose of 5.0 μg, depending on how well the patient tolerated the treatment. After each inhalation, the residual volume in the nebulizer was discarded. This maneuver was repeated six or nine times daily, with an overnight break. The frequency of inhalation and the dose were individually determined within the first eight days of therapy according to a predefined dosing algorithm.

Right-heart catheterization was performed in all patients at base line and after 12 weeks. The acute effects of inhaled iloprost were evaluated after 12 weeks in all patients, but not at base line, to avert unblinding. At base line and after 4, 8, and 12 weeks, patients completed a six-minute walk test, the Mahler Dyspnea Index questionnaire,[24] the EuroQol questionnaire,[25] and the 12-item Medical Outcomes Study Short-Form General Health Survey.[26]

Patients were removed from the study if they met two or more of the following predefined criteria for a deterioration in their condition: refractory systolic arterial hypotension (blood pressure, less than 85 mm Hg); worsening right ventricular failure (e.g., as indicated by the development of refractory edema or ascites); rapidly progressing cardiogenic, hepatic, or renal failure; a decrease of at least 30 percent in the distance walked in six minutes; and a decline in measures of hemodynamic function, such as central venous pressure and mixed venous oxygen saturation.

Outcome Measures

The primary end point of the study consisted of an increase of at least 10 percent in the distance walked in six minutes and an

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	ILOPROST GROUP (N=101)	PLACEBO GROUP (N=102)
Age — yr	51.2±13.2	52.8±12.0
Weight — kg	71.3±14.6	72.6±13.9
Sex — %		
Male	31.7	33.3
Female	68.3	66.7
Underlying disease — no. (%)		
Primary pulmonary hypertension	51 (50.5)	51 (50.0)
Nonprimary pulmonary hypertension	50 (49.5)	51 (50.0)
Appetite suppressants	4 (4.0)	5 (4.9)
Collagen vascular disease	13 (12.9)	22 (21.6)
Chronic thromboembolic pulmonary hypertension	33 (32.7)	24 (23.5)
Oral vasodilator therapy — no. (%)	52 (51.5)	58 (56.9)
NYHA functional class — no. (%)		
III	60 (59.4)	59 (57.8)
IV	41 (40.6)	43 (42.2)
Mahler Dyspnea Index†	4.14±1.8	4.27±1.8
6-Minute walk distance — m	332±93	315±96
Hemodynamic variables‡		
Pulmonary-artery pressure — mm Hg	52.8±11.5	53.8±14.1
Cardiac output — liters/min	3.8±1.1	3.8±0.9
Pulmonary vascular resistance — dyn·sec·cm^{-5}	1029±390	1041±493
Systemic vascular resistance — dyn·sec·cm^{-5}	1872±673	1827±503
Central venous pressure — mm Hg	9.2±5.3	8.2±5.0
Pulmonary-artery wedge pressure — mm Hg	7.5±3.3	7.6±3.9
Arterial oxygen saturation — %	92.6±4.4	92.2±5.0
Mixed venous oxygen saturation — %	60.4±7.5	60.5±8.2
Heart rate — beats/min	83.9±12.2	81.8±15.4

*Plus–minus values are means ±SD. NYHA denotes New York Heart Association. There were no significant differences between the iloprost and the placebo groups. Data on all variables were available for all patients except in the following categories: pulmonary-artery pressure, 1 patient in each group; cardiac output, 1 patient in the iloprost group and 6 in the placebo group; pulmonary vascular resistance, 10 and 6, respectively; systemic vascular resistance, 11 and 14; central venous pressure, 5 and 7; pulmonary-artery wedge pressure, 8 and 3; arterial oxygen saturation, 35 and 31; mixed venous oxygen saturation, 16 and 18; and heart rate, 2 and 3.

†On this 12-point scale, higher scores indicate less dyspnea.

‡Patients who were receiving long-term oxygen therapy received nasal oxygen during the measurement of base-line hemodynamic variables.

improvement in the NYHA functional class in the absence of a deterioration in the clinical condition or death during the 12 weeks of the study. Secondary efficacy variables were changes in the values for the six-minute walk test, the NYHA class, Mahler Dyspnea Index scores, hemodynamic variables, and the quality of life; clinical deterioration; death; and the need for transplantation.

Statistical Analysis

The primary evaluation of efficacy included all randomized patients. Data are presented as means ±SD, unless otherwise stated. We included data on patients who prematurely discontinued the study using a last-observation-carried-forward analysis for the six-minute walk test. Patients who died were assigned a value of 0 m. All statistical tests for efficacy variables were two-tailed, with an alpha level of 0.05.

To analyze the primary efficacy end point and the improvement criteria, we used the Mantel–Haenszel test,[27] stratified according to the type of pulmonary hypertension (primary or nonprimary) and NYHA class (III or IV). Patients with missing data on the primary end point at week 12 were considered not to have had a response.

Changes in the results of the six-minute walk were evaluated with use of nonparametric analysis of covariance stratified according to the type of pulmonary hypertension (primary or nonprimary) and the NYHA class (III or IV), with use of the base-line value as the covariate (analysis of covariance), and the Wilcoxon signed-rank test.

Changes from base line in hemodynamic values were analyzed with t-statistics. The investigators had full access to the data and performed the analyses independently of the sponsor.

RESULTS

Base-line demographic and hemodynamic data are given in Table 1. The mean frequency of inhalation was 7.5 times per day. Ninety-one percent of patients received 5.0 μg per inhalation, and 9 percent received 2.5 μg, corresponding to a median inhaled dose of 30 μg per day.

Primary Efficacy End Point

For the primary end point, we found a significant effect of treatment in favor of iloprost ($P=0.007$) (Fig. 1). The estimated odds of an effect in the iloprost group, as compared with the placebo group, were 3.97 (95 percent confidence interval, 1.47 to 10.75, by the Mantel–Haenszel test), with no significant heterogeneity among the four subgroups categorized according to type of pulmonary hypertension and NYHA class at base line ($P=0.79$ by the Breslow–Day test). The secondary analysis of the primary end point was a logistic-regression model that included treatment assignment, demographic data, and base-line characteristics. Only treatment assignment ($P=0.01$) contributed significantly to the probability of a response.

Secondary End Points

Six-Minute Walk Test

The percentage of patients who had an increase of at least 10 percent in the distance walked in six minutes at week 12 was slightly, but not significantly, higher in the iloprost group than in the placebo group ($P=0.06$) (Table 2). The type of pulmonary hypertension had no significant effect on the outcome in either group ($P=0.90$). A higher percentage of patients in the placebo group than in the iloprost group had a decrease in the distance walked of at least 10 percent or did not complete the study (Table 2).

The absolute change in the distance walked in six minutes was significantly larger (by 36.4 m) in the

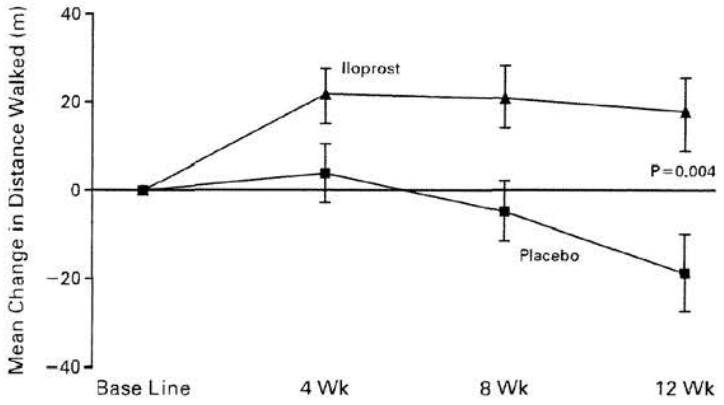

Figure 1. Effect of Inhaled Iloprost and Placebo on the Mean (±SE) Change from Base Line in the Distance Walked in Six Minutes, According to an Intention-to-Treat Analysis.
The P value was obtained with Wilcoxon's test for two independent samples.

iloprost group than in the placebo group (P=0.004) (Fig. 1): 58.8 m among those with primary pulmonary hypertension and 12 m among those with nonprimary pulmonary hypertension. A parametric analysis of covariance, which included the absolute value on the six-minute walk test at week 12 as a dependent variable and the treatment assignment (P=0.02), type of pulmonary hypertension (P=0.06), and distance walked at base line (P<0.001) did not show a statistically significant interaction between treatment and type of pulmonary hypertension (P=0.09).

NYHA Class

More patients in the iloprost group than in the placebo group had an improvement in the severity of heart failure, as assessed by the NYHA class (P=0.03) (Table 2). The type of pulmonary hypertension had no effect on the outcome in either group (P=0.39). The percentage of patients with a deterioration in NYHA class did not differ significantly between the groups, but the analysis did not include patients who left the study early owing to death or other reasons. A larger proportion of patients in the placebo group than in the iloprost group did not complete the study (Table 2 and Fig. 2). Reasons included death, discontinuation of study medication, and withdrawal of consent, mostly owing to clinical deterioration, insufficient clinical benefit, or both.

Hemodynamics and Gas Exchange

In the placebo group, cardiac output, systemic arterial oxygen saturation, and mixed venous oxygen saturation decreased significantly after 12 weeks and pulmonary vascular resistance and right atrial pressure increased significantly (Table 3). In the iloprost group, values assessed at 12 weeks, before the first morning dose of inhaled iloprost, were largely unchanged from base line, whereas values assessed after inhalation were significantly decreased (in the case of pulmonary-artery pressure, pulmonary vascular resistance, systemic arterial pressure, and systemic arterial oxygen saturation) or increased (in the case of carbon monoxide and pulmonary-artery wedge pressure). At the completion of the 12-week study, acute hemodynamic responsiveness to inhaled iloprost was equivalent in the placebo group and the iloprost group, though the latter group had been exposed to daily iloprost inhalation for three months (data not shown).

Mahler Dyspnea Index

The mean Mahler Dyspnea Index transition score was significantly better at week 12 in the iloprost

TABLE 2. EFFECTS OF 12 WEEKS OF THERAPY WITH INHALED ILOPROST OR PLACEBO ON THE NEW YORK HEART ASSOCIATION (NYHA) CLASS AND THE SIX-MINUTE WALK TEST.

VARIABLE	ILOPROST GROUP			PLACEBO GROUP		
	ALL PATIENTS	PATIENTS WITH PRIMARY PULMONARY HYPERTENSION	PATIENTS WITH NONPRIMARY PULMONARY HYPERTENSION	ALL PATIENTS	PATIENTS WITH PRIMARY PULMONARY HYPERTENSION	PATIENTS WITH NONPRIMARY PULMONARY HYPERTENSION
	percentage of patients					
Change in NYHA class						
Improved by 2 classes	1.0*	1.9	0.0	0.0	0.0	0.0
Improved by 1 class	23.8*	22.6	25.0	12.7	7.3	19.1
Unchanged	64.4	66.0	62.5	65.7	69.1	61.7
Worsened	5.9	3.8	8.3	7.8	10.9	4.3
Data missing	1.0	1.9	0.0	0.0	0.0	0.0
Noncompletion of study	4.0	3.8	4.2	13.7	12.7	14.9
Death	1.0	1.9	0.0	3.9	3.6	4.3
Other	3.0†	1.9	4.2	9.8‡	9.1	10.6
Change in 6-minute walk distance						
≥10% increase	37.6§	49.1	25.0	25.5	30.9	19.1
<10% increase to <10% decrease	42.6	37.7	47.9	32.4	20.0	46.8
≥10% decrease	13.9	5.7	22.9	25.5	32.7	17.0
Data missing	5.9	7.5	4.2	16.7	16.4	17.0
Combined end point	16.8¶	20.8	12.5	4.9	5.5	4.3

*P=0.03 for the comparison of rates of improvement (by one or two classes) with the placebo group.

†Treatment was discontinued in all three patients.

‡Treatment was discontinued in seven patients, and three patients withdrew their consent.

§P=0.06 for the comparison with the placebo group.

¶P=0.007 for the comparison with the placebo group.

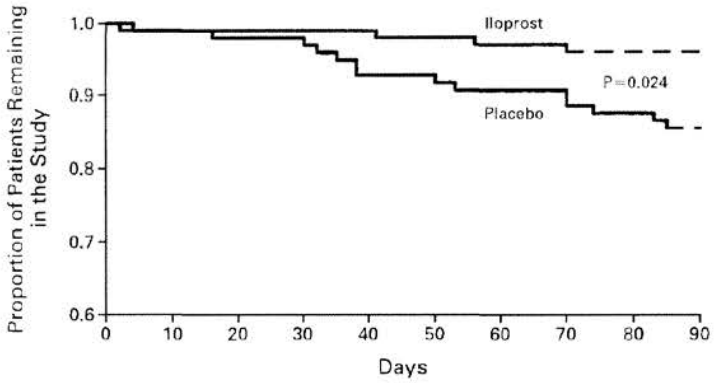

Figure 2. Kaplan–Meier Estimates of the Likelihood of Completing the 12-Week Study.
Reasons for not completing the study included death, discontinuation of study medication, and withdrawal of consent (see Table 2).

TABLE 3. MEAN (±SD) CHANGE FROM BASE LINE IN HEMODYNAMIC VALUES DURING 12 WEEKS OF THERAPY WITH INHALED ILOPROST OR PLACEBO.*

VARIABLE	PLACEBO GROUP	ILOPROST GROUP	
		BEFORE INHALATION	AFTER INHALATION
		mean ±SD	
Pulmonary-artery pressure (mm Hg)	−0.2±6.9	−0.1±7.3	−4.6±9.3†
Cardiac output (liters/min)	−0.19±0.81‡	+0.05±0.86	+0.55±1.1†
Pulmonary vascular resistance (dyn·sec·cm^{-5})	+96±322‡	−9±275§	−239±279†
Systemic arterial pressure (mm Hg)	−0.2±12.4	−1.7±12.8	−4.3±13.6¶
Right arterial pressure (mm Hg)	+1.4±4.8‡	+0.5±4.6	−0.8±4.6
Pulmonary-artery wedge pressure (mm Hg)	+0.7±3.6	+1.1±4.7‡	+1.8±5.3¶
Arterial oxygen saturation (%)	−1.6±4.4‡	−0.4±3.7	−1.4±3.7‡
Mixed venous oxygen saturation (%)	−3.2±6.7†	−1.1±7.6	+1.8±8.3
Heart rate (beats/min)	−1.2±9.5	−1.8±12.4	−2.25±12.6

*For the iloprost group, both preinhalation and postinhalation values after 12 weeks are compared with the base-line values at study entry.

†$P<0.001$ for the difference from base-line values.

‡$P<0.05$ for the difference from base-line values.

§$P<0.01$ for the comparison with the placebo group.

¶$P<0.01$ for the difference from base-line values.

group than in the placebo group (change, +1.42±2.59 vs. +0.30±2.45; P=0.015). The type of pulmonary hypertension had no effect on this outcome.

Quality of Life

Mean scores on the EuroQol visual-analogue scale improved significantly (from 46.9±15.9 to 52.8±19.1) in the iloprost group but were virtually unchanged in the placebo group (dropping from 48.6±16.9 to 47.4±21.1, P=0.026 by analysis of covariance). The EuroQol health-state score improved from 0.49±0.28 to 0.58±0.27 in the iloprost group and was unchanged in the placebo group (0.56±0.29 to 0.56±0.31, P=0.11 by analysis of covariance). None of the other measures of the quality of life were significantly different between the groups.

Clinical Deterioration and Death

One patient died in the iloprost group during the 12-week study, as compared with four patients in the placebo group (P=0.37) (Table 2). Criteria for clinical deterioration were met in 4.9 percent of patients in the iloprost group and 8.8 percent of those in the placebo group (P=0.41). This indicated that fewer patients either died or deteriorated in the iloprost group than in the placebo group (4.9 percent vs. 11.8 percent, P=0.09). The type of pulmonary hypertension had no effect on the outcome. During the study period, none of the patients received a lung transplant.

Safety

The total number of patients who had serious adverse events did not differ significantly between the groups (Table 4). Right ventricular failure and edema were more than twice as frequent in the placebo group as in the iloprost group. The total number of syncopal events in each of the two groups was similar (eight in the iloprost group and five in the placebo group), but these events were more often considered serious in the iloprost group. Syncope was not associated with clinical deterioration or premature withdrawal from the study. Syncopal events occurred more than two hours after the last inhalation (often after an overnight break), were exercise-induced in two patients, were induced by bradycardia in two patients (associated with gastroenteritis in one patient and with verapamil therapy in the other), and resulted in head trauma in one patient. Flushing and jaw pain were more common in the iloprost group, but these adverse effects were mostly transient and mild and were not considered to be serious in any patient.

DISCUSSION

The results of this clinical trial demonstrate that long-term inhaled administration of aerosolized iloprost, a stable analogue of prostacyclin, improves a clinically important combined end point consisting of exercise capacity, NYHA class, and clinical deterioration in patients with selected forms of pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. Moreover, iloprost improved several secondary end points.

Since intravenous epoprostenol was shown to improve survival among the most severely ill patients with primary pulmonary hypertension, it has been unethical to perform randomized clinical trials among patients with pulmonary hypertension in which survival is used as an end point. We chose a combined rather than a single end point (e.g., the distance walked in six minutes) in order to make a more rigorous determination of whether inhaled iloprost was efficacious. Nearly 40 percent of all patients who were treated with iloprost increased their six-minute walking distance by at least 10 percent. However, only half as many patients also had improvement in the NYHA class; conversely, not all patients with an improvement in NYHA class had an increase of at least 10 percent in the distance walked in six minutes. Thus, although only 17 percent of patients in the iloprost group reached the combined end point, a substantial number of the remaining patients met less strict criteria for clinical improvement that would warrant continued therapy. Furthermore, significantly fewer patients in the iloprost group than in the placebo group prematurely discontinued the study as a result of lack of efficacy or other reasons, suggesting that even when iloprost therapy does not produce substantial improvement, it may stabilize the clinical condition.

The mean inhaled dose of iloprost corresponded to 0.37 ng per kilogram of body weight per minute, which is considerably lower than an effective intravenous or subcutaneous dose.[2,28] Thus, targeted delivery of prostanoids to the pulmonary vasculature by means of inhalation may substantially reduce the drug requirements.

TABLE 4. INCIDENCE OF SERIOUS AND OTHER ADVERSE EVENTS.*

VARIABLE	ILOPROST GROUP (N=101)	PLACEBO GROUP (N=102)	P VALUE
	no. of patients (%)		
Serious adverse event			
Any event	28 (27.7)	25 (24.5)	0.63
Right ventricular failure and edema	4 (4.0)	10 (9.8)	0.16
Syncope	5 (5.0)	0	0.03
Other†	33 (32.7)	35 (34.3)	0.88
Adverse event‡			
Any event	91 (90.1)	90 (89.2)	0.82
Increased cough	39 (38.6)	26 (25.5)	0.05
Headache	30 (29.7)	20 (19.6)	0.11
Flushing	27 (26.7)	9 (8.8)	0.001
Influenza-like syndrome	14 (13.9)	10 (9.8)	0.39
Peripheral edema	13 (12.9)	16 (15.7)	0.69
Nausea	13 (12.9)	8 (7.8)	0.26
Jaw pain	12 (11.9)	3 (2.9)	0.02
Hypotension	11 (10.9)	6 (5.9)	0.22
Diarrhea	9 (8.9)	11 (10.8)	0.81
Vertigo	7 (6.9)	11 (10.8)	0.46
Syncope	8 (7.9)	5 (4.9)	0.41
Other adverse events§	296	277	

*The most common adverse events are listed.

†These events included an aggravation reaction (an event causing concern about possible deterioration) in four patients in the iloprost group and five patients in the placebo group, hypoxemia in two patients in the placebo group, pneumonia in two patients in the iloprost group, tachycardia in two patients in the iloprost group and one in the placebo group, laboratory-test abnormalities in two patients in the iloprost group, chest pain in two patients in each group, and dyspnea in two patients in each group.

‡Data were available for 101 patients in the placebo group.

§The number is the total number of other adverse events.

Like other investigators, we found that the benefit was greatest among patients with primary pulmonary hypertension and was similar to that of epoprostenol[1] and bosentan.[29] Although patients with nonprimary pulmonary hypertension had improvement in the scores for the Mahler Dyspnea Index and quality-of-life measures that were similar to those achieved in patients with primary pulmonary hypertension, fewer such patients reached the combined end point, and they also had a smaller absolute change in the distance walked in six minutes. Similar results have been obtained with the use of other drugs for pulmonary hypertension, including epoprostenol,[30] beraprost,[31] and treprostinil.[28]

Hemodynamic assessments of preinhalation values showed that values stabilized in the iloprost group, whereas they deteriorated in the placebo group. The degree of deterioration may be underestimated, since patients who discontinued treatment prematurely did not undergo follow-up hemodynamic examination. Postinhalation assessments of hemodynamic variables demonstrated a significant improvement in the iloprost group, as was anticipated on the basis of previous reports.[4,11,13,16] Since the acute hemodynamic response did not differ between the groups, it appears unlikely that tolerance developed over the 12-week course of iloprost treatment. During long-term treatment, the patients' hemodynamic status is somewhere between preinhalation and postinhalation values. In comparison, continuous intravenous therapy may result in a more sustained hemodynamic improvement[32]; however, continuous intravenous therapy also poses considerable risks, including relapse after the interruption of therapy and complications, and is difficult to administer.

With respect to adverse events, flushing was more common in the iloprost group, but the frequency of most of the other inhalation-associated side effects was similar. There were more syncopal episodes in the iloprost group than in the placebo group (eight vs. five), and these episodes were more frequently defined as serious adverse events, but they were not associated with clinical deterioration. Since syncope occurred a relatively long time (two to nine hours) after the last inhalation, the loss of an effect of iloprost may have caused these events. However, the same side effect was observed with bosentan therapy, suggesting that these drugs may have a more pronounced effect on blood pressure during exercise. Alternatively, patients who had clinical improvement with therapy may have become more physically active, challenging the limits of their cardiac reserve. We would advise patients to increase their physical activity gradually after the initiation of therapy for pulmonary hypertension.

The inhalation device that we used provided accurate doses of iloprost. However, it is not battery-driven, and inhalation commonly required 10 minutes. Different techniques of administering aerosolized iloprost result in similar acute hemodynamic effects as long as identical doses are delivered to the respiratory tract in a particle size suitable for alveolar deposition.[14,33] With other techniques, the duration of inhalation may be shortened considerably.[14]

In conclusion, this large, placebo-controlled trial demonstrates the efficacy and safety of inhaled iloprost for the treatment of severe primary pulmonary hypertension and selected forms of pulmonary arterial and chronic thromboembolic pulmonary hypertension. The advantages of intermittent inhaled therapy over intravenous therapy, coupled with the improvement in a number of clinically meaningful variables, suggest that inhaled iloprost therapy is effective. It may be a suitable alternative to continuous intravenous prostacyclin, especially in patients who do not derive a clear survival benefit with intravenous therapy.

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APPENDIX

The members of the AIR study group were as follows: *Steering Committee* — W. Seeger (chair), N. Galiè, T. Higenbottam, S. Nikkho, R. Naeije, H. Olschewski, L.J. Rubin, G. Simonneau; *Other Investigators* — H. Fabel and E. Spiekerkötter (Medizinische Hochschule, Hannover, Germany); F. Grimminger and R. Wiedemann (University Clinic, Giessen, Germany); H. Leuchte (University Clinic Großhadern, Munich, Germany); M. Aquilina (Università di Bologna, Bologna, Italy); K. Amsha and R. Lawson (Royal Hallamshire Hospital, Sheffield, United Kingdom); R. Alcock (Freeman Hospital, Newcastle-upon-Tyne, United Kingdom); A. Pforte (Universitätsklinik Eppendorf, Hamburg, Germany); J. Schauer (Medizinische Klinik und Poliklinik Universitätsklinik, Leipzig, Germany); W. Budts (Gasthuisberg University Clinic, Leuven, Belgium); P. Escribano and M. Lázaro (Hospital 12 de Octubre, Madrid); E. Huchalla (Hôpital Claude-Huriez, Lille, France); M. Borst (Ludolf-Krehl-Klinik, Heidelberg, Germany); C.M. Black (Royal Free Hospital, London); C. Bravo, A. Román, and V. Monforte (Centro Sanitario, Vall d'Hebron, Barcelona, Spain); A. Peacock (West Infirmary, Glasgow, United Kingdom); A. Boonstra (Academic Hospital, Free University, Amsterdam); C. Fracchia (Fondazione Salvatore Maugeri, Montescano, Italy); C. Marini (Istituto di Fisiologia Clinica Consiglio Nazionale della Ricerche, Pisa, Italy); L. Nicod (Hôpital Cantonal Universitaire, Geneva); J. Pepke-Zaba (Papworth Hospital, Cambridge University, Cambridge, United Kingdom); G. Sybrecht and H. Wilkens (Pneumologie Uniklinik, Homburg, Germany); A. Torbicki (National Institute of Tuberculosis and Lung Disease, Warsaw, Poland); P. Diot (Hôpital Bretonneau, Tours, France); T. Mota (Hospital de Pulido Valente, Lisbon, Portugal); J.L. Pennaforte (Centre Hospitalier Universitaire [CHU] Reims, Reims, France); T. Perez and F. Radenne (CHU Lille, Lille, France); C. Pison (CHU Hôpital Nord, Grenoble, France); J.L. Vachiery (Hôpital Erasme, Brussels, Belgium); P. Hallgren (Kardiologkliniken, Goteborg, Sweden); E-X. Kleber (Unfallkrankenhaus, Berlin, Germany); L. Providencia (Hospitais de Universidade de Coimbra, Coimbra, Portugal); V.R.G. Ribeiro (Vila Nova de Caia, Portugal); M. Soler (Kantonsspital, Basel, Switzerland); and H. Stricker (Ospedale Regionale di Locarno La Carità, Locarno, Switzerland).

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EXHIBIT 3

Drugs

Priority NDA and BLA Approvals in 2004

Priority New Drug Application (NDA) Approvals:

NDA Number	ProprietaryName	Established Name	Applicant	Chemical Type	Review Classification	Approval Date	Indication
N021539	Acetadote	Acetylcysteine	Cumberland Pharms	3	P, O	23-Jan-04	Acetadote is indicated to be administered intravenously within 8 to 10 hours after ingestion of a potentially hepatotoxic quantity of acetaminophen, to prevent or lessen hepatic injury.
N021462	Alimta	Pemetrexed Disodium	Eli Lilly	1	P, O	04-Feb-04	Alimta is indicated in the treatment of patients with malignant pleural mesothelioma whose disease is either unresectable or who are otherwise not candidates for curative surgery.
N021688	Sensipar	Cinacalcet Hydrochloride	Amgen	1	P	08-Mar-04	Sensipar is indicated for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease on dialysis, and the treatment of hypercalcemia in patients with parathyroid carcinoma.
N021256	Human Secretin	Human Secretin	Chirhoclin	1	P, O	09-Apr-04	Human Secretin is indicated for (1) Stimulation of pancreatic secretions, including bicarbonate, to aid in the diagnosis of pancreatic exocrine dysfunction, (2) Stimulation of gastrin secretion to aid in the diagnosis of gastrinoma, and (3) Stimulation of pancreatic secretions to facilitate the identification of the ampulla of Vater and accessory papilla during endoscopic retrograde cholangiopancreatography (ERCP).
N021264	Apokyn	Apomorphine Hydrochloride	Bertek	1	P	20-Apr-04	Apokyn is indicated for the acute, intermittent treatment of hypomobility, "off" episodes ("end-of-dose wearing off" and unpredictable "on/off" episodes) associated with advanced Parkinson's disease.
N021640	Vitrase	Ovine Hyaluronidase	Ista Pharms	1	P	05-May-04	Vitrase is indicated as an adjuvant to increase the absorption and dispersion of other injected drugs; for hypodermoclysis; and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents.
N050794	Vidaza	Azacitidine	Pharmion	1	P, O	19-May-04	Vidaza is indicated for the treatment of patients with the following myelodysplastic syndrome subtypes: refractory anemia or refractory anemia with ringed sideroblasts (if accompanied by neutropenia or thrombocytopenia and requiring transfusions), refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia.
N021497	Alinia	Nitazoxanide	Romark	3	P	21-Jul-04	Alinia is indicated for the treatment of diarrhea caused by Giardia Lamblia in patients 12 years and older.
N021431	Campral	Acamprosate Calcium	Lipha	1	P	29-Jul-04	Campral is indicated for the maintenance of abstinence from alcohol in patients with alcohol dependence who are abstinent at treatment initiation.
N021752	Truvada	Emtricitabine; Tenofovir Disoproxil Fumarate	Gilead Sciences	4	P	02-Aug-04	Truvada is indicated in combination with other antiretroviral agents (such as non-nucleoside reverse transcriptase inhibitors or protease inhibitors) for the treatment of HIV-1 infection in adults.
N021749	Pentetate Calcium Trisodium	Pentetate Calcium Trisodium	Pharma Hameln GmbH	1	P	11-Aug-04	Pentetate Calcium Trisodium is indicated for the treatment of internal contamination with plutonium, americium or curium to increase the rates of elimination.
N021751	Pentetate Zinc Trisodium	Pentetate Zinc Trisodium	Pharma Hameln GmbH	1	P	11-Aug-04	Pentetate Zinc Trisodium is indicated for the treatment of internal contamination with plutonium, americium or curium to increase the rates of elimination.

N021563	Clarinex	Desloratadine	Schering	3	P	01-Sep-04	Clarinex is indicated for the relief of the nasal and non-nasal symptoms of perennial allergic rhinitis, and the symptomatic relief of pruritus, reduction in the number of hives, and size of hives, in patients with chronic idiopathic urticaria in children 6 months to 2 years of age.
N021683	Manoplex	Insoluble Prussian Blue	Degussa Limited	5	P	14-Oct-04*	Manoplex is indicated for the treatment of patients with known or suspected internal contamination with radioactive cesium and/or radioactive or non-radioactive thallium to increase their rates of elimination.
N021665	Amphadase (hyaluronidase)	Amphadase (hyaluronidase)	Amphastar Pharms	1	P	26-Oct-04	Amphadase is indicated as an adjuvant to increase the absorption and dispersion of other injected drugs; for hypodermoclysis; and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents.
N021743	Tarceva (erlotinib)	Tarceva (erlotinib)	OSI Pharms	1	P	18-Nov-04	Tarceva is indicated for the treatment of locally advanced or metastatic Non Small-Cell Lung Cancer (NSCLC) after failure of at least one prior chemotherapy regimen.
N021786	Kelacal	Pentetate Calcium Trisodium	CIS-US	5	P	01-Dec-04*	Kelacal is indicated for the treatment of internal contamination with plutonium, americium, or curium.
N021787	Kelazin	Pentetate Zinc Trisodium	CIS-US	5	P	01-Dec-04*	Kelazin is indicated for the treatment of internal contamination with plutonium, americium, or curium.
N021670	Vision Blue	Trypan Blue	DORC	1	P	16-Dec-04	Vision Blue is indicated as an aid in ophthalmic surgery by staining the anterior capsule of the lens.
N021756	Macugen	Pegaptanib sodium	Eyetech	1	P	17-Dec-04	Macugen is indicated for the treatment of neovascular (wet) age-related macular degeneration.
N021785	Invirase	Saquinavir Mesylate	Hoffman-La Roche	3	P	17-Dec-04	Invirase is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults.
N021060	Prialt	Ziconotide	Elan Pharms	1	P	28-Dec-04	Prialt is indicated for the management of severe chronic pain in patients for whom intrathecal (IT) therapy is warranted and who are intolerant of or refractory to other treatment, such as systemic analgesics, adjunctive therapies, or IT morphine.
N021673	Clolar	Clofarabine	Genzyme	1	P, O	28-Dec-04	Clolar is indicated for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lymphoblastic leukemia after at least two prior regimens.
N021779	Ventavis	Iloprost	CoTherix	1	P, O	29-Dec-04	Ventavis is indicated for the treatment of pulmonary arterial hypertension.
N021446	**Lyrica**	**Pregabalin**	**Pfizer**	**1**	**P**	**30-Dec-04**	**Lyrica is indicated for the management of neuropathic pain associated with diabetic peripheral neuropathy.**

Priority Biologic License Application (BLA) Approvals:

BLA Number	ProprietaryName	Proper Name	Applicant	Review Classification	Approval Date	Indication
BL125084	Erbitux	Cetuximab	ImClone Systems	P	12-Feb-04	Erbitux is indicated for the treatment of EGFR-expressing, metastatic colorectal carcinoma in patients who are refractory to irinotecan-based chemotherapy (in combination with irinotecan); Treatment of EGFR-expressing, metastatic colorectal carcinoma in patients who are intolerant to irinotecan-based chemotherapy (administered as a single agent).
BL125085	Avastin	Bevacizumab	Genentech	P	26-Feb-04	Avastin is indicated for the first-line treatment of patients with metastatic carcinoma of the colon and rectum (in combination with intravenous 5-fluorouracil-based chemotherapy).
BL125104	Tysabri	Natalizumab	Biogen Idec	P	23-Nov-04	Tysabri is indicated in the treatment of patients with relapsing forms of multiple sclerosis (MS) to reduce the frequency of clinical exacerbations.
BL125103	Kepivance	Palifermin	Amgen	P	15-Dec-04	Kepivance is indicated to decrease the incidence and duration of severe oral mucositis in patients with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell support.

NDA Chemical Type:
1 - New molecular entity
2 - New ester, new salt, or other noncovalent derivative
3 - New formulation
4 - New combination
5 - New manufacturer
7 - Drug already marketed, but without an approved NDA

Review Classification:
P - Priority Review - Significant improvement compared to marketed products, in the treatment, diagnosis, or prevention of a disease.
O - Orphan Designation - Pursuant to Section 526 of the Orphan Drug Act (Public Law 97-414 as amended).

* NDA 21683, Manoplex was tentatively approved on October 14, 2004.
* NDA 21785, Kelacal was tentatively approved on December 1, 2004.
* NDA 21787, Kelazin was tentatively approved on December 1, 2004.

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EXHIBIT 4

REVIEW

Current treatment strategies for pulmonary arterial hypertension

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Abstract. Lee SH, Rubin LJ (University of California, San Diego, La Jolla, CA, USA). Current treatment strategies for pulmonary arterial hypertension (Review). *J Intern Med* 2005; **258**: 199–215.
Pulmonary arterial hypertension (PAH) is a disease characterized by an elevation in pulmonary artery pressure that can lead to right ventricular failure and death. Although there is no cure for PAH, newer medical therapies have been shown to improve a variety of clinically relevant end-points including survival, exercise tolerance, functional class, haemodynamics, echocardiographic parameters and quality of life measures. Since the introduction of continuous intravenous prostacyclin, the treatment armamentarium of approved drugs for PAH has expanded to include prostacyclin analogues with differing routes of administration, a dual endothelin receptor antagonist, and a phosphodiesterase-5 inhibitor. Selective endothelin-A receptor antagonists have shown promise in clinical trials and are likely to be added to the list of options. As the number of medications available for PAH continues to increase, treatment decisions regarding first-line therapy, combination treatments, and add-on strategies are becoming more complex. This article reviews the current treatments strategies for PAH and provides guidelines for its management.

Keywords: drug therapy, hypertension, pulmonary.

Introduction

Until the introduction of intravenous (i.v.) epoprostenol in 1995, the prognosis of pulmonary arterial hypertension (PAH) was dismal as treatment was limited only to supportive measures. The median survival was 2.8 years with an estimated 5-year survival of 34% [1]. Epoprostenol was a therapeutic breakthrough that brought new hope to those with PAH. However, treatment decisions for PAH were relatively uncomplicated as they were limited to this one medication. The situation today is quite different: the last decade has witnessed considerable growth in clinical interest in PAH that has been paralleled by scientific advances in our understanding of the pathobiology of this disease (Fig. 1). Reflecting this expansion, the first expert consensus statement on primary pulmonary hypertension (PPH) published by the American College of Chest Physicians in 1993 was a 14-page document [2]. It has now evolved into a 92-page updated, evidence-based monograph [3].

There are now three classes of medications that have shown efficacy in the treatment of PAH: prostanoids, endothelin receptor antagonists (ERAs), and phosphodiesterase-5 (PDE-5) inhibitors (Fig. 2). These medications differ in terms of their pathway targets and mechanisms of action, indications for use, routes of delivery, and side-effect profiles. The challenge lies in integrating the available information

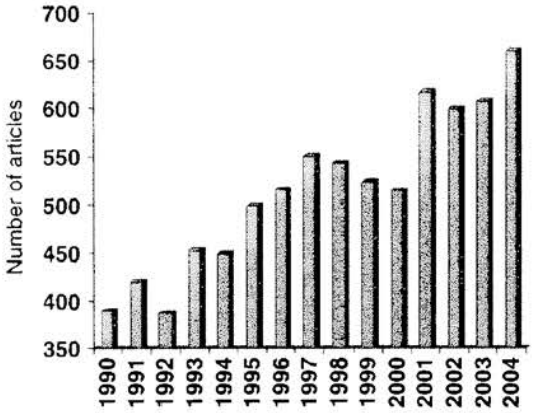

Fig. 1 Number of published articles per year listed in PubMed under the MeSH heading 'hypertension, pulmonary'. http://www.pubmed.gov (accessed 4/5/05).

into a sound treatment and management plan that provides optimal care for patients with PAH.

Establishing the diagnosis

The classification scheme for pulmonary hypertension was recently revised in 2003 at the Third World Symposium on Pulmonary Hypertension held in Venice and is shown in Table 1. Group 1 pulmonary hypertension encompasses PAH, the focus of this review, whilst a variety of secondary causes of pulmonary hypertension are grouped into groups 2–5. Effective treatment of PAH is dependent upon establishing a definitive diagnosis. Pulmonary hypertension resulting from secondary causes should be excluded as many of these conditions are treated with alternative approaches. Recommendations for systematic work-up have been recently reviewed [4, 5]. Published expert guidelines suggest that all patients with suspected PAH undergo formal right heart catheterization (RHC) prior to initiation of treatment. RHC provides important diagnostic and prognostic information based on a thorough characterization of the cardiopulmonary system.

Many secondary causes of pulmonary hypertension can be most thoroughly investigated by RHC. An elevated pulmonary artery occlusion pressure suggests the presence of left-sided heart disease caused by systolic or diastolic dysfunction, or valvular heart disease. Findings of large v-waves suggest significant mitral regurgitation. A significant systolic pressure gradient across the pulmonic

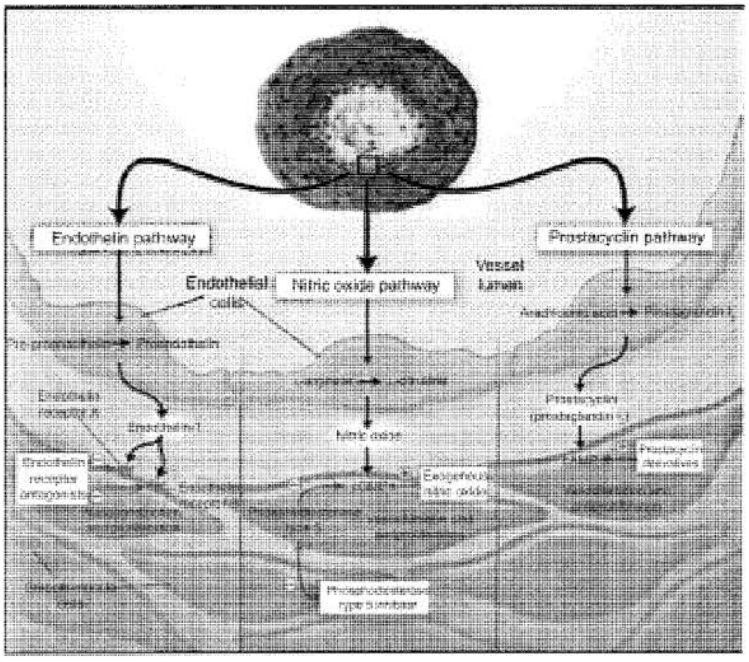

Fig. 2 Targets for current or emerging therapies in pulmonary arterial hypertension. Three major pathways involved in abnormal proliferation and contraction of the smooth muscle cells of the pulmonary artery in patients with pulmonary arterial hypertension are shown. These pathways correspond to important therapeutic targets for the medications used to treat this condition: endothelin receptor antagonists, phosphodiesterase-5 inhibitors, and prostanoids. Plus signs denote an increase in the intracellular concentration; minus signs blockage of a receptor, inhibition of an enzyme, or a decrease in the intracellular concentration (Reproduced with permission from Humbert *et al.* [100]; Copyright 2005 Massachusetts Medical Society. All rights reserved).

Table 1 Classification of pulmonary hypertension (Venice 2003: revised from Evian 1998)

Group 1. Pulmonary artery hypertension (PAH)
1.1 Idiopathic (IPAH)
1.2 Familial (FPAH)
1.3 Associated with (APAH)
1.3.1 Collagen vascular disease
1.3.2 Congenital systemic-to-pulmonary shunts
1.3.3 Portal hypertension
1.3.4 HIV infection
1.3.5 Drugs and toxins
1.3.6 Other (thyroid disorders, glycogen storage disease, Gaucher disease, splenectomy, hereditary haemorrhagic telangiectasia, haemoglobinopathy)
1.4 Associated with significant venous or capillary involvement
1.4.1 Pulmonary veno-occlusive disease
1.4.2 Pulmonary capillary haemangiomatosis
1.5 Persistent pulmonary hypertension of the newborn
Group 2. Pulmonary hypertension with left heart disease
Group 3. Pulmonary hypertension associated with lung disease and/or hypoxaemia
Group 4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease
Group 5. Miscellaneous (sarcoidosis, histiocytosis X, lymphangiomyomatosis, compression of pulmonary vessels)

valve is indicative of pulmonic valve stenosis. Sampling of blood to measure oxygen saturation from the vena cavae and right heart chambers could lead to the diagnosis of an intracardiac left-to-right shunt by demonstrating the presence and location of a 'step-up'.

Echocardiograms alone should not be used to diagnose or monitor the course of PAH. The diagnosis of PAH requires documentation of a mean pulmonary artery pressure >25 mmHg at rest or >30 mmHg with exercise with a normal pulmonary artery occlusion pressure. The echocardiogram directly measures neither, and only provides an estimate of the right ventricular systolic pressure based on the tricuspid regurgitant velocity. This can underestimate or overestimate the true pulmonary artery systolic pressure, depending on a number of factors, including the quality of the echo window obtained and presence or absence of pulmonary outflow obstruction. Underestimation can lead to delays in treatment, whilst overestimation may expose patients to incorrect diagnoses and unnecessary treatment. Finally, the haemodynamic responses to acute vasodilator testing cannot be reliably assessed with echocardiography.

General measures

Physical activity

Although we recommend an active lifestyle that promotes general cardiovascular health, PAH patients should be counselled against activities that abruptly increase the work of the heart during exertion [6]. Patients with mild PAH may have only minimal symptoms with exertion, whilst those with more advanced disease may experience dyspnoea at rest, exertional lightheadedness, syncope or chest pain, which are indicative of impaired right ventricular performance. Grading of functional capacity in PAH is usually based upon the World Health Organization classification scheme, which is a modification of the well-known New York Heart Association heart failure functional classification system (Table 2). Functional class is an important prognostic marker and has been used as an endpoint in PAH clinical trials.

Diuretics

Loop diuretics and potassium-sparing aldosterone inhibitors can be used to control signs and symptoms

Table 2 World Health Organization classification of functional status of patients with pulmonary hypertension

	Description
I	Patients with pulmonary hypertension in whom there is no limitation of usual physical activity. Ordinary physical activity does not cause increased dyspnoea, fatigue, chest pain, or syncope.
II	Patients with pulmonary hypertension who have mild limitation of usual physical activity. There is no discomfort at rest, but normal physical activity causes increased dyspnoea, fatigue, chest pain, or presyncope.
III	Patients with pulmonary hypertension who have a marked limitation of physical activity. There is no discomfort at rest, but less than ordinary activity causes increased dyspnoea, fatigue, chest pain, or presyncope.
IV	Patients with pulmonary hypertension who are unable to perform any physical activity at rest and who may have signs of right ventricular failure. Dyspnoea and/or fatigue may be present at rest and symptoms are increased by almost any physical activity.

Adapted from Rich *et al.* [101]. Primary pulmonary hypertension: Executive Summary. Evian, France: World Health Organization, 1998.

of volume overload from right ventricular failure, such as hepatic congestion, ascites and lower extremity oedema. Diuretics should be used cautiously to avoid precipitous reductions in preload.

Supplemental oxygen

Hypoxia is a potent pulmonary vasoconstrictor, leading to increased pulmonary arterial pressure both acutely and chronically [7]. In patients with PAH complicated or caused by chronic hypoxaemia, supplemental oxygen can improve haemodynamics by decreasing the mean pulmonary artery pressure and increasing the cardiac index, thus decreasing the calculated pulmonary vascular resistance [8]. A relatively high incidence of sleep-disordered breathing, in a pattern similar to the Cheyne–Stokes respiration pattern seen in congestive heart failure, has also been observed in idiopathic pulmonary arterial hypertension (IPAH) [9]. This nocturnal periodic breathing pattern can produce or aggravate hypoxaemia and can be markedly improved with supplemental oxygen. The use of supplemental oxygen to maintain arterial oxygen saturation above 90% both at rest and with exercise is recommended.

Cardiac glycosides

The role of cardiac glycosides (e.g. digoxin) in PAH is unclear. When administered intravenously to patients with IPAH and right ventricular failure, there is a modest, but significant, acute increase in cardiac output (3.49–3.81 L min^{-1}) [10]. However, long-term benefits of chronic cardiac glycoside administration in PAH have not been reported. Well-designed clinical trials are needed in order to further assess the role of cardiac glycosides in the management of PAH.

Anticoagulation

Although there have been no prospective, randomized, placebo-controlled trials (RCT), evidence from several studies suggests that the use of chronic anticoagulation in patients with PAH improves survival [11–13]. Patients with PAH are likely at higher risk for thromboembolic complications because of their decreased activity level, slower blood blow, dilated right-sided heart chambers, and for some, the presence of an implanted central catheter for administering PAH medications. The fragile haemodynamic state and limited cardiopulmonary reserve of patients with PAH place them at risk for death even from a small thromboembolism. Anticoagulation may also reduce the propensity for *in situ* microvascular thrombosis in the distal pulmonary arterial circulation that is commonly observed pathologically in PAH.

In the absence of contraindications, chronic anticoagulation should be a standard component of the treatment regimen in patients with PAH. Expert guidelines recommend a goal international normalized ratio of 1.5 to 2.5 times control [14].

Specific treatment of PAH

Calcium channel blockers

Treatment of PAH with calcium channel blockers (CCBs) is reserved for patients who demonstrate evidence of acute vasoreactivity, currently defined as a reduction in mean pulmonary artery pressure ≥10 mmHg to a level that is ≤ 40 mmHg, with a normal cardiac output during testing with an acute, short-acting vasodilator such as inhaled nitric oxide or iloprost or i.v. epoprostenol or adenosine [4, 15]. Although the definition of vasoreactivity has changed somewhat over the years, the underlying principle remains the same: only those with significant haemodynamic reversal of pulmonary hypertension during acute vasodilator testing should be considered candidates for chronic CCB treatment. The rationale for this stems from the thought that the primary driving force for PAH in these patients is significant reversible vasoconstriction, rather than a structural pathological vasculopathy due to chronic remodelling changes.

About 13% of IPAH patients exhibit acute vasoreactivity, and of this group, only half experience sustained benefit from chronic CCB treatment [16]. The same can roughly be said about patients with anorexigen-associated PAH. Therefore, although those with acute vasoreactivity may benefit from chronic CCB therapy, a significant number of these patients do not. Acute vasoreactivity is unlikely to be found in patients with other forms of PAH, and amongst these, the likelihood of sustained benefit from CCB treatment is exceedingly rare [17] (Fig. 3). Accordingly, CCB therapy has

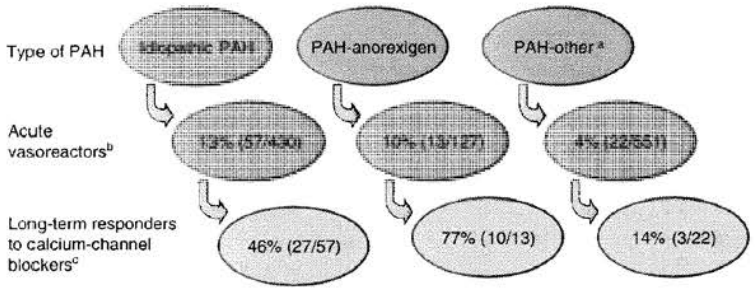

Fig. 3 Breakdown of long-term responders to calcium channel blocker (CCB) monotherapy amongst those who are acutely vasoreactive, by type of PAH (data adopted from Sitbon *et al.* [17]).
[a]Other includes PAH associated with connective tissue disease, veno-occlusive disease, pulmonary capillary haemangiomatosis, human immunodeficiency virus, portopulmonary hypertension, familial and congenital heart defects.
[b]Acute vasoreactors were defined by a fall in both mean pulmonary artery pressure and pulmonary vascular resistance >20%.
[c]Long-term responders were defined as those being in functional class I or II after at least 1 year on CCB monotherapy.

been relegated for use only in a handful of patients with PAH.

All patients treated with a CCB should be closely followed for evidence of benefit as those who do not respond have a survival rate that approximates that of untreated PAH [12]. This is particularly important as newer and more widely effective therapies that specifically target the pathogenic processes of PAH are now available. Their initiation should not be delayed when treatment with CCBs is not proving effective.

Prostanoids

Epoprostenol

In 1995, epoprostenol, or prostacyclin (Flolan), became the first drug approved by the United States Food and Drug Administration (FDA) for the treatment of pulmonary artery hypertension. Epoprostenol is a potent, short-acting vasodilator and antiproliferative agent whose efficacy and safety have been well documented in numerous short and long-term clinical trials and observational studies [18–22]. It is the only medication for PAH that has shown a survival benefit in a randomized clinical trial [19].

Epoprostenol is widely considered to be the most potent and efficacious treatment for PAH. Although it has been most extensively studied in IPAH and PAH associated with the scleroderma spectrum of disease, epoprostenol is also useful in PAH associated with systemic lupus erythematosis [23], HIV infection [24], portopulmonary hypertension [25], and Eisenmenger's syndrome [26]. Epoprostenol is indicated for use in functional classes III and IV PAH.

The beneficial acute effects of epoprostenol stem from its potent vasodilatory and, probably, inotropic actions, whilst the long-term effects are likely attributable to its antithrombotic properties and effects on vascular remodelling [27–29]. With chronic administration, epoprostenol lowers pulmonary vascular resistance to a level beyond that achieved during acute vasodilator testing [30]. Reports of successful withdrawal of chronic epoprostenol therapy suggest that reversal of the vasculopathic process may be achievable in some patients [31].

In a long-term follow-up study of patients with IPAH treated with epoprostenol, McLaughlin *et al.* observed survival rates of 88%, 76% and 63% at 1, 2 and 3 years respectively [21]. In a similar study, Sitbon *et al.* reported survival rates of 85%, 70% and 63% [20]. Survival rates in both studies were significantly better than those predicted by the National Institutes of Health Primary Pulmonary Hypertension (NIH-PPH) Registry equation [1].

Of the therapies available for PAH, epoprostenol is the most complex to administer. It requires a portable infusion pump for continuous i.v. administration through a central catheter because of its short half-life (<5 min) and high pH of the diluent. Ice packs must be used to keep the infusate cold as the drug is unstable at room temperature. The most common side-effects are jaw pain, flushing, headache,

diarrhoea and arthralgias. Many of these side-effects are dose dependent and respond to conservative measures or decreases in dose. Patients are also at increased risk for catheter-related complications such as infection and thrombosis.

Prostacylin analogues

The complexity of continuous i.v. epoprostenol therapy and its attendant risks has led to the development of stable prostacyclin analogues that can be administrated by simpler routes. In contrast to epoprostenol, the prostacyclin analogues are stable at room temperature and can be diluted in physiological saline without inactivation [32]. These characteristics allow them to be delivered by the inhaled or subcutaneous (s.c.) routes.

Treprostinil

Treprostinil is a stable prostacyclin analogue with a half-life of 55–117 min [32]. It can be administered subcutaneously using a minipump, similar to that used to deliver insulin. The infusion site is typically rotated every few days to minimize local skin reactions.

The therapeutic efficacy of s.c. treprostinil was investigated in a large, 12-week RCT involving patients with functional classes II–IV IPAH or PAH associated with connective tissue disease or congenital systemic-to-pulmonary shunt [33]. The treprostinil group had a small, but significant increase in the 6-minute walk test (6MWT) compared with placebo (+16 m). The magnitude of effect appeared to be dose related. Although effective, the improvement in 6MWT seen with treprostinil appeared to be relatively modest when compared with studies that used epoprostenol [19, 20]. Improvements were also seen in haemodynamic parameters and the Borg dyspnoea score. However, deaths and study discontinuations because of clinical worsening were not significantly different between the two treatment groups.

Notably, 85% of patients reported pain at the infusion site and 83% had an infusion site reaction, leading to discontinuation of the study by 8%. Other commonly reported side-effects included headache, diarrhoea, nausea and rash. Five patients (2%) who were receiving treprostinil were transitioned to epoprostenol for worsening clinical status. Long-term data from an open-label extension study has shown continued efficacy of s.c. treprostinil after 24 months of therapy [34].

Treprostinil can also be delivered as a continuous i.v. infusion. It is dosed similarly to s.c. treprostinil as they are bioequivalent at steady state [35]. Twelve-month data from a prospective transition study of i.v. epoprostenol to i.v. treprostinil has shown it to be effective in this form [36]. This was accomplished without evidence of deterioration, suggesting that epoprostenol's benefits were being maintained by treprostinil. It is important to note that no RCT using i.v. treprostinil as initial treatment has been performed. Its efficacy in this situation is extrapolated from the bioequivalence data to s.c. treprostinil and the fact that s.c. treprostinil has been found to be effective as an initial treatment regimen. In general, experience with i.v. treprostinil is relatively limited.

One advantage of treprostinil over epoprostenol is that it does not require constant cooling as it is stable at room temperature. The longer half-life may also theoretically allow unintentional dose interruptions to be better tolerated than epoprostenol, which has a very short half-life. The s.c. route also offers the advantage of a less complex delivery system compared with the i.v. route as it does not require an implanted i.v. catheter; however, it suffers from a high incidence of skin infusion site complications that may limit its usefulness. Treprostinil is also being investigated as an inhalation treatment.

Iloprost

Iloprost has been marketed as both an i.v. and inhaled medication. It is stable at room temperature and ambient light and has a plasma half-life of almost 30 min [37]. Most of the attention has focused on iloprost as an inhalation drug. This route allows it to selectively promote vasodilation in the pulmonary artery circulation whilst minimizing the systemic effects commonly associated with i.v. prostanoids [38, 39]. The inhaled route also promotes drug deposition and selective action to those areas that are well ventilated, thereby minimizing ventilation–perfusion mismatch. This may be especially important in patients with PAH who have underlying parenchymal lung disease. In contrast, i.v. prostacyclin increases the shunt fraction in this setting [38].

The safety and efficacy of inhaled iloprost was studied in a pivotal 12-week RCT involving 203 patients with functional classes III–IV IPAH or PAH associated with appetite suppressants, scleroderma, or inoperable chronic thromboembolic pulmonary hypertension (Aerosolized Iloprost Randomized, AIR) [40]. Those receiving inhaled iloprost showed significant improvement in the combined primary end-point of 6MWT, functional class, and absence of clinical deterioration, in addition to the dyspnoea and quality of life scores. Notably, this study produced positive results for inhaled iloprost despite including a significant proportion of class IV patients. The greatest benefit on the 6MWT occurred in those with IPAH compared with non-IPAH and the magnitude of change was similar to that seen in studies using i.v. epoprostenol [19]. A subgroup analysis of AIR showed no benefit of inhaled iloprost in those with chronic thromboembolic pulmonary hypertension.

The inhaled route theoretically provides the advantage of minimizing systemic side-effects associated with infused prostanoid therapy. However, the incidence of reported adverse events from the pivotal s.c. treprostinil RCT [33] and the inhaled iloprost RCT [40] shows mixed results, respectively: headache (27% vs. 30%), diarrhoea (25% vs. 9%), nausea (22% vs. 13%), jaw pain (13% vs. 12%), vasodilation/flushing (11% vs. 27%), dizziness/vertigo (9% vs. 7%), and oedema (9% vs. 13%). As expected, increased cough was a commonly reported side-effect in the inhaled iloprost study.

Iloprost requires administration six to nine times a day, with each inhalation taking 5–10 min through a special nebulizer device.

Beraprost

Beraprost is an orally active prostacyclin analogue that has been used in Japan since 1995 for the treatment of PAH [41]. It was not until 2002, however, that the first RCT of beraprost was published which showed short-term efficacy in improving exercise capacity and symptoms [42]. A similar, but longer RCT of 12 months duration was subsequently performed in the US [43]. This study confirmed the short-term benefits of beraprost seen in the previous trial; however, these improvements were no longer evident at either 9 or 12 months. Therefore, as monotherapy, beraprost has found little use in the management of PAH.

This trial highlighted the importance of performing longer-term pivotal PAH drug trials to investigate the durability of short-term gains. A primary end-point measured at 12 weeks, which is not uncommon in the published PAH literature, may not accurately reflect what happens to that end-point in the long-term. In the case of beraprost, although it may not be useful as monotherapy in the chronic management of PAH, the prospect of having an orally active prostanoid in the treatment armamentarium is appealing. Further studies may be warranted to determine whether an oral prostanoid could be effective as part of a combination treatment regimen. Beraprost is currently approved for use only in Japan.

Endothelin receptor antagonists

Since the characterization of endothelin (ET-1) in 1988 as a potent vasoconstrictor [44], numerous lines of scientific evidence have pointed to a prominent role of ET in the regulation of pulmonary vasomotor tone and possible role in vascular remodelling, processes which are important in the pathogenesis of PAH. Antagonism of ET receptors is now firmly established as a therapeutic target for patients with PAH.

The endothelins represent a family of 21-amino acid proteins derived from vascular endothelial cells with three known isoforms in humans, ET-1, ET-2 and ET-3 [45]. All three isoforms are characterized by two intramolecular disulphide bonds between cysteine amino acids at residues 1–15 and 3–11. ET-1, the endothelin that is thought to play the most prominent role in PAH, exerts its actions via two receptor subtypes: ET_A, which is located on vascular smooth muscles cells, and ET_B, which is found on both vascular smooth muscle cells and vascular endothelium [46]. Activation of ET_A by ET-1 leads to potent vasoconstriction due to an increase in cytosolic calcium levels via influx of extracellular calcium [47, 48] and release of intracellular calcium stores [49].

The actions of ET_B are more complicated. Like ET_A, activation of ET_B on vascular smooth muscles cells leads to vasoconstriction [50]. Furthermore, some studies suggest that blockade of both ET_A and ET_B is necessary to achieve maximal vasodilation

in the pulmonary hypertensive state [51–53]. Conversely, other studies suggest a protective role of ET_B in pulmonary hypertension by producing nitric oxide and prostacyclin and clearing circulating ET-1 [54–58]. Therefore, the overall net effect of ET_B in regulating pulmonary vasomotor tone is unclear. There may be theoretical benefit in selectively blocking ET_A whilst leaving ET_B unopposed.

Dual endothelin receptor antagonism

Bosentan is an oral, nonselective ERA that has proved its efficacy for the treatment of PAH in two pivotal RCTs [59, 60]. The second and larger trial, Bosentan Randomized trial of Endothelin Antagonist Therapy (BREATHE-1), confirmed the benefits of bosentan given at a dose of 125 mg twice daily in improving the 6MWT, Borg dyspnoea index, and functional class, whilst increasing the time to clinical worsening [59]. The higher dose of 250 mg twice daily was associated with a higher incidence of aminotransferase abnormalities without a significant increase in efficacy. Consistent with a prior study showing a poorer prognosis in scleroderma-associated PAH patients [61], a subgroup analysis of BREATHE-1 showed that bosentan increased the 6MWT in those with IPAH, whereas it prevented deterioration in those with scleroderma (compared with each group's respective placebo arms).

It is important to keep in mind that lack of absolute improvement in clinical parameters does not necessarily equate to treatment failure. The main treatment effect may be one of disease stabilization or decreasing the rate of deterioration rather than overt improvement. Whilst this may not be an optimal response, bosentan is still clearly exerting a beneficial effect by delaying the time to clinical worsening. For this reason, we do not recommend withdrawing bosentan therapy once it has been started unless clinical deterioration is thought to be directly attributable to bosentan or there are intolerable side-effects.

Long-term extension study data with bosentan show Kaplan–Meier survival estimates of 96%, 89% and 86% at 1, 2 and 3 years respectively [62]. These are significantly higher than those predicted by the NIH-PPH Registry equation [1]. At 2 years, 70% of patients remained on bosentan monotherapy. Clearly, the majority of patients experience long-term benefits from bosentan alone; however, there is certainly a subset of patients who will require other agents as add-on therapy as the disease continues to progress.

Data regarding use of bosentan in other forms of PAH are limited. BREATHE-4, a small, prospective, noncomparative cohort study of human immunodeficiency virus (HIV)-associated PAH patients showed significant improvements in a variety of clinical end-points including the 6MWT and Borg dyspnoea index, indicating less dyspnoea despite increased walk distance [63]. Bosentan had no impact on control of the HIV infection. Additionally, despite the fact that several of the patients were co-infected with either hepatitis B or hepatitis C virus and the majority were receiving potentially hepatotoxic antiretroviral therapy, elevated liver function tests were seen only in a minority (12.5%).

Studies investigating the use of bosentan in PAH associated with Eisenmenger's syndrome (BREATHE-5 trial) and functional class II patients with PAH (EARLY trial) are ongoing and should shed further light into the role of bosentan in these conditions.

The most important adverse effect associated with bosentan is hepatocellular injury. Aminotransferase elevations at least three times above the upper limit of normal occurred in about 5–10% of patients treated with bosentan in the pivotal RCTs [59, 64]. Long-term safety data up to 2 years from a European postmarketing surveillance system (TRAX) of patients treated with bosentan showed that the cumulative incidence of abnormal transaminases was about 7% [65]. There were no fatal outcomes related to liver injury.

The likelihood of first aminotransferase elevation appears to diminish over time; however, it can develop at any time. Therefore, it is important to continue monthly monitoring of liver enzymes throughout the duration of treatment. If needed, most patients can be successfully managed with dose reduction or temporary cessation of treatment. Guidelines are available in the packaging insert. Other commonly reported side-effects include flushing and headache, with a few patients experiencing unexplained decreases in haemoglobin concentration.

Bosentan is a pregnancy category X drug. Women of childbearing potential must be monitored with pregnancy tests before and regularly

during treatment with bosentan. In addition, women using a hormonally based method of contraception must use a second form of birth control as bosentan decreases hormone levels. Bosentan should not be co-administered with glyburide or cyclosporin because of a pharmacological interaction that increases the risk of liver enzyme abnormalities.

Selective ET_A receptor antagonism

Selective antagonism of the ET_A receptor has the theoretical advantage of blocking the deleterious vasoconstrictive and vascular smooth muscle proliferative effects mediated through ET_A, whilst maintaining the vasodilatory and ET-1 clearance actions of ET_B.

The safety and efficacy of sitaxsentan, an orally active, selective ET_A receptor antagonist, was originally shown in a small, open-label pilot study [66]. This was followed by a larger RCT involving functional classes II–IV PAH patients randomized to placebo or either of two sitaxsentan dosing groups (Sitaxsentan to Relieve Impaired Exercise, STRIDE-1) [67]. Patients receiving sitaxsentan had significant improvements in 6MWT, functional class, and haemodynamic parameters. Unlike the studies with bosentan, however, there was no difference seen in the time to clinical worsening. A small 1-year follow-up study showed persistent improvement in several clinical parameters compared with baseline [68]. Although potentially useful in treating PAH, the data to date suggest that selectivity for the ET_A receptor does not confer superior effects in PAH compared with dual receptor antagonism.

The incidence of liver enzyme abnormalities with sitaxsentan does not appear to be noticeably different from that seen with bosentan, although direct comparisons are not yet available. The dose of warfarin may need to be decreased as sitaxsentan can cause an increase in the protime international normalized ratio.

Several other studies are underway with sitaxsentan. STRIDE-2 is investigating the safety and efficacy of sitaxsentan compared with placebo and open-label bosentan. STRIDE-6 is studying the use of sitaxsentan in patients who have failed therapy with bosentan because of clinical deterioration or liver enzyme abnormalities. Ambrisentan, another selective ET_A-receptor blocker, is currently in phase III trials.

Phosphodiesterase-5 inhibitors

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger that is responsible for mediating the vasodilatory activity of nitric oxide [69]. cGMP is rapidly inactivated by PDE-5, an enzyme abundantly found in lung tissue [70]. In the pulmonary circulation, PDE-5 inhibition promotes vascular relaxation by inhibiting the breakdown of cGMP. Numerous studies have suggested a beneficial effect of sildenafil, a PDE-5 inhibitor, in patients with pulmonary hypertension from a variety of causes, including interstitial lung disease, thromboembolism, and hypoxia [7, 71–73].

A cross-over RCT of patients with PAH showed significant improvements in exercise duration, cardiac index, and dyspnoea and fatigue scores with sildenafil treatment [14]. More recently, the results of a 12-week RCT involving patients with PAH predominantly in functional classes II–III (96%) were reported in a late-breaking clinical trials session at the American College of Chest Physicians' meeting in October 2004 (Sildenafil Use in Pulmonary Arterial Hypertension, SUPER-1). The pooled sildenafil dosing groups showed significant improvements in 6MWT and functional class. A more thorough evaluation and interpretation of SUPER-1 are not possible until the full results are published; however, the US FDA has already announced approval of sildenafil 20 mg three times daily for the treatment of PAH without functional class restriction. The regulatory approval process in other countries is ongoing.

Further supporting the efficacy of sildenafil in PAH, preliminary data from a 1-year open-label extension of SUPER-1 presented at the 2005 International Conference of the American Thoracic Society (SUPER-2) showed continued benefit of sildenafil on 6MWT and functional class [74], in addition to survival [75].

Sildenafil appears to be well tolerated with headache as the most commonly reported side effect. Others include dyspepsia, sinus congestion, epistaxis and back pain. No specific laboratory monitoring is recommended; however, its use is contraindicated in those taking nitrate medications because of potentiation of hypotensive effects. A summary of the

currently approved medications for PAH is presented in Table 3.

Treatment decisions

A treatment algorithm for PAH is presented in Fig. 4. All patients with PAH should be treated with anticoagulants and, if indicated, diuretics, and supplemental oxygen. For acutely vasoreactive patients with functional class I to early III IPAH or PAH associated with anorexigen use, an initial strategy using a CCB is reasonable. Still, these patients need to be closely monitored for evidence of improvement as up to 50% will not have a beneficial long-term response and thus have poorer survival. Those who do not show evidence of benefit after 3–6 months of CCB therapy should start treatment with a PDE-5 inhibitor, ERA, or prostanoid.

Although the most recently published evidence-based treatment algorithms recommend CCB therapy as first-line therapy in all patients with PAH who are acutely vasoreactive, many experts are re-considering whether such a broad management strategy is appropriate. For example, it may be reasonable to reserve use of CCBs only to those with acutely vasoreactive IPAH or PAH associated with anorexigen use, as the incidence of long-term response to CCB therapy in other forms of PAH is exceedingly low (Fig. 3). For several reasons, strong consideration should also be given to using a PDE-5 inhibitor, ERA or prostanoid instead of a CCB as first-line therapy for those in advanced functional classes, despite being acutely vasoreactive. First, these patients have little cardiopulmonary reserve and may deteriorate quickly if they turn out to be nonresponders to chronic CCB therapy. Secondly, numerous proven PAH-specific therapies for advanced functional classes are available. Thirdly, there are now less invasive and simpler methods to deliver these effective medications. Thus, in an era in which the number of definitive treatment options for PAH are expanding, the role of CCBs is diminishing.

Nevertheless, it is still currently recommended that acute vasoreactivity testing be performed in all patients with PAH. The presence and magnitude of vasoreactivity may have some prognostic implication, although this is controversial [76–78].

At present, early functional classes of PAH may potentially be treated with sildenafil or continuously administered s.c./i.v. treprostinil, although the latter

Table 3 Summary of medications approved for use in pulmonary arterial hypertension. (a) Approval may be limited to certain PAH subgroups, depending on the country; (b) approved for classes III–IV in the US and III in Europe

Drug (class)	Route	Dose range	Major class	Functional side-effects	Contraindications	Regulatory approval (a)
Epoprostenol (prostanoid)	i.v.	2 ng kg^{-1} min^{-1} and up	III–IV	Flushing, headache, nausea, diarrhoea, jaw pain, lightheadedness, arthralgias	None	US, Europe, Canada, Australia
Iloprost (prostanoid)	inh.	2.5–5 mcg 6–9 times daily during waking hours; total daily dose usually <45 mcg	III–IV (b)	Flushing, cough, headache, jaw pain, insomnia, nausea, hypotension	None	US, Europe, New Zealand, Australia
Treprostinil (prostanoid)	s.c.	1.25 ng kg^{-1} min^{-1} and up, i.v. usually <40 ng kg^{-1} min^{-1} (s.c. and i.v. routes are bioequivalent)	II–IV	Infusion site pain and reaction (i.v./s.c.), headache, diarrhoea, nausea, jaw pain, flushing	None	US, Europe, Canada
Bosentan (Dual ERA)	p.o.	62.5 mg q.d. ×4 weeks, then 125 mg b.i.d.	III–IV	Hepatocellular injury, flushing, headache, oedema, sinus congestion, haemoglobin decrease	Concurrent use of cyclosporin or glyburide; pregnancy; moderate-to-severe pre-existing liver impairment	US, Europe, Canada, Australia, Japan
Sildenafil (PDE-5 inhibitor)	p.o.	20 mg t.i.d.	I–IV	Headache, dyspepsia, epistaxis, back pain, sinus congestion	Concurrent use of organic nitrate medication	US, under review in Europe

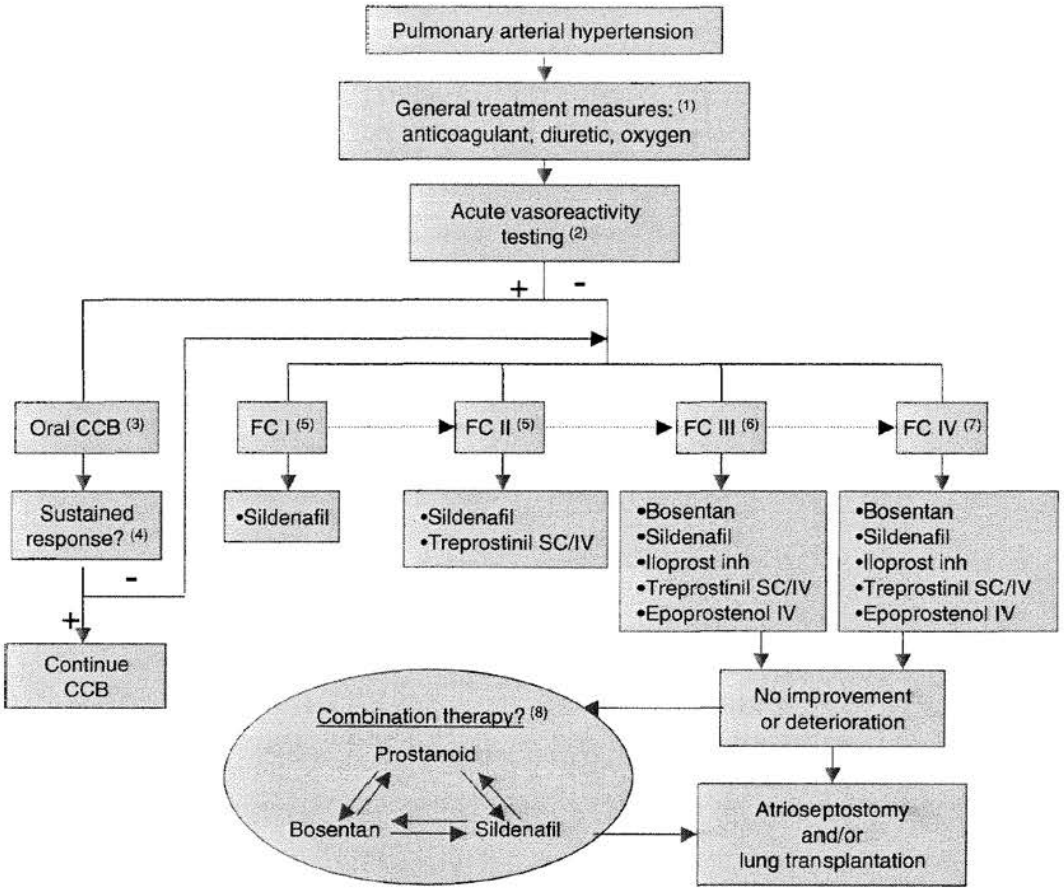

Fig. 4 Treatment algorithm for pulmonary arterial hypertension. The recommended therapies presented in this algorithm have been evaluated mainly in those with idiopathic pulmonary arterial hypertension (IPAH), or PAH associated with connective tissue disease or anorexigen use. Extrapolation to other forms of PAH should be made with caution. Medications are listed in order of increasing invasiveness. Country-specific regulatory agency approval status and functional class indications for PAH medications vary. (1) All patients should receive treatment with an anticoagulant. Diuretics and oxygen should be added as necessary. (2) A positive acute vasodilator response is defined as a fall in the mean pulmonary artery pressure from ≥10 to ≤ 40 mmHg with a normal or increased cardiac output when challenged with inhaled nitric oxide, i.v. epoprostenol or i.v. adenosine. (3) Consideration should be given to using a PAH-specific medication such as a PDE-5 inhibitor, endothelin receptor antagonist (ERA), or prostanoid as first-line treatment instead of a calcium channel blocker (CCB) in those with PAH that is not IPAH or PAH associated with anorexigen use, or in those in an advanced FC given the exceedingly low long-term response rate to CCB monotherapy in the former and poor prognosis in the latter. (4) Sustained response to CCB therapy is defined as being in FC I or II with normal or near-normal haemodynamics after several months of treatment. (5) The risks and benefits of treatment in early PAH should be considered. (6) First-line therapy for FC III includes bosentan, epoprostenol, inhaled iloprost, sildenafil, and s.c./i.v. treprostinil. (7) Most experts recommend i.v. epoprostenol as first-line treatment for unstable patients in FC IV. (8) RCTs studying add-on combination treatment regimens are underway.

is rarely used in these instances due to the potential serious risks and complications related to its administration. Given its oral availability, sildenafil is an attractive option for early PAH, especially in patients who are class II. There is actually very little information about sildenafil's efficacy in functional class I patients. In fact, only one patient in the sildenafil SUPER-1 trial was from this class. Oral bosentan is also currently being studied for use in functional class II patients in the EARLY trial.

Patients in functional classes III and IV pose a significant challenge in choosing an initial treatment

regimen. For patients in class IV, most experts recommend first-line treatment with i.v. epoprostenol given the extensive experience with its use, proven efficacy, survival benefit, and rapidity of action. This is consistent with the most current evidence-based treatment algorithms published [15, 79].

For the rest of the patients in class III or IV, treatment with inhaled iloprost, treprostinil s.c./i.v., bosentan, and sildenafil are available. Because direct, prospective comparisons between different PAH medications are not available (with the exception of one study, discussed below), the decision to use one treatment over another in the majority of cases will ultimately be influenced by the clinical scenario, availability of medication, preferred route of administration, medication side-effect profile, patient preference and provider experience. Still, there are situations in which it may be rational to use one medication over another.

Inhaled iloprost is an attractive option for PAH as it comes from the powerful prostanoid class of medication and administration is noninvasive. Compared with systemic therapies, the inhaled route may also particularly be useful in situations in which the ventilation–perfusion relationship is significantly altered, e.g. those with parenchymal lung disease complicating PAH associated with connective tissue disease, although more studies are needed to investigate this. The downside to inhaled iloprost includes the relatively short duration of action requiring repeated treatments six to nine times a day. Additionally, long-term efficacy data are not yet available.

In terms of administration, treprostinil has some advantages over epoprostenol in that it does not have to be continually cooled and, at least for the subcutaneous form, an implanted central venous catheter is not required. However, long-term efficacy data for s.c. treprostinil are not yet available. As for i.v. treprostinil, it has been shown to be effective as a transition therapy from i.v. epoprostenol; however, data are not available about its efficacy as initial treatment. This must be extrapolated from the bioequivalence data to s.c. treprostinil.

With respect to bosentan, it should be kept in mind that the beneficial effects probably take at least 8 weeks to manifest. Therefore, bosentan is not appropriate for use as monotherapy in unstable class IV patients. Additionally, haemodynamic evidence of right heart failure on initial RHC may help predict poor response to bosentan and could persuade one to use a prostanoid as first-line treatment in this situation [80]. For those in functional class III, there are data to suggest that survival estimates up to 36 months are similar between those initially treated with bosentan compared with those initially treated with epoprostenol [81]. Using an initial treatment combination of bosentan with epoprostenol may provide some additional benefit compared with using epoprostenol alone in class III/IV patients (BREATHE-2 trial) [82].

Sildenafil's place within the treatment algorithm is most firmly established for functional classes II and III as 96% of the patients in the SUPER-1 trial were from these classes; however, general clinical experience with its use in PAH is still in its infancy. Certainly, for those in whom oral therapy is being considered and/or bosentan is contraindicated, sildenafil is a viable option. A more thorough evaluation will be possible when the SUPER-1 results are published. Additionally, more complete follow-up data from SUPER-2 are needed regarding its long-term efficacy. In this respect, at least in comparison with the only other oral therapy available for PAH, bosentan may have an advantage as data regarding 3-year survival and need for add-on therapy are already available [62]. It is unknown whether the difference in dosing frequency between bosentan (twice daily) and sildenafil (three times daily) could potentially affect treatment compliance.

Keeping these factors in mind, one small, double-blind RCT has already been published investigating the efficacy of bosentan versus sildenafil over a 16-week period in patients with class III IPAH or PAH associated with connective tissue [83]. One patient in the sildenafil group died unexpectedly. When analysed by intention-to-treat, there were no significant differences between the treatment groups with respect to changes in right ventricle mass, 6MWT, echocardiographic parameters, brain natriuretic peptide, or Borg dyspnoea index.

Combination/add-on therapy

Unfortunately, not everyone responds to the initial drug treatment regimen chosen. The addition of a second PAH drug may be reasonable for patients who deteriorate or have a suboptimal response to monotherapy. Potential candidates include those

with worsening symptoms or deteriorating exercise capacity, functional class, or haemodynamics. In those initially treated with epoprostenol, persistence of an advanced functional class or lack of improvement in certain haemodynamic parameters at follow-up portend a poor prognosis [20, 62, 84]. These are also appropriate candidates for combination drug therapy.

An approach that uses a combination of drugs that targets different pathways has been successfully employed in systemic hypertension and congestive heart failure, and a similar strategy in PAH may increase efficacy whilst minimizing toxicity. Although prostanoids, ERAs and PDE-5 inhibitors work through different intracellular pathways, there may be important interactions between them. For example, the stable prostacyclin analogue cicaprost inhibits the release of ET-1 from pulmonary artery smooth muscle cells, whilst the antiproliferative effects of cicaprost are attenuated by ET-1 [32, 85]. Similarly, PDE-5 inhibitors increase the intracellular levels of cyclic adenosine monophosphate, a mediator for the cardiovascular effects of prostanoids [32].

A handful of case series and observational cohort studies preliminarily have shown promising results using various combinations of sequential add-on therapy including prostanoids + sildenafil [73, 86, 87], prostanoids + bosentan [88], and bosentan + sildenafil [89]. However, rigorous RCTs, several of which are currently ongoing, are needed to clarify definitively the proper timing and appropriate combination of drugs to use [90].

Switching therapies

Transitioning therapies has been made possible with the availability of less invasive and more convenient treatment options. The concept of transitioning therapy is one of de-evolution: going from a more invasive and complex treatment to one that is less invasive and simpler. This should be accomplished without clinical deterioration. Preliminary data suggest that transitioning patients from chronic i.v. epoprostenol to i.v. treprostinil [36] or s.c. treprostinil [91] can be carried out safely and without clinical deterioration. For patients who have made adequate improvement with epoprostenol or treprostinil, transitioning to oral therapies such as an ERA or PDE-5 inhibitor may also be possible. As there are no guidelines available for the selection of candidates for transitioning therapies, the timing, or the choice of agents, these decisions should be reserved for highly experienced physicians.

Lung or heart-lung transplantation

Lung or heart-lung transplantation for PAH remains the treatment of last resort when medical therapy has failed. Atrial septostomy can be used as a bridge to transplantation in patients with refractory right heart failure or as an alternative when transplantation is not a viable option. Septostomy is a high-risk procedure that should only be performed in centres with expertise [92].

Between January 1995 and June 2002, the Registry of the International Society for Heart and Lung Transplantation reported that 427 lung transplants were performed around the world for IPAH [93]. The majority were bilateral lung transplants (85%). Compared with other conditions for which transplantation was performed, those with IPAH had the highest risk of death within the first year of transplantation. However, for those who survived the first year, the prognosis improved considerably compared with the outcome of other recipient groups. The median survival after transplantation for IPAH was 4 years.

We recommend considering transplantation for PAH patients in class III or IV who are deteriorating on medical therapy [92]. For those being treated with epoprostenol, the presence of right-sided heart failure, persistence of NYHA functional classes III–IV, or the absence of a significant fall in total pulmonary resistance >30% relative to baseline after 3 months of therapy is associated with poor survival and may be useful in the consideration and timing of transplantation [20].

Monitoring treatment

Pulmonary arterial hypertension is a progressive disease for which no single therapy may suffice. Accordingly, ongoing and methodic monitoring of the responses to treatment is crucial in order to optimize outcomes. We reassess the clinical status every 2–3 months using noninvasive assessments such as functional class and 6MWT. Decisions regarding dose changes or add-on therapy depend on subjective and objective criteria. In general, our

goal is to improve the 6MWT to >380 m and functional class to I/II whilst on treatment, given their prognostic significance [20]. In instances when add-on therapy is being considered or the haemodynamic status is unclear, we perform repeat RHC. Echocardiography can also noninvasively provide useful measurements that have prognostic significance [94–99].

Conclusions

Until quite recently, PAH was an untreatable condition that invariably progressed to premature death. Whilst i.v. epoprostenol, the first medication introduced specifically for PAH, is still widely considered the 'gold standard' of therapy, newly studied prostacyclin analogues, ERAs and PDE-5 inhibitors provide alternative means of treatment that are less complex yet still efficacious for many patients with PAH.

Each advance, however, raises new questions about first-line treatment strategies and proper use of combination regimens. Certainly, treatment and management decisions are becoming increasingly complex. Referral of PAH patients to centres that have physicians and clinical support staff with particular expertise in managing patients with PAH may be necessary. In the meantime, as more data become available, the treatment algorithm will continue to evolve to optimize the evidence-based decision-making process.

Conflict of interest statement

Stephen H. Lee MD does not have a financial relationship with a commercial entity that has an interest in the subject of this article. Lewis J. Rubin MD has served as an investigator and consultant for the following commercial entities with an interest in the subject of this manuscript: Actelion, Pfizer, Schering, United Therapeutics, CoTherix, Myogen, MondoBiotech and Nitrox.

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EXHIBIT 5

Getting Started on VENTAVIS® (iloprost) Inhalation Solution

A Patient's Guide to Taking VENTAVIS

INDICATION

What is VENTAVIS?

VENTAVIS is a prescription medicine used to treat adults with certain kinds of severe pulmonary arterial hypertension (PAH), a condition in which blood pressure is too high in the blood vessels between the heart and the lungs. VENTAVIS may improve your ability to exercise and your symptoms for a short time by lowering your blood pressure and opening up the blood vessels in your lungs.

The study showing VENTAVIS is effective included mainly patients with NYHA Functional Class III-IV PAH. In these patients, PAH was caused by unidentified or hereditary factors (65%) or connective tissue diseases (23%).

VENTAVIS has not been studied in children younger than 18 years old.

Please see accompanying full Prescribing Information and Patient Information, and Important Safety Information on pages 3 and 4.

INHALED Ventavis® (iloprost) INHALATION SOLUTION

Understand Your PAH Therapy with

Your doctor has prescribed VENTAVIS to treat your pulmonary arterial hypertension (PAH)

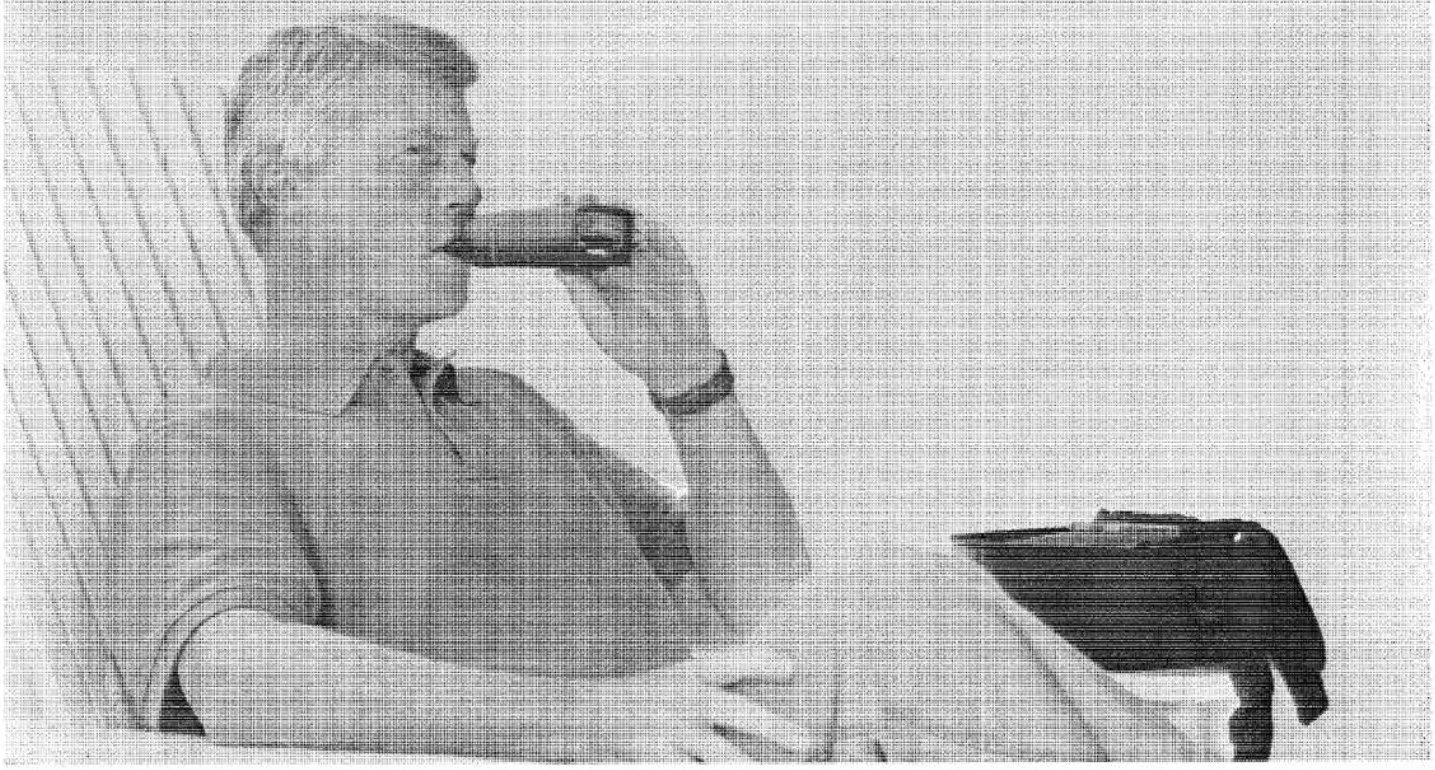
VENTAVIS is an inhaled PAH therapy that can be given to help patients improve some symptoms of PAH.

VENTAVIS has been shown to help some patients[1]:

- Improve some PAH symptoms (NYHA Functional Class III-IV)
- Walk farther in a timed test
- Slow down the progression of PAH

This brochure will help you to:

- Understand VENTAVIS
- Learn how to take VENTAVIS
- Use the I-neb® AAD® System
- Find and use resources for help and information
  - Actelion Services and Support—If you enroll, Actelion can provide you with services and support such as helping you with:
    - Answering your questions about filling your prescription
    - Coordinating with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medicine or if additional information is needed
    - Informing you of possible financial assistance programs based on your eligibility
  - Actelion Patient Services—trained Nurse Educators who can help answer your questions about VENTAVIS and the I-neb AAD System.



VENTAVIS® (iloprost) Inhalation Solution

INDICATION

What is VENTAVIS?

VENTAVIS is a prescription medicine used to treat adults with certain kinds of severe pulmonary arterial hypertension (PAH), a condition in which blood pressure is too high in the blood vessels between the heart and the lungs. VENTAVIS may improve your ability to exercise and your symptoms for a short time by lowering your blood pressure and opening up the blood vessels in your lungs.

The study showing VENTAVIS is effective included mainly patients with NYHA Functional Class III-IV PAH. In these patients, PAH was caused by unidentified or hereditary factors (65%) or connective tissue diseases (23%).

VENTAVIS has not been studied in children younger than 18 years old.

IMPORTANT SAFETY INFORMATION

What should I tell my doctor before taking VENTAVIS?

VENTAVIS may not be right for you. Before taking VENTAVIS, tell your doctor about all of your medical conditions, including if you:

- have liver or kidney problems. Your doctor may need to give you a lower dose of VENTAVIS.
- are pregnant, or plan to become pregnant. It is not known if VENTAVIS can harm your unborn baby. VENTAVIS should only be used during pregnancy if the benefit to you is worth the possible risk to your baby.
- are breast-feeding. It is not known if VENTAVIS passes into your breast milk. You and your doctor should decide if you will take VENTAVIS or breast feed.

Tell your doctor about all the medicines you take, including prescription and nonprescription medicines, vitamins, and herbal supplements.

VENTAVIS and other medicines may affect each other causing side effects. VENTAVIS may affect the way other medicines work, and other medicines may affect how VENTAVIS works.

Especially tell your doctor if you take:

- medicines used to treat high blood pressure or heart problems
- medicines that lessen blood clotting (warfarin, Coumadin, Jantoven)

Know the medicines you take. Keep a list of your medicines and show it to your doctor and pharmacist when you get a new medicine.

How should I take VENTAVIS?

- Take VENTAVIS exactly as your doctor tells you to take it. Your doctor may change your dose if needed.
- You should not take VENTAVIS more than every 2 hours. The benefits of VENTAVIS may not last 2 hours, so you may adjust the times that you use it to cover planned activities.
- Do not drink VENTAVIS.
- Do not let VENTAVIS solution come into contact with your skin or eyes. If it does, rinse your skin or eyes with water right away.

Please see accompanying full Prescribing Information and Patient Information.
Please see the Important Safety Information continued on the next page.

IMPORTANT SAFETY INFORMATION (continued)

- Do not allow other people to be exposed to VENTAVIS while you are breathing it, especially babies and pregnant women.
- If you take too much VENTAVIS, you may have a headache, red face, dizziness, nausea, vomiting and diarrhea. If this happens stop taking VENTAVIS. If your symptoms do not go away, call your doctor or get emergency help right away.

What are the possible side effects of VENTAVIS?

VENTAVIS may cause side effects, including feeling dizzy, lightheaded and faint. If you have any of these side effects, you should stand up slowly when you get out of chairs or bed. Tell your doctor if your fainting gets worse during treatment with VENTAVIS. Your doctor may need to change your dose or your treatment.

Do not drive a car or operate any tools or machines if dizziness or fainting from low blood pressure is a problem for you.

You may have trouble breathing after taking VENTAVIS because it may cause the muscles around your airway to tighten (bronchospasm). Get emergency help right away if you have trouble breathing.

Other important side effects of VENTAVIS include:

- bleeding
- red face (flushing)
- increased cough
- low blood pressure
- headaches
- nausea
- spasm of your jaw muscles that makes it hard to open your mouth

Talk to your doctor if you have any side effect that bothers you or that does not go away.

These are not all the possible side effects of VENTAVIS. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to the FDA at 1-800-FDA-1088.

Please see accompanying full Prescribing Information and Patient Information.

More about how VENTAVIS can help

VENTAVIS is delivered right to the lungs—the site of the disease. VENTAVIS is an inhaled therapy that can be given alone to help patients walk farther and breathe easier with daily activities.

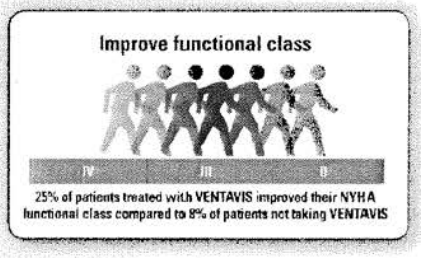
In clinical studies, VENTAVIS has been shown to lower high blood pressure and resistance in the pulmonary artery (main blood vessel) leading to the lungs to allow the heart to pump better.[1]

VENTAVIS is the only inhaled PAH therapy which has shown that patients have clinical improvement with treatment—defined as the combination of 3 different clinical measurements. The clinical study showed that PAH patients treated with VENTAVIS*:

Improved functional class[1]

- **Patients felt better doing daily activities and improved their NYHA Class.**

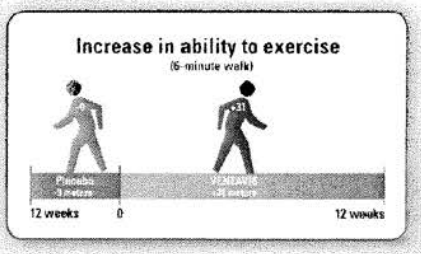
  Many patients who felt short of breath (even when sitting still) found that they were able to be more active.



Increased their ability to exercise*[1]

- **Also, patients were able to walk farther in a timed test.**

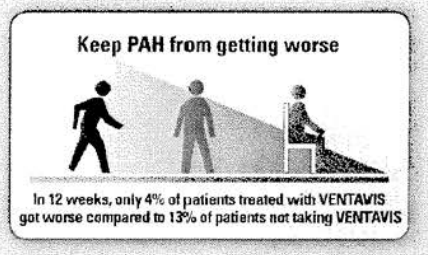
  Patients who took VENTAVIS were able to walk farther by at least 10% in a 6-minute walk test. On average, patients who took VENTAVIS could walk 40 meters farther than those who did not take VENTAVIS.



Experienced less worsening of PAH symptoms[1]

- **VENTAVIS decreased the worsening of PAH symptoms.**

  PAH is a progressive disease, which means it tends to get worse if not treated. Many patients who took VENTAVIS had less worsening of PAH.



It is important to remember that each person responds differently to therapy.

*In a study of 146 patients with NYHA Class III or IV PAH, researchers compared 2 groups of patients for 12 weeks: 1 group received VENTAVIS inhaled 6 to 9 times per day, while the other group inhaled placebo (no active medicine). About 5 times as many patients taking VENTAVIS had clinical improvement compared to those who took placebo during the study (19% vs 4%).[1]

Connect with *Actelion Pathways*™...

Getting your VENTAVIS® (iloprost) Inhalation Solution prescription

Your VENTAVIS prescription comes with helpful services brought to you by *Actelion Pathways*. *Actelion Pathways* is your one point of contact for access to VENTAVIS, answers to questions about filling your prescription, and help in coordinating with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medicine.

Here's how it happens...

1. Your healthcare provider will send your prescription form directly to *Actelion Pathways*. An Actelion Patient Services Counselor will coordinate with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medicine.

2. Then, *Actelion Pathways* will work with the specialty pharmacy to help you get your prescription filled as soon as possible.

3. Your specialty pharmacy contacts you to set up a meeting between you and a VENTAVIS-trained specialty pharmacy nurse. The specialty pharmacy will send your VENTAVIS prescription and the I-neb® AAD® System directly to you. Then the specialty pharmacy nurse meets with you at your doctor's office or your home to show you how to take VENTAVIS with the I-neb AAD System.

Please see accompanying full Prescribing Information and Patient Information.

Actelion Pathways is with you on your journey

When your VENTAVIS prescription is written, it goes to Actelion. A VENTAVIS prescription cannot be filled at your neighborhood pharmacy. It must be dispensed through a specialty pharmacy that is part of the VENTAVIS network.

Is VENTAVIS covered by insurance?

Every insurance company is different. Our Actelion Patient Services Counselors will help in coordinating with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medicine.

If you have any questions about the services and support offered by Actelion, call toll-free, **1-866-ACTELION** (1-866-228-3546) Monday through Friday, 12 PM-8 PM (ET)/ 9 AM-5 PM (PT).

Please see pages 3 and 4 for Important Safety Information.

Taking VENTAVIS® (iloprost) Inhalation Solution

How is VENTAVIS taken?

VENTAVIS is inhaled through a special system called the I-neb® AAD® System, which is compact, portable, and lightweight. The I-neb AAD System is small—about the size of a box of kitchen matches—and it has an internal rechargeable battery like a cell phone, so you can take your medication almost anywhere at any time. VENTAVIS should be inhaled as your doctor prescribes, usually 6-9 times a day, but not more often than every 2 hours.[1]

What does AAD stand for?

Adaptive **A**erosol **D**elivery.

- The word "Adaptive" is important because it means that this system adjusts to fit your breathing pattern each time you use it. It releases the medicine (as a mist) only when you breathe in. This device was designed to deliver the right amount of medication.[1]
- "Aerosol" means fine mist.

Why is VENTAVIS inhaled?

Inhaling VENTAVIS gets it right to the lungs—the site of the disease. There are other ways to deliver medications like VENTAVIS into the body that require pumps, needles and catheters.

Can I take VENTAVIS only with the I-neb AAD System?

VENTAVIS must be taken with the I-neb AAD System because it is the only system approved by the FDA and available for use with VENTAVIS. This special handheld system turns VENTAVIS liquid medicine into a fine mist (or "aerosol") that you breathe in. Its advanced technology provides direct-to-lung delivery of VENTAVIS.

Please see accompanying full Prescribing Information and Patient Information.

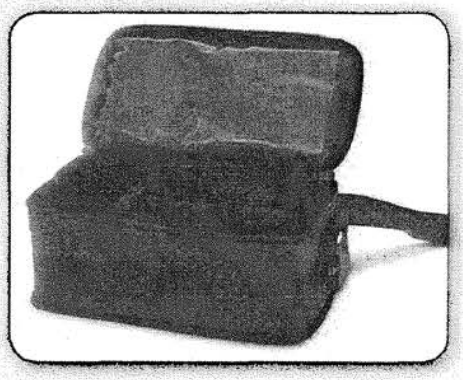
A high-tech handheld system

The I-neb AAD System has been developed with high-tech features so that it:

- Adjusts to fit your breathing pattern each time you use it (unlike other nebulizers which make you adjust when and how you breathe)
- Produces a fine mist that can reach into the tiny airways throughout the lungs
- Makes sure dosing is accurate every time you take VENTAVIS
- Records treatment information to help your doctor follow your progress

Handy carrying case

The I-neb AAD System is compact, portable, and comes with a convenient over-the-shoulder carrying case, making your treatments accessible and easy when you're on the go.



Learning how to use the I-neb AAD System

After you receive your VENTAVIS prescription and I-neb AAD System from your specialty pharmacy, they will schedule a meeting between you and a VENTAVIS-trained nurse educator. The nurse educator will meet with you (either at home or in your doctor's office) to show you how to take your VENTAVIS treatments, and how to use and clean the I-neb AAD System:

- Each treatment should take about 4-10 minutes.
- If you notice that your treatments are starting to take longer than usual, call your specialty pharmacy or your healthcare professional to ask for help.
- Or you can call Actelion's team of Registered Nurses and Respiratory Therapists, who will be glad to help answer your questions. Call **1-866-ACTELION** (1-866-228-3546) Monday through Friday, 12 PM-8 PM (ET)/ 9 AM-5 PM (PT).

To learn more about VENTAVIS and watch videos about using the I-neb AAD System, visit the I-neb AAD Learning Center at: **www.VENTAVIS.com**.

Please see pages 3 and 4 for Important Safety Information.

Understanding Your I-neb® AAD® System

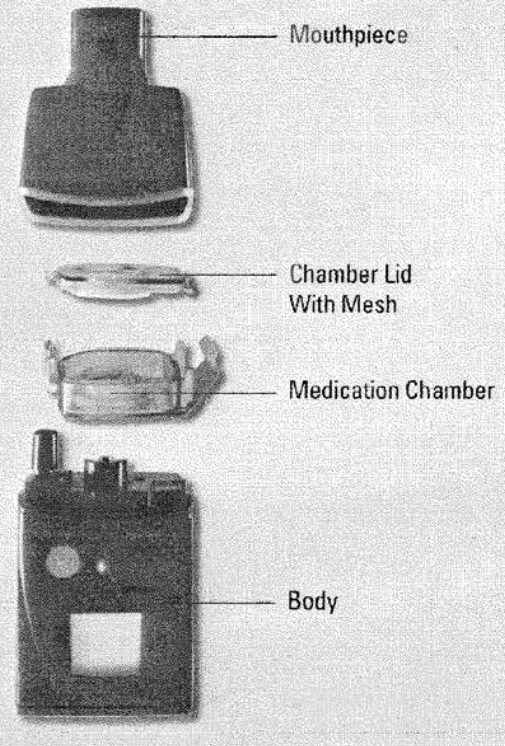
Help when you start

A VENTAVIS-trained nurse educator from your specialty pharmacy will be with you to help you get started on VENTAVIS® (iloprost) Inhalation Solution. The nurse educator will show you, step-by-step, how to use and clean your I-neb AAD System. You will also get a more detailed instruction booklet.

Your I-neb AAD System[2]

Mobility and portability. That's what you get with the I-neb AAD System. You can take the I-neb AAD System with you for treatments almost anywhere at any time.*

The I-neb AAD System comes with two convenient carrying cases for your chamber lids with mesh.



For full details on how to use the I-neb AAD System, see the user manual that accompanies your device.

*Usually 6 to 9 times a day but no more than once every 2 hours.[1]

Please see accompanying full Prescribing Information and Patient Information.

Setting up is simple and easy

- Make sure the battery is charged. The I-neb AAD System, when fully charged, will last for up to 40 treatments before recharging is needed.

- Hold the VENTAVIS ampule with the blue dot facing away from you and align with dot on ampule breaker to open the ampule.

- After removing the medication lid, put the dosing guide over the medication chamber. Use the pipette to draw VENTAVIS out of the ampule. Carefully squeeze the liquid into the medication chamber.

- Replace the lid, cover the latch, and attach the mouthpiece.

If you have any questions about VENTAVIS or the I-neb AAD System, help is just a phone call away. Contact Actelion Patient Services at **1-866-ACTELION** (1-866-228-3546) Monday through Friday, 12 PM-8 PM (ET)/ 9 AM-5 PM (PT). Or call your specialty pharmacy.

Please see pages 3 and 4 for Important Safety Information.

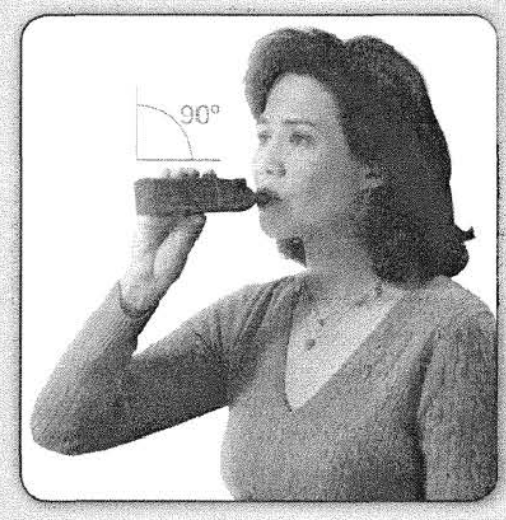
The ABCs of Using the I-neb® AAD® System

Learn the ABCs—**Angle, Breathing, Cleaning**—to help you manage your VENTAVIS® (iloprost) Inhalation Solution treatment. These are the three keys to taking VENTAVIS with the I-neb AAD System.

Angle:

Holding the I-neb AAD System at the right angle ensures the best drug delivery.

- Sit in a comfortable, upright position.
- Hold the I-neb AAD System at a 90-degree angle to your mouth, like you would when eating a hamburger; resting your elbows on the table while holding your I-neb AAD System makes it easy.
- The I-neb AAD System will remind you with 4 short beeps if you are not holding it at a 90-degree angle.
- Holding the I-neb AAD System at the wrong angle will increase the length of treatment time.



Practice your ABCs every day

Please see accompanying full Prescribing Information and Patient Information.

Breathing:

Proper breathing is the single most important part of your treatment.

Keep your lips closed around the I-neb AAD System mouthpiece as you breathe in and out.

- Breathe in and out through your mouth, not your nose.
- The I-neb AAD System will take the first 3 breaths in and out to adapt to your own breathing pattern. As you begin your 4th breath in, VENTAVIS will be delivered.
- The I-neb AAD System will vibrate as you begin to take a breath in to let you know VENTAVIS is being delivered.

Relax and breathe in and out in a slow and steady manner

- While breathing in a slow and steady manner, try counting one one-thousand, two one-thousand, three one-thousand.
- The longer you can breathe in, the more VENTAVIS is delivered and your treatment times may decrease.
- If you need a break, take one. Rest a minute or 2, then restart your treatment. Remember, the I-neb AAD System will take 3 breaths in and out to adapt to your breathing pattern before VENTAVIS will be delivered again.

Cleaning:

Keeping your I-neb AAD System clean is important to treatment success. Thorough cleaning keeps the I-neb AAD System working well. The parts must be cleaned once a day and boiled once a week. See the "Cleaning the I-neb AAD System" section for more details on cleaning.

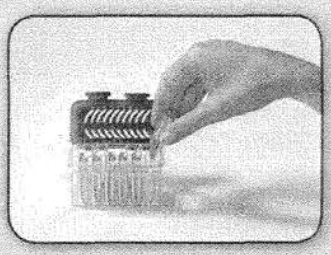
Please see pages 3 and 4 for Important Safety Information.

Once-Daily Cleaning...Easy as 1-2-3

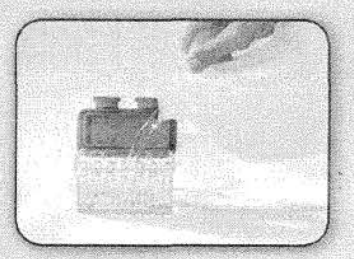
To keep your I-neb® AAD® System in the best working condition, clean it once a day and boil it weekly.

1. Starting the day

- Load the blue case with 6 clean, dry chamber lids with mesh—the blue case is always for clean chamber lids with mesh.
- Fill the orange case with distilled water and secure the lid—the orange case is now ready to store up to 6 used chamber lids with mesh.



Load blue case with clean, dry chamber lids with mesh.



Fill orange case with distilled water and secure the lid.

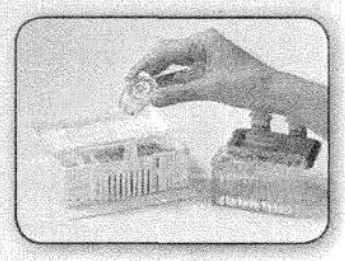
2. During the day

- Remove a chamber lid with mesh from the blue carrying case and place it on top of the I-neb AAD System medication chamber. Close the latch and attach the mouthpiece.
- Take your VENTAVIS® (iloprost) Inhalation Solution treatment.
- When finished with your treatment, remove the used chamber lid with mesh and place it in the orange carrying case.
- Repeat the steps above each time you take a VENTAVIS treatment.

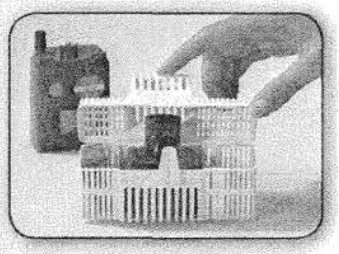
Please see accompanying full Prescribing Information and Patient Information.

3. Ending the day

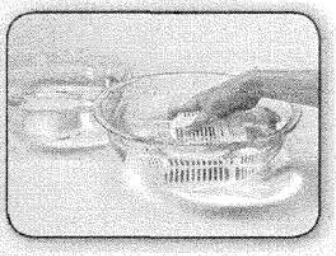
- When your orange case is filled with used chamber lids with mesh from the day's treatments, you're ready for once-daily cleaning.
- Remove the chamber lids with mesh and place them in the mesh wash basket.



- Place the mouthpiece, medication chamber, chamber lids with mesh, and drug guide in the main wash basket.
- Using only one drop of dishwashing liquid,* wash all of the pieces in distilled water.



- Rinse the pieces with more distilled water (never reuse the distilled water).
- Shake off the water and then air dry the pieces for 2 hours before using again.
- Be sure to clean the orange used chamber lid with mesh carrying case with soapy water at least once a week.



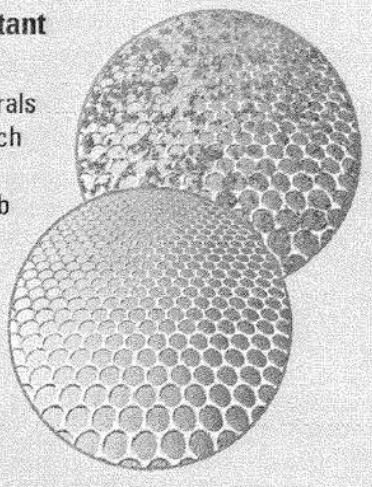
Note: If you want, you can wash each chamber lid with mesh after each treatment instead of all at once at the end of the day. Use the same cleaning method described above.

*Use any liquid detergent without bleach, fragrance, or antibacterial ingredients—examples are Original Dawn®, Ultra Dawn®, Palmolive® Original, Method® Go Naked Dish Soap, and Seventh Generation® Free & Clear Natural Dish Liquid. Only one drop is needed per cleaning.

Please see pages 3 and 4 for Important Safety Information.

Distilled Water

Why cleaning with distilled water is so important



Your I-neb® AAD® System contains chamber lids with mesh that has very tiny holes. Tap water contains minerals that can build up and collect on the mesh over time. Each chamber lid has over 5000 holes smaller than a human hair. If the chamber lids with mesh are blocked, the I-neb AAD System will not work as it should. As a result, it may take longer to do your treatments.

It is important to use ONLY distilled water for your daily cleaning and weekly boil. Do not reuse your distilled water. Keeping the mesh clear of minerals is important to maintaining the I-neb AAD System and to helping you manage your VENTAVIS® (iloprost) Inhalation Solution treatments.

Once-Weekly Cleaning

Weekly boiling

- Put all of the parts in the cleaning basket (do not boil the main body).
- Boil for 6-10 minutes—do not microwave since some of the parts are metal (also, do not wash in the dishwasher as this may damage the parts).
- Rinse with distilled water.
- Shake off the water and then allow to air dry.

Tip: Keep all of your supplies together to make it as easy as possible.

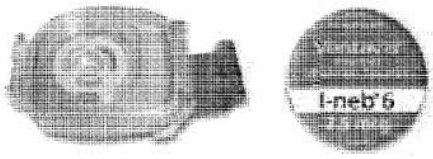
If you have any questions about the use, care, and cleaning of your I-neb AAD System, just call Actelion Patient Services, toll-free, at **1-866-ACTELION** (1-866-228-3546). Monday through Friday, 12 PM-8 PM (ET)/9 AM-5 PM (PT). Or call your specialty pharmacy.

Please see accompanying full Prescribing Information and Patient Information.

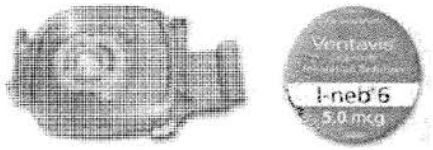
VENTAVIS Dosing Options

VENTAVIS is available in 3 dosing options

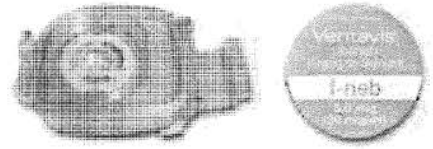
2.5 mcg with 10 mcg/mL – Red*



5.0 mcg with 10 mcg/mL – Purple*



5.0 mcg with 20 mcg/mL – Gold*



You will start with this dose, which uses the red-latched medication chamber.

If tolerated

Nearly all patients transition to this dose, which uses the purple-latched medication chamber.

If you are experiencing long treatment times, your doctor may transition you to this dosing option, which may decrease your treatment time.

Take your VENTAVIS treatments as prescribed by your doctor. FDA-recommended dosing is 6-9 times each day, at least 2 hours apart, or as prescribed by your doctor.

VENTAVIS 20 mcg/mL:

Your doctor may prescribe VENTAVIS 20 mcg/mL if you have long treatment times and are maintained at the 5 mcg dose. The higher concentration of medicine lowers the amount of solution you need to take by 50% which gives you shorter treatment time.[1]

*Different colored chambers and dosing discs are NOT interchangeable. Do not use the 20 mcg/mL ampule with the purple- or red-latched medication chambers.

Please see pages 3 and 4 for Important Safety Information.

Actelion Services for VENTAVIS® (iloprost) Inhalation Solution Patients

Actelion Pathways™

Your VENTAVIS prescription comes with support from *Actelion Pathways.*

A dedicated support team

Actelion Patient Services provides a dedicated team of Registered Nurses and Respiratory Therapists who are available to answer your questions about VENTAVIS or the I-neb® AAD® System, including:

- Use of the I-neb AAD System
- Cleaning and maintenance of the I-neb AAD System

If you would like to enroll in *Actelion Pathways,* you will receive information updates from *Actelion Pathways* about changes or improvements in the I-neb AAD System or new or expanded information about treatment with VENTAVIS.

If you have any questions about VENTAVIS or the I-neb AAD System, call Actelion Patient Services at **1-866-ACTELION** (1-866-228-3546), Monday through Friday, 12 PM-8 PM (ET)/9 AM-5 PM (PT).

Please see accompanying full Prescribing Information and Patient Information.

Understanding Your Support Team

As you can see, getting your VENTAVIS involves a team of people and several organizations. Here is a brief outline of the various groups you'll interact with:

Your Healthcare Team: Diagnoses and treats PAH. They're a great resource to answer your questions about PAH, VENTAVIS, and your I-neb AAD System.

Your Specialty Pharmacy: VENTAVIS is supplied through specialty pharmacies only. The specialty pharmacy will deliver VENTAVIS and the I-neb AAD System to you, and show you how to use them. You can call the specialty pharmacy with questions about your VENTAVIS drug shipment.

Your Actelion Patient Services Team: We are dedicated to helping you make your treatment a success. Remember, our team of Registered Nurses and Respiratory Therapists is waiting to help you with answers to your questions about VENTAVIS and the I-neb AAD System.

***Actelion Pathways*:** Coordinates with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medication. Call **1-866-ACTELION** (1-866-228-3546) Monday through Friday, 12 PM-8 PM (ET)/9 AM-5 PM (PT).

References:
1. VENTAVIS (iloprost) full prescribing information. Actelion Pharmaceuticals US, Inc. May 2013.
2. I-neb AAD System user guide. Koninklijke Philips Electronics N.V. 2010.

Please see pages 3 and 4 for Important Safety Information.

Actelion Pathways™

If you enroll, Actelion can provide you with services and support such as helping you with:

- Answering your questions about filling your prescription
- Coordinating with your doctor, insurance company, and specialty pharmacy to find out whether you have coverage for your medicine or if additional information is needed
- Informing you of possible financial assistance programs based on your eligibility

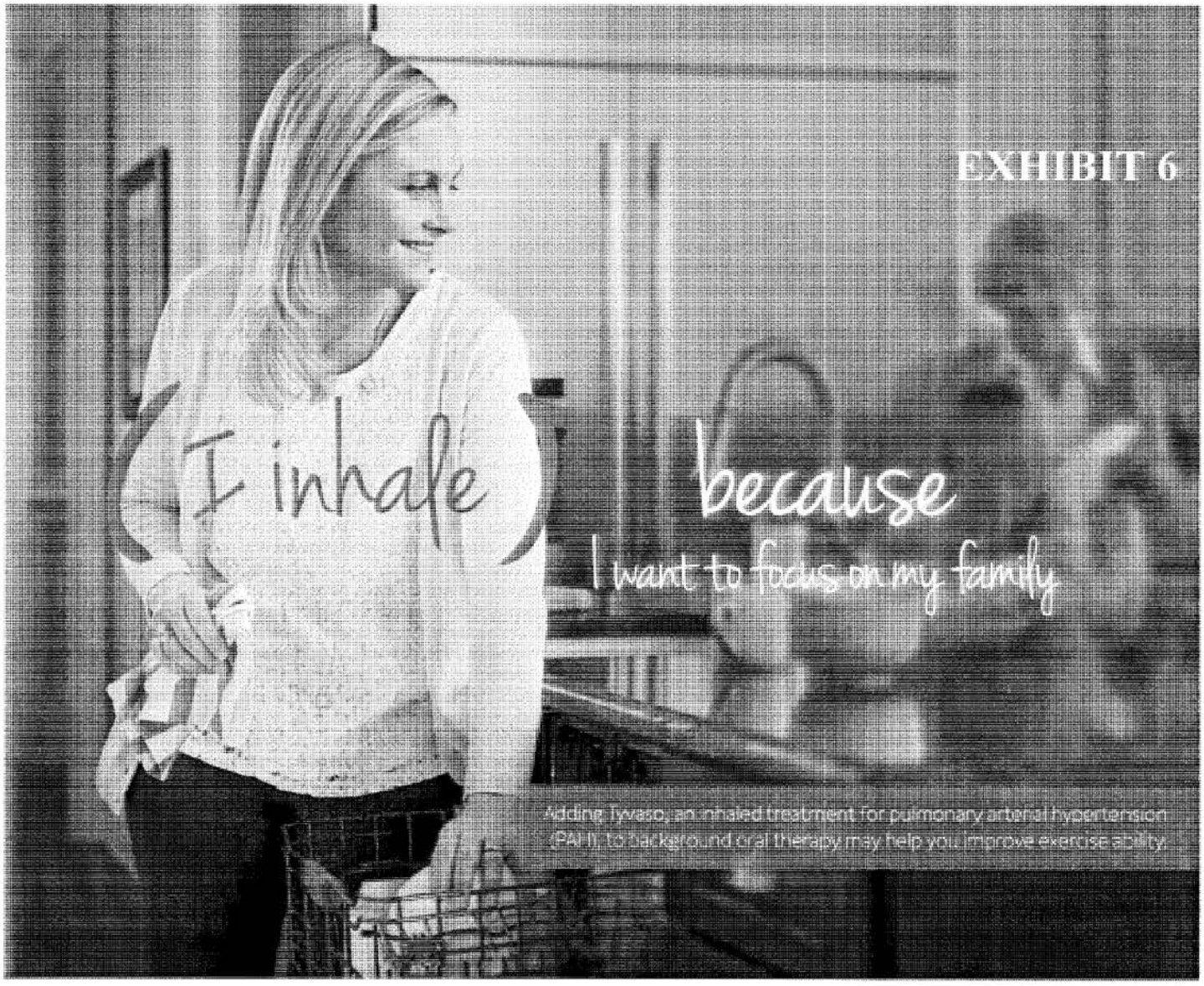
Through Actelion's Patient Services Department, you'll also have access to a dedicated team of nurse educators—including Registered Nurses and Respiratory Therapists.

An Actelion Nurse Educator can be reached from Monday through Friday, 12 PM-8 PM (ET)/9 AM-5 PM (PT) at **1-866-ACTELION** (1-866-228-3546).

Please see accompanying full Prescribing Information and Patient Information, and Important Safety Information on pages 3 and 4.

ACTELION

Ventavis® (iloprost) INHALATION SOLUTION



Selected Important Safety Information

- Tyvaso is breathed in (inhalable) through your mouth into your lungs. Tyvaso should only be used with the Tyvaso Inhalation System
- The effects of Tyvaso are unknown in patients with lung disease (such as asthma or chronic obstructive pulmonary disease) and in patients under 18 years of age

Please see complete Important Safety Information and Indication on page 18.

TYVASO® (treprostinil) INHALATION SOLUTION

Tyvaso Patient Brochure

Why Inhale?

Pulmonary arterial hypertension (PAH) is high blood pressure in the arteries of the lungs. The symptoms of PAH may make activities like walking to the mailbox or walking up stairs difficult. As PAH progresses, it may become harder to carry out daily tasks.

Even if you're doing okay on your oral therapy, adding Tyvaso may help you improve
Adding Tyvaso has been shown to help patients with PAH (WHO Group 1) already taking tosentan (an endothelin receptor antagonist [ERA]) or sildenafil (a phosphodiesterase-5 [PDE-5] inhibitor) improve their exercise ability by an average of 20 meters, as shown by their 6-minute walk distance (6MWD).

Tyvaso is an inhaled (breathed in) medication that can be scheduled around your daily activities

Indication
Tyvaso is a prescription medicine used in adults to treat pulmonary arterial hypertension (PAH) (WHO Group 1), which is high blood pressure in the arteries of your lungs. Tyvaso can improve exercise ability in people who also take tosentan (an endothelin receptor antagonist) or sildenafil (a phosphodiesterase-5 inhibitor). The effects decrease over 4 hours; treatment timing can be adjusted for planned activities.

Studies establishing effectiveness included predominately patients with NYHA Functional Class III symptoms and etiologies of idiopathic or heritable PAH (66%) or PAH associated with connective tissue diseases (33%).

Selected Important Safety Information
- If you have low blood pressure, Tyvaso may cause symptomatic hypotension (low blood pressure)
- Because Tyvaso reduces the ability of your blood to clot (coagulate), it may increase your risk for bleeding if you are taking blood thinners (anticoagulants), such as warfarin or heparin

Please see complete Important Safety Information on page 18.

TYVASO® treprostinil

What Is Tyvaso?

Tyvaso is a synthetic (man-made) form of prostacyclin called a prostacyclin analogue. Prostacyclin is a chemical made naturally by the body. People with PAH lack enough natural prostacyclin to keep the vessels of the lungs open and working properly.

Tyvaso is an inhaled medication, meaning it is breathed in through the mouth and directly into the lungs. In the body, Tyvaso mimics some of the effects of natural prostacyclin, helping to open up the arteries in the lungs and make it easier for the heart to pump blood through them. In the clinical trial, patients who added Tyvaso to their oral therapy were able to increase their exercise ability by an average of 20 meters, as shown by their 6MWD.

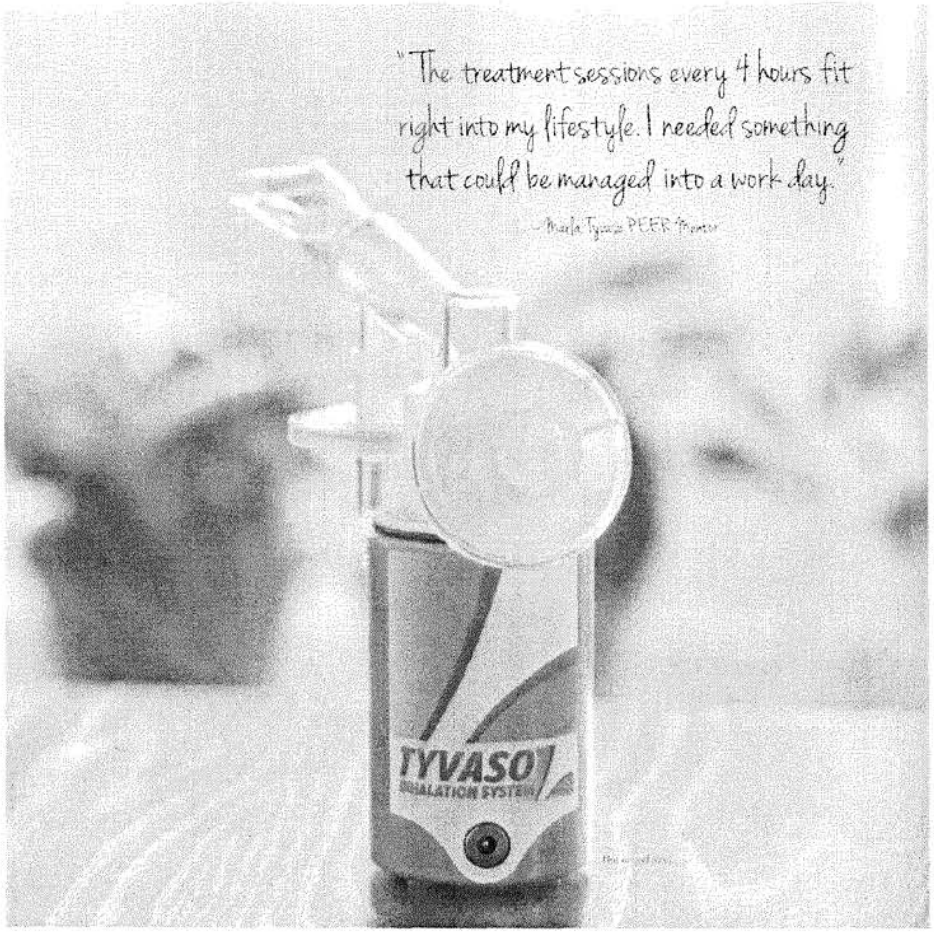
Tyvaso is the only inhalable PAH treatment studied solely as an add-on therapy to be used with oral medications such as bosentan (an ERA) or sildenafil (a PDE-5 inhibitor). No therapy is without risk. Common side effects of Tyvaso include coughing, headache, and throat irritation and pain.

Selected Important Safety Information

- Because Tyvaso reduces the ability of your blood to clot (coagulate), it may increase your risk for bleeding if you are taking blood thinners (anticoagulants), such as warfarin or heparin.
- The use of Tyvaso with diuretics (water pills), antihypertensives (medications used to treat high blood pressure or heart disease), or other vasodilators (medications that lower blood pressure) may increase your risk for hypotension (low blood pressure).

Please see complete Important Safety Information on page 18.

TYVASO® (treprostinil) INHALATION SOLUTION



Why Add Tyvaso?

You may feel okay with oral PAH treatment alone, but could you improve by adding another treatment? Tyvaso has been shown to help patients with PAH who are already taking bosentan (an ERA) or sildenafil (a PDE-5 inhibitor) improve their exercise ability by an average of 20 meters, as measured by the 6MWD. This could mean improvement in your ability to take a short stroll in the neighborhood or walk your kids to the bus stop, individual results may vary.

Make improvement the goal

It is important to work with your doctor to determine which treatment goals are right for you, and then develop a plan to help you work toward them. Physical symptoms may not always reflect whether PAH is progressing or how it is affecting the heart. Making sure you are meeting your treatment goals is one way to assess your progress. The 6MWD is one of several measures doctors use to determine how well you're doing and the effects of your PAH treatment.

Do you know what your 6MWD is?

Talk to your doctor and see if adding Tyvaso could help you go farther.

Selected Important Safety Information

Other medical conditions and medicines may affect your use of Tyvaso by increasing the risk of side effects or decreasing effectiveness. It is important to tell your doctor about your medical conditions and any medicines you may be taking, including:

- If you are taking gemfibrozil (for high cholesterol) or rifampin (for infection), your Tyvaso dosage may need adjustment
- If you have liver or kidney problems, your ability to tolerate Tyvaso may be affected
- If you are pregnant, breast-feeding, or planning to become pregnant, talk with your healthcare provider about whether you should take Tyvaso

Please see complete Important Safety Information on page 12.

TYVASO (treprostinil) inhalation solution

Taking Tyvaso

Tyvaso is a medication you inhale (or breathe in) using the Tyvaso Inhalation System. It is inhaled during 4 treatment sessions each day, approximately 4 hours apart, and can be scheduled around your daily activities.

Tyvaso Inhalation System
The Tyvaso Inhalation System is a lightweight, portable device (under 14 oz) that allows you to inhale Tyvaso Inhalation Solution directly through your mouth into your lungs.

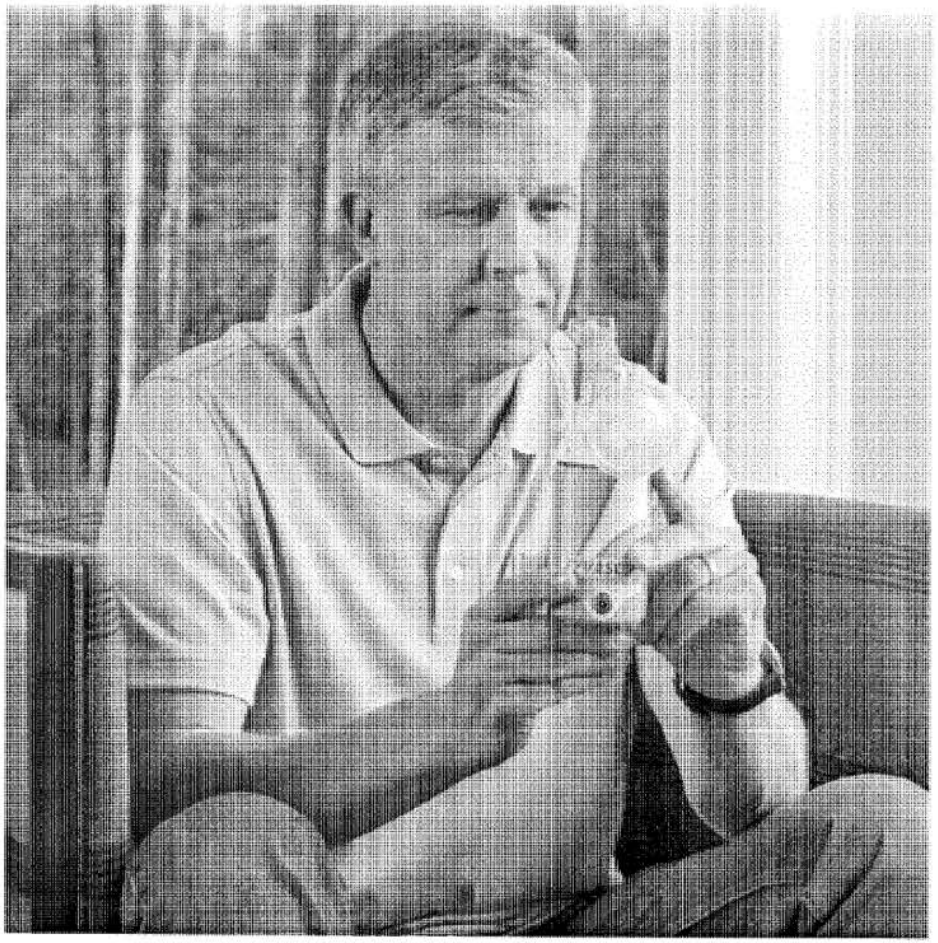
Treatments can be worked around your daily activities:
- 4 treatments per day, approximately every 4 hours, during waking hours
- Only 2 to 3 minutes for each treatment session
- 1 set-up daily

Coordinate treatment sessions with other things you do each day
For example, take before breakfast, lunch and dinner and at bedtime.

Selected Important Safety Information
The most common side effects of Tyvaso are coughing, headache, throat irritation and pain, nausea, reddening of the face and neck (flushing), and fainting or loss of consciousness. These are not all the possible side effects of Tyvaso. Tell your doctor about any side effects that bother you or do not go away. Your doctor may be able to help you manage the side effects.

Please see complete Important Safety Information on page 18.

TYVASO (treprostinil) INHALATION SOLUTION



Get Support

Tyvaso Support Program

The Tyvaso Support Program is a free program for people who are considering or taking Tyvaso. By signing up for the program, you'll receive a series of e-mails designed to deliver important information and tips, wherever you are on your journey with PAH, including:

- Information about PAH
- How adding Tyvaso to oral therapy (bosentan [an ERA] or sildenafil [a PDE-5 inhibitor]) could help you improve your exercise ability
- Potential side effects of Tyvaso and approaches to help manage them
- Tips to help you get the most from your treatment
- Support information, resources, and more

TYVASO
(Support Program)

SIGN UP NOW
Mail in the registration card in the back pocket of this brochure.

OR VISIT
www.MyTyvasoSupport.com

Tyvaso PEER Mentors

Tyvaso PEER Mentors are Tyvaso patients who have volunteered their time to help people like you. If you are new to Tyvaso, or are considering taking Tyvaso and are seeking support from someone who has been down a similar path, we encourage you to connect with a Tyvaso PEER Mentor. For more information, visit www.peernetwork.net or call 1-866-505-PEER (7337).

Financial Support

There are a number of financial assistance programs available to help you obtain your medication, should you qualify. To learn more about the programs, call 1-877-UNITHER (1-877-864-8437).

TYVASO (treprostinil) inhalation solution

What to Expect When Starting Tyvaso

If your doctor has just prescribed Tyvaso to treat your PAH, you may be wondering what comes next. Once your doctor has put in a referral, or application, for your Tyvaso prescription, a Specialty Pharmacy Services (SPS) provider will be responsible for working with your insurance company and healthcare provider to process the referral.

What is an SPS provider?
SPS providers are different from the local pharmacy you normally visit. They will work with you and your healthcare team to coordinate many aspects of your care. This includes step-by-step training on how to use Tyvaso and monthly delivery of your prescription and supplies. SPS is a service designed to provide you with ongoing support. Through SPS, you can expect to receive phone calls, refills, and access to a 24-hour hotline.

Tyvaso is available only through the following Specialty Pharmacies:

accredo
1-866-344-4874

CVS CAREMARK
1-847-484-8491

Please see complete Important Safety Information on page 18.

"My Specialty Pharmacy nurse was always there if I had questions. I felt like I wasn't alone while I was getting adjusted to Tyvaso."

– Ben Tyvaso PCEP Patient

TYVASO® (treprostinil) INHALATION SOLUTION

Here is what you can expect while you wait for your treatment to begin.
Note: This is a general overview of the SPS process and may vary based on individual circumstances or need.

Referral
Your doctor submits your referral form to an SPS provider.

Insurance
Your SPS provider contacts your insurance company to discuss coverage.

Confirmation
Your insurance company may require confirmation that you have PAH in order for you to start Tyvaso. This may include documentation of procedures, such as a right heart catheterization (RHC).

Demonstration
Your SPS nurse may contact you to schedule an appointment at your doctor's office or at your home to teach you how to use Tyvaso (do not start treatment until you have completed your training).

Approval
Your SPS provider will notify you of your insurance company's approval and discuss any uncovered costs.

Start
Your healthcare or SPS provider will contact you to coordinate the date and location for you to start using Tyvaso.

TYVASO® (treprostinil) INHALATION SOLUTION

because
I want to be in the moment

Indication

Tyvaso is a prescription medicine used in adults to treat pulmonary arterial hypertension (PAH) (WHO Group 1), which is high blood pressure in the arteries of your lungs. Tyvaso can improve exercise ability in people who also take bosentan (an endothelin receptor antagonist) or sildenafil (a phosphodiesterase-5 inhibitor). The effects decrease over 4 hours; treatment timing can be adjusted for planned activities.

Studies establishing effectiveness included predominately patients with NYHA Functional Class III symptoms and etiologies of idiopathic or heritable PAH (56%) or PAH associated with connective tissue diseases (33%).

Important Safety Information for Tyvaso

- Tyvaso is breathed in (inhalable) through your mouth into your lungs. Tyvaso should only be used with the Tyvaso Inhalation System
- The effects of Tyvaso are unknown in patients with lung disease (such as asthma or chronic obstructive pulmonary disease) and in patients under 18 years of age
- If you have low blood pressure, Tyvaso may cause symptomatic hypotension (low blood pressure)
- Because Tyvaso reduces the ability of your blood to clot (coagulate), it may increase your risk for bleeding if you are taking blood thinners (anticoagulants), such as warfarin or heparin
- The use of Tyvaso with diuretics (water pills), antihypertensives (medications used to treat high blood pressure or heart disease), or other vasodilators (medications that lower blood pressure) may increase your risk for hypotension (low blood pressure)

Other medical conditions and medicines may affect your use of Tyvaso by increasing the risk of side effects or decreasing effectiveness. It is important to tell your doctor about your medical conditions and any medicines you may be taking, including:

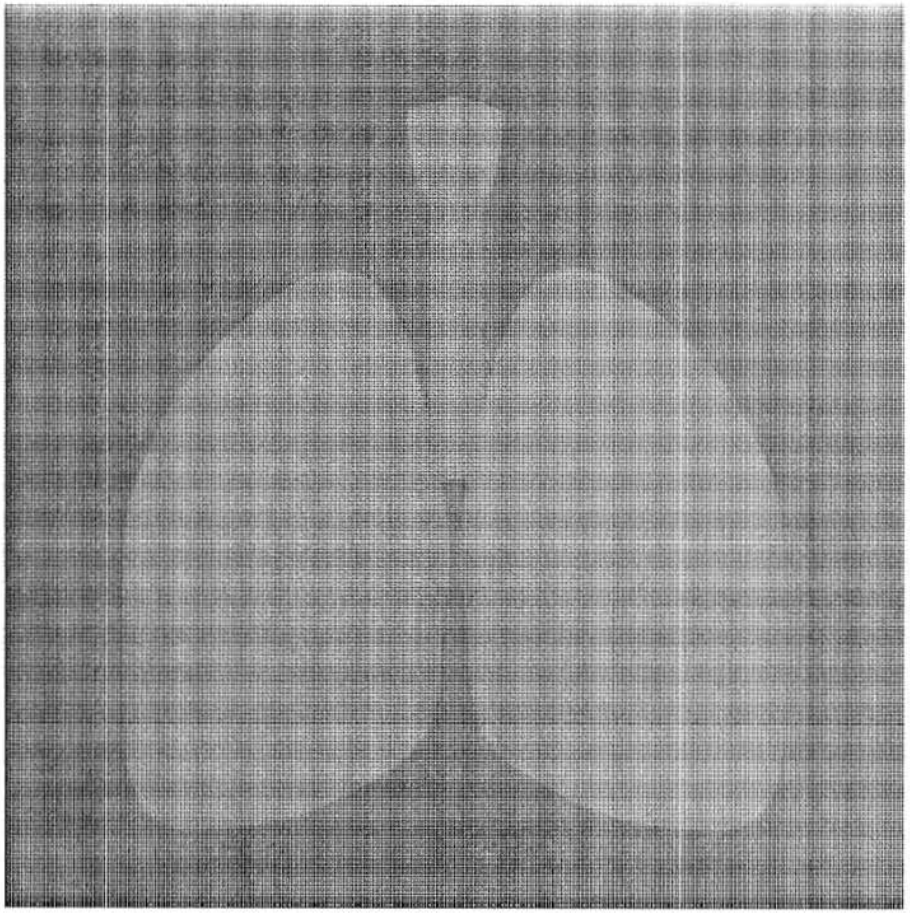
- If you are taking gemfibrozil (for high cholesterol) or rifampin (for infection), your Tyvaso dosage may need adjustment
- If you have liver or kidney problems, your ability to tolerate Tyvaso may be affected
- If you are pregnant, breast-feeding, or planning to become pregnant, talk with your healthcare provider about whether you should take Tyvaso

The most common side effects of Tyvaso are coughing, headache, throat irritation and pain, nausea, reddening of the face and neck (flushing), and fainting or loss of consciousness. These are not all the possible side effects of Tyvaso. Tell your doctor about any side effects that bother you or do not go away. Your doctor may be able to help you manage the side effects.

Please see the accompanying Full Prescribing Information, Patient Package Insert, and the Tyvaso Inhalation System Instructions for Use manual. For additional information about Tyvaso, visit www.tyvaso.com or call 1-877-864-8437.

Watch the Getting Started With Tyvaso DVD to learn more about Tyvaso.

TYVASO® treprostinil INHALATION SOLUTION



Talk to your doctor today about adding Tyvaso.

To learn more about Tyvaso,
visit tyvaso.com or call 1-877-UNITHER (1-877-864-8437).

United Therapeutics Corporation

PATIENT PACKAGE INSERT

Tyvaso (Tī-vāsō)

(treprostinil)

Inhalation Solution

Read this Patient Package Insert before you start taking Tyvaso and each time you get a refill. There may be new information. This leaflet does not take the place of talking with your healthcare provider about your medical condition or your treatment.

What is Tyvaso?

Tyvaso is a prescription medicine used in adults to treat pulmonary arterial hypertension (PAH), which is high blood pressure in the arteries of your lungs. Tyvaso can improve the ability to do exercise in people who also take bosentan (an endothelin receptor antagonist (ERA)) or sildenafil (a phosphodiesterase-5 (PDE-5) inhibitor). Your ability to do exercise decreases 4 hours after taking Tyvaso.

It is not known if Tyvaso is safe or effective in people under 18 years of age.

What should I tell my healthcare provider before taking Tyvaso?

Before taking Tyvaso, tell your healthcare provider about all of your medical conditions, including if you:

- have lung disease, such as asthma or chronic obstructive pulmonary disease (COPD)
- have a lung infection
- have liver problems or kidney problems
- have low blood pressure
- are pregnant or plan to become pregnant. It is not known if Tyvaso will harm your unborn baby. Women who can become pregnant should use effective birth control while taking Tyvaso.
- are breast-feeding or plan to breast-feed. It is not known if Tyvaso passes into your breast milk. Talk to your healthcare provider about the best way to feed your baby while taking Tyvaso.

Tell your healthcare provider about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements. Tyvaso and other medicines may affect each other.

Especially tell your healthcare provider if you take any of these medicines:

- medicines that decrease blood clotting
- water pills (diuretics)
- medicines used to treat high blood pressure or heart disease
- gemfibrozil (Lopid) (for high cholesterol)
- rifampin (Rimactane, Rifadin, Rifamate, Rifater) (for infection)

Know the medicines you take. Keep a list of them and show it to your healthcare provider and specialty pharmacist when you get a new medicine.

How should I take Tyvaso?

- Take Tyvaso each day exactly as your healthcare provider tells you.
- See the detailed Tyvaso Inhalation System Instructions for Use.
- Tyvaso is breathed in (inhaled) through your mouth into your lungs. Tyvaso should only be used with the Tyvaso Inhalation System.
- Tyvaso is taken in 4 treatment sessions each day during waking hours. The sessions should be at about 4 hours apart.
- At the beginning of each day, it will take about 5 minutes to prepare the Tyvaso Inhalation System. Each treatment session will take 2 to 3 minutes.
- Take your first Tyvaso treatment session in the morning and take your last treatment session before bedtime.
- Your healthcare provider may change your dose if needed.
- If you miss a dose of Tyvaso take it as soon as you remember.
- Do not let Tyvaso solution get into your eyes or onto your skin. If it does, rinse your skin or eyes right away with water.
- Using the Treatment Tracker, record the number of breaths you inhale during each treatment session (4 times a day). You should bring your Treatment Tracker to your medical appointments, as your doctor may want to review it with you.

What are the possible side effects of Tyvaso?

Tyvaso can cause serious side effects, including:

- Tyvaso may increase the risk of bleeding in people who take blood thinners (anticoagulants).
- If you have low blood pressure, Tyvaso may lower your blood pressure further.

Ask your healthcare provider if you are not sure if this applies to you.
The most common side effects of Tyvaso include:

- coughing
- headache
- nausea
- reddening of your face and neck (flushing)
- throat irritation and pain
- fainting or loss of consciousness

Tell your healthcare provider if you have any side effect that bothers you or that does not go away. These are not all the possible side effects of Tyvaso. For more information, ask your healthcare provider or specialty pharmacist.

Call your healthcare provider for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How should I store Tyvaso?

- Store Tyvaso ampules in the unopened foil pack between 59°F to 86°F (15°C to 30°C) until ready to use.
- When the foil pouch is opened, Tyvaso ampules should be used within 7 days.
- Tyvaso is sensitive to light. The unopened Tyvaso ampules should be stored in the foil pouch.
- After a Tyvaso ampule is opened and put into the medicine cup in the Tyvaso Inhalation System, Tyvaso can be kept in the medicine cup for no more than 1 day (24 hours).
- Tyvaso that is left in the medicine cup at the end of the day must be thrown away.
- The Tyvaso Inhalation System can be stored in the carrying case when not in use (Example: between treatment sessions or overnight). If storing between treatment sessions, ensure that the plugs are firmly in place in the dome assembly to prevent spillage of Tyvaso. See the Instructions for Use for additional information regarding storage of your Tyvaso Inhalation System.

Keep Tyvaso and all medicines out of the reach of children.

General information about the safe and effective use of Tyvaso.

Medicines are sometimes prescribed for conditions that are not mentioned in a patient information leaflet. Do not use Tyvaso for a condition for which it was not prescribed. Do not give Tyvaso to other people, even if they have the same symptoms you have. It may harm them.

This patient information leaflet summarizes the most important information about Tyvaso. You can ask your healthcare provider or specialty pharmacist for information about Tyvaso that is written for health professionals.

For more information, go to www.tyvaso.com or call 1-877-UNITHER (1-877-864-8437).

What are the ingredients in Tyvaso?

Active ingredient: treprostinil
Inactive ingredients: sodium chloride, sodium citrate, sodium hydroxide, hydrochloric acid, and water for injection.

Tyvaso is a registered trademark of United Therapeutics Corporation.

Literature issued May 2013.

United Therapeutics Corp.
Research Triangle Park, NC 27709 USA

TYVASO®
INHALATION SYSTEM
Instructions for Use

TYVASO®

Table of Contents

General Instructions

The TYVASO Inhalation System should be handled with care.

Follow these important instructions to ensure proper use:

- Always unplug the device after each use.
- Do not immerse the device in water or other liquids.
- Do not place the device in a microwave or regular oven.
- Do not place the device or use the device in the presence of strong electric or magnetic fields (e.g., microwave oven, magnetic imaging equipment).
- Do not leave the device alone with a small child.
- Do not use the device near flammable liquids and materials or heated surfaces.
- Read the instructions carefully and completely to prevent damage to your TYVASO Inhalation System and help you get the best results.
- This device should only be used on the order of your doctor or licensed healthcare practitioner.
- Ensure the breath counter is correctly programmed prior to beginning a treatment (see page 14).

Introduction

Your doctor has prescribed TYVASO® (treprostinil) Inhalation Solution. Please see the accompanying Patient Package Insert for important safety information on TYVASO.

TYVASO is breathed in (inhaled) using the TYVASO Inhalation System, which consists of the inhalation device and its accessories.

These Instructions for Use (IFU) for the TYVASO Inhalation System provide important safety information. It is important that you read these instructions and the TYVASO Patient Package Insert (PPI) before setting up and using the TYVASO Inhalation System. If you have any questions, talk to your doctor or specialty pharmacy provider.

Before beginning treatment with TYVASO, you will receive a Patient Starter Kit containing a 28-day supply of TYVASO and 2 complete inhalation devices (all accessories and supplies included). When you refill your prescription for TYVASO each month, you will receive a Refill Kit that contains a 28-day supply of TYVASO and new accessories. You will receive a replacement device every 2 years.

IMPORTANT

- **Do not start treatment with TYVASO until you have been trained to use the TYVASO Inhalation System. Make sure you understand all of the directions. Always ask your doctor or specialty pharmacy provider if you have any questions or are unsure of anything you are taught.**
- **TYVASO is for use only with the TYVASO Inhalation System.**

Preparing for Treatment With TYVASO

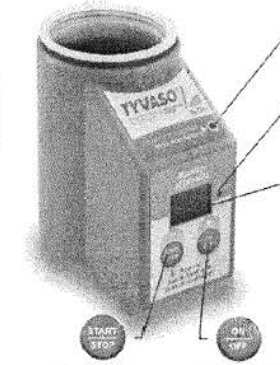
Preparing the Use Environment for Treatment

Follow these important instructions before setting up your treatment.

- Use the device in a quiet, distraction-free area.
- Try to use the device at times when your treatment will not be interrupted. If you encounter any distractions during treatment, you can pause your treatment (see page 19).
- Use the device in a comfortable space where you can stand or sit in an upright position that allows you to take deep breaths.
- Use the device in an area that provides enough space for the TYVASO Inhalation System and its accessories.
- Gather all necessary supplies before starting to prepare for treatment (see pages 6 and 7).
- The TYVASO Inhalation System is temperature-sensitive. Be sure to use and store the inhalation device in environments that match the specified temperature and humidity ranges (see Specifications on page 36).

Markings, Indicators and Symbols

Inhalation device



"START/STOP" Treatment Button - Begins or pauses treatment.

"ON/OFF" Button - Switches between fully on and fully off power states.

The device is equipped with a multicolored status light and a red separate control light for inhalation.

Control light for inhalation

- Green light on – Inhale
- Green light off – Exhale, then breathe normally

Status light

- Yellow – ready for operation
- Green – device in operation
- Red – device error indication

Device Display Messages

- 09 – Displays the number of breaths in an active cycle.
- PA – (PA) Pause - Displayed if user pauses a treatment session.
- End – (End) End - Displayed when a treatment session is complete.
- LH – (LH) Low Hydrogen (i.e., Low Water) - Displayed if device detects empty or missing cartridge (used or canceled).
- LB – (LB) Low Battery - Displayed if device battery does not have enough power to start a treatment.

Device markings

- The TYVASO Inhalation System complies with the requirements of Protection Class II, Class B equipment (provides additional precautions, over and above basic insulation, to provide protection against electric shock).
- Please read the accompanying instructions and labels for important information regarding the TYVASO Inhalation System.
- The TYVASO Inhalation System has a Type B Applied part. Type B Applied parts comply with strict requirements to provide protection against shock and are not suitable for direct cardiac applications.

! Rx Only – The TYVASO Inhalation System should only be used on the advice of your doctor or licensed healthcare provider.

12Vdc – The TYVASO Inhalation System requires a 12V DC power supply.

Gathering Supplies

Gather the following supplies before starting treatment. Use only the parts and accessories that are provided with your starter kit or in the monthly refill kit. Note: Supplies are not shown to scale.

1. Inhalation device

2. Water Level Cup with 45 mL of distilled water

Use only distilled water to fill your inhalation device once per day.

3. Pack of TYVASO ampules

Use one ampule per day.

4. One of the provided power sources

AC wall plug (2)

Rechargeable battery

12V DC adapter

5. Accessories

Medicine cups*

Drone assembly with baffle plate insert*

Inhalation piece*

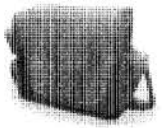
Mouthpiece*

2 Filter shells*

Filter inserts/discs (use 2 per day)*

Plugs*

Treatment Tracker*



Carrying case

Distilled water carrier (optional)

Note: Supplies are not shown to scale.

* These accessories are replaced every month. Replacement accessories are included in the Monthly Refill Kit.

Setting Up Your TYVASO Inhalation System
Filling the Inhalation Device Chamber and Medicine Cup

Before using the TYVASO Inhalation System, you should:

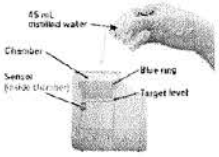
- Wash your hands

- Make sure the device is **NOT** connected to a power source
- Make sure the device is resting on a stable, flat surface during assembly

ONLY USE DISTILLED WATER in the device. Distilled water is highly purified water that can be purchased at most grocery stores and pharmacies. It is necessary for the device to function properly. If you use another type of water (such as bottled or tap water), the device may not function properly.

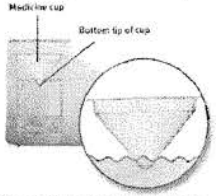
1
Fill the white chamber inside the device with approximately 45 mL of distilled water (about 15 ounces), using the water level cup provided. Fill the cup until water level is between the two arrow markings.

There is a short sensor on the inside wall of the chamber. The water level should be above the short sensor, but below the blue ring in the device chamber. DO NOT OVER-FILL the chamber or the medicine cup will not fit correctly. Check the water level after filling the chamber.



2
Obtain one medicine cup and inspect it for any damage, holes, cracks, or dents. Do not use the medicine cup if it is damaged.

Insert the empty medicine cup into the chamber of the device, in a way such that the cup's bottom tip is in the distilled water. The cup will sit on the blue ring.



⚠ CAUTION
Make sure you insert only one medicine cup. Inserting multiple cups will prevent the flow of medicine.

3
Carefully tear open the top of the foil pouch, making sure not to cut the ampules. Each pouch contains four (4) ampules.

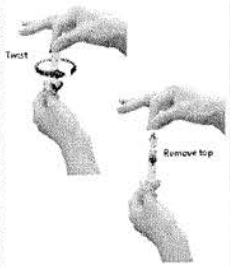
Remove only 1 ampule of TYVASO. Keep unused ampules in the foil pouch because the TYVASO medicine is sensitive to light.

One ampule contains enough medicine for one day of treatment no matter how many breaths your doctor has prescribed.

⚠ CAUTION
Ampules must be used within 7 days of opening foil pouch.

Open only 1 pouch at a time. Discard any unused ampules after 7 days.

4
Gently hold the ampule in the upright (up-right) position and twist off the top.

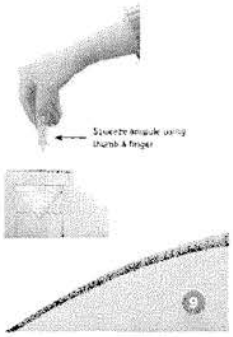


⚠ CAUTION
If any medicine from the ampule spills on your hands, wash your hands immediately.

Medicine contact with the skin can cause irritation.

5
Point the ampule straight down (vertical) into the medicine cup's center, to avoid spills.

Gently squeeze the ampule to push out all the medicine into the medicine cup. Squeeze until it is empty. Check to see that all of the medicine is in the medicine cup.



Assembling the Inhalation Device

The TYVASO Inhalation System is designed so the parts only fit together properly one way.

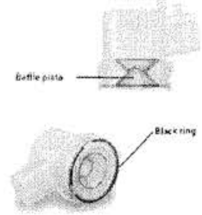
When the device is assembled correctly, the parts should fit together easily.

Do not force the parts together.

1

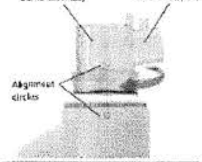
Visually check to make sure the blue plastic on the plate and black ring are correctly placed in the dome assembly. They should appear as they do in the images below.

In the unlikely event that the baffle plate is loose or disconnected, use a new dome assembly. If you need to order a new dome assembly, contact your specialty pharmacy provider.



2

Align the raised circle on the side of the dome assembly with the circle on the side of the device. Push down and screw the dome assembly onto the device clockwise (right) until you hear a click, indicating the dome assembly is fully connected to the medicine cup. When the dome assembly is properly aligned, the filter shell port will point to the back of the device.

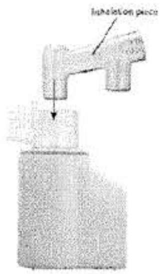


IMPORTANT

The dome assembly "clicks" only the first time it connects to the medicine cup. If you then realign the dome assembly you will not hear another click.

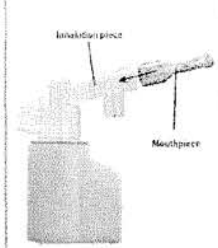
3

Insert the inhalation piece into the upper opening of the dome assembly and rotate toward the front of the device. Gently push down the inhalation piece to make sure it is securely inserted in the dome assembly.



4

Insert the mouthpiece into the inhalation piece.



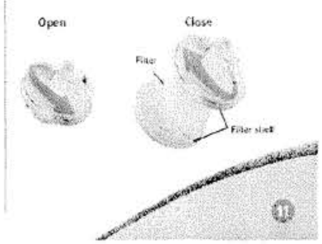
5

Each day you will need to use a new filter membrane in each filter shell.

To install a new filter membrane:

- Open the filter shell by [illegible] the two halves.
- Place a new filter membrane in one of the filter shell halves.
- Close the filter shell by screwing the two halves together until you can twist no further.

Note: New filter shells come with [illegible] already installed.

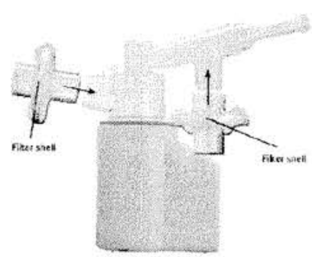


Assembling the Inhalation Device (continued)

6

Insert one of the filter shells into the filter shell port on the side of the dome assembly and insert the other filter shell into the port on the bottom of the inhalation piece. The two filter shells are identical and can be used in either opening.

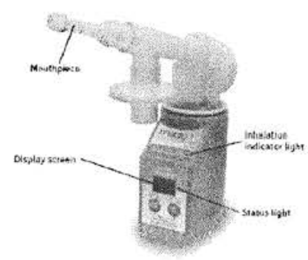
Make sure to insert the filter shells straight into their ports, rather than at an angle. If necessary, rotate the inhalation piece so you can insert the filter shell without the device getting in the way.



7

When the device is fully assembled, it should appear as it does in the photo below.

For convenience, rotate the inhalation piece so you can best see the indicator lights and display screen, which provide important prompts during your treatment.



Turning On the Inhalation Device

1

Connect your device to one of the power source options by plugging one end of the power cord into the back of the inhalation device and the other end into the power source.

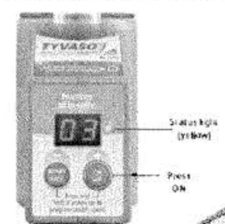
If using the rechargeable battery, make sure it is not also plugged into the AC wall plug.

2

To turn on the device, press and hold the ON/OFF button for approximately 3 seconds. When you hear a short beep and the yellow status light is illuminated, release the ON/OFF button. Remember to press the center of the ON/OFF button when powering the device on or off.

When the power is on, the display screen will show the last number of breaths programmed and a yellow light will appear next to the screen.

Make sure the number matches the prescribed number of breaths for that treatment session. If it does not match, see the next page for instructions on setting the number of breaths in a treatment session.

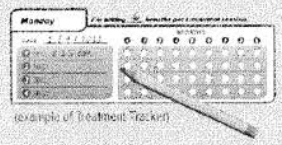


Setting the Number of Breaths in a Treatment Session

You will inhale TYVASO during 4 treatment sessions each day. During each treatment session, you will take a series of breaths through the mouthpiece of the TYVASO Inhalation System.

Your doctor will prescribe the number of breaths you should inhale in each treatment session.

You should record the number of breaths in your Treatment Tracker.



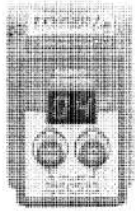
(example of Treatment Tracker)

1

Make sure the device is powered off. To power on the device, press and hold the ON/OFF button for 3 seconds. When you hear a short beep and the display screen turns on, release the ON/OFF button.

To set the number of breaths, press and hold both the START/STOP and ON/OFF buttons at the same time until the display flashes and the device emits three (3) short beeps.

The flashing display indicates the inhalation device is in the programming mode. You cannot begin a treatment while the device is flashing.

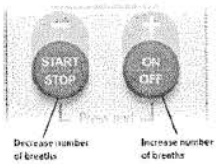


2

To increase the number of breaths press the + (ON/OFF) button.

To decrease the number of breaths press the − (START/STOP) button.

Remember to press in the center of the buttons.

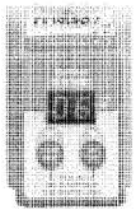


3

Once you have entered your desired number of breaths, hold the unit still for 10 seconds.

After approximately 10 seconds, the display will stop flashing and the device will emit three short beeps, indicating that it has saved the new number of breaths. The new number of breaths will appear on the display.

Make sure the number of breaths on the display matches the number of breaths in your prescription. If the numbers do not match, repeat steps 1-3.

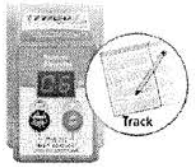


Inhaling Your Medicine, TYVASO® (treprostinil) Inhalation Solution

You will inhale TYVASO during 4 treatment sessions each day. During each treatment session, you will take 4 puffs of breaths through the mouthpiece of the TYVASO Inhalation System.

IMPORTANT

Before inhaling your medicine, check the number on the display screen to make sure it matches the prescribed number of breaths for that treatment session. Record this value in your Treatment Tracker.



If the number of breaths on the display do not match the number of breaths in your prescription go to page 14 "Setting the Number of Breaths in a Treatment Session" and repeat steps 1-3.

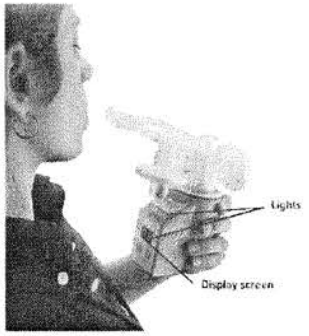
Inhalation Tips

Technique:
When breathing each TYVASO treatment, be sure to keep the device level, directing the flow of medicine into the throat and not toward the rest of the mouth.

Seal your lips around the mouthpiece to ensure that you can inhale the full amount of TYVASO after it is produced by the device.

Attention:
Each breath should last approximately 3 seconds, breathing "normal full breaths." Do not hold your breath. Exhale normally and prepare for the next breath.

1
Hold the device upright as shown below. Make sure you can see the display screen and lights clearly and that your hands or hair cover the display screen or lights while holding device. If the inhalation piece or mouthpiece blocks your view of the display screen or lights, move the inhalation piece so you can best see them.



Inhaling Your Medicine, TYVASO™ (treprostinil) Inhalation Solution (continued)

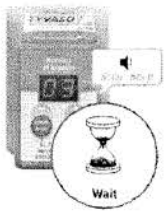
2

Perform steps A-G to complete one treatment session.
Follow the instructions exactly to make sure you receive the correct medication dose.

A

Press the START/STOP button to begin treatment.

B

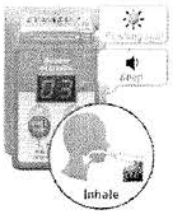


The status light turns green and the device emits two (2) short beeps.

C

When the device emits one (1) long beep, exhale completely.

D

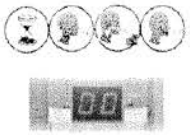


After the device emits a short beep and while the inhalation medium (light is flashing green), place your lips securely around the mouthpiece and inhale slowly for the duration of the beep (at least 3 seconds). Exhale normally, mark a circle on the Treatment Tracker, and prepare for the next breath.

⚠ CAUTION

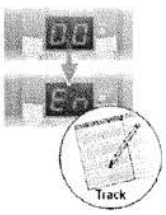
If medicine does not appear to be flowing properly, the system might be set up incorrectly. See Troubleshooting section, pages 30-35, for details.

E



Repeat steps B through D for the number of inhs (deep breaths) and until the display counts down to "00."

F



After the final breath, the display screen will show "00," then "En," indicating that treatment session is complete.

Check the number of breaths you inhaled on the Treatment Tracker.

G

To turn off the device, press and hold the ON/OFF button for 3 seconds and then release.

If the device is left in "En" mode for more than 60 seconds, it will turn off automatically.

3

Storing the TYVASO Inhalation System Between Treatment Sessions

If you have more treatment sessions left in the day, perform the following steps.

If you have completed your last treatment session of the day, skip to *Cleaning and Maintenance of the TYVASO Inhalation System* (see page 24).

Be sure to pack all parts, including a backup power supply, in the carry case whenever transporting your device.

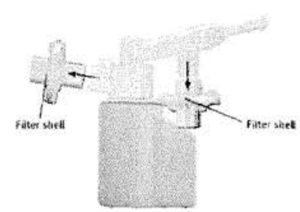
1

Disconnect the device from its power source.

2

Remove both filter shells.

Do NOT remove the filter membranes from filter shells until after the last treatment session of the day.

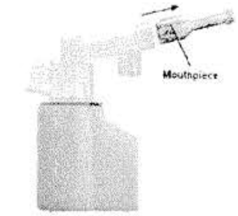


IMPORTANT

When removing accessories between treatment sessions, hold the device by its base to avoid spilling the medicine.

3

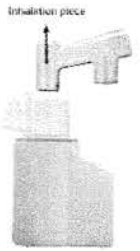
Remove the mouthpiece.



Storing the TYVASO Inhalation System Between Treatment Sessions (continued)

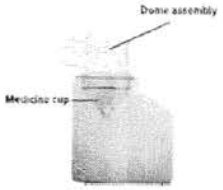
4

Remove the inhalation piece.



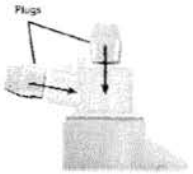
5

Leave the dome assembly and medicine cup (with the medicine still in it) connected to the device.



6

Insert 3 plugs into each of the valve holes on the dome assembly to prevent the medicine from spilling out.

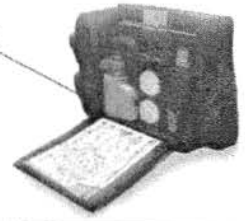


IMPORTANT

If the plugs are not in place, the medicine may spill. If you spill any medicine, start over with a new ampule.

7

The inhalation device with the plugged dome assembly and all the accessories can be stored in the carrying case between treatment sessions.



IMPORTANT

Store the inhalation device in an upright position until the next treatment session. See page 36 for additional storage and transport information.

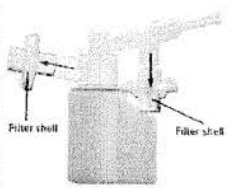
Cleaning and Maintenance of the TYVASO Inhalation System
End of Day Cleaning of the Accessories

1

Disconnect the device from the power source.

2

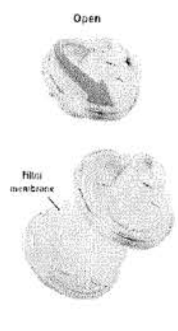
Remove both filter shells.



3

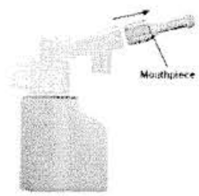
Open the filter shells by twisting in opposite directions.

Remove and discard the used filter membranes.



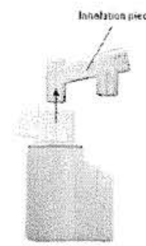
4

Remove the mouthpiece.



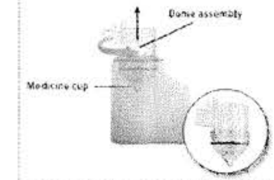
5

Remove the inhalation piece.



6

Remove the dome assembly by turning it counterclockwise (to the left). The medicine cup should stay attached to the dome assembly.



⚠ CAUTION

If any medicine from the medicine cup spills on your hands, wash your hands immediately.

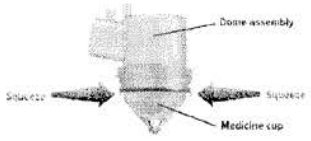
Medicine contact with the skin can cause irritation.

End of Day Cleaning of the Accessories (continued)

7

Remove the medicine cup by gently squeezing on the sides where it is attached to the dome assembly. Empty the medicine cup into a sink or waste basket, then discard the medicine cup.

Be careful not to spill the medicine when removing or discarding the medicine cup.



IMPORTANT

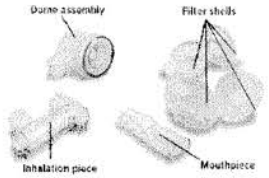
Discard the remaining TYVASO® (treprostinil) Inhalation Solution in an appropriate waste receptacle. Throw away the plastic medicine cup. Do not reuse the medicine cup. Do not recycle the medicine cup.

8

Empty the distilled water from the chamber and let the inhalation device air dry upside down. You can wipe the chamber with a soft cloth or paper towel to collect water.

9

Clean the accessories (pictured below) by using a mild, soapy, warm water, then rinse them thoroughly with water. Allow accessories to air dry.



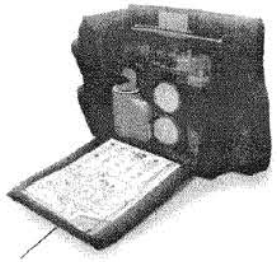
IMPORTANT

Do not place the inhalation device in water or in a dishwasher.

Do not place the inhalation device or its accessories in a microwave or conventional oven.

10

Once all the accessories (e.g., the filter shells, inhalation piece, mouthpiece, dome assembly, and inhalation device) can be stored in the carrying case until the next day's treatment sessions.



Weekly Cleaning

- Once a week, use a clean cloth to wipe the interior of the inhalation device chamber. Be sure to wipe the disc in the bottom of the device. You may wipe the exterior of the device if the lights or buttons become difficult to see. Proper cleaning will help to avoid corrosion and leaks, and keep your device working properly.

Monthly Refill Kit

- Once a month, you will receive a refill kit with a new set of accessories from your home infusion pharmacy provider. Inspect the shipment to be sure all parts are included (see page 7). Once the new kit has arrived, discard the used dome assembly, inhalation piece, mouthpiece, and 2 filter shells. Do not recycle the used accessories.

Device Replacement

- The inhalation device should be replaced every four (4) years from your first day of use. Replacement inhalation devices will be supplied by your specialty pharmacy provider.

IMPORTANT

All service of the TYVASO Inhalation System must be performed by the manufacturer. Attempting to alter the device in any way voids the warranty and may cause a malfunction.

Charging Your TYVASO Inhalation System Rechargeable Battery

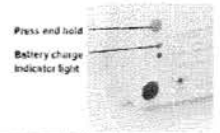
The rechargeable battery is supplied with its own instructions for use. Refer to these instructions for more information.

- Your TYVASO Inhalation System is supplied with a portable, rechargeable battery. **Do NOT** use other batteries.

- Your battery can only be charged by using one of the AC wall plugs that comes with your inhalation device.

- To check the battery's voltage level, press and hold the blue button on the front of the battery. You can only check the battery's voltage level when the battery is not plugged into a power source.

  The light below the button will turn on and indicate the battery's charge level. A red light indicates the battery needs to be charged. A green light indicates the battery is ready to operate the device.

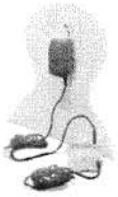


IMPORTANT

A new battery might not be fully charged when you receive it. Always charge a new battery before you first use it.

- To charge the battery, connect the AC wall plug to the battery pack and plug the AC wall plug into an outlet.

  A yellow light on the front of the battery will turn on, indicating that the battery is charging.



- Your battery may take up to 10 hours to fully charge. It is not possible to use the TYVASO Inhalation System with the battery while it is recharging.

  A fully charged battery will typically last up to 20[illegible] minutes. A battery charged for one night (about 10 hours) will typically last up to one day of therapy (about 48 inhalations).

IMPORTANT

Make sure the battery is not connected to the inhalation device while the battery is charging. The battery will charge more slowly and require a longer time to fully charge if plugged into the inhalation device.

- You should connect the charged battery to the TYVASO Inhalation System only for your treatment. When using the battery pack to power the device, make sure the battery is not plugged into a power source.

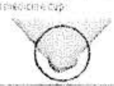
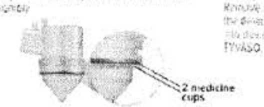
- After your treatment session is complete, remove the power plug of the battery from the device.
- Charge the battery whenever you are not using it to prevent the inhalation device.
- Always have your AC wall plug or 12V DC adapter available as backup, in case the battery is not charged.

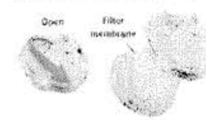
Troubleshooting the TYVASO Inhalation System

Problem	Possible causes	Corrective actions
Low battery (LB) 	• Low battery	• Unplug the battery from the device. Charge the battery by attaching it to the AC wall plug and outlet • Use an alternative power source, such as the AC wall plug or 12V DC adapter for treatment
	• Defective adapter (AC wall plug or 12V DC adapter)	• Ensure that the plug is properly connected to an outlet
	• AC wall plug is still plugged into battery and battery is connected to device	• Turn off device (see page 14) • Disconnect AC wall plug from battery • Check battery status, following instructions on page 25
		• If device is properly powered and still does not function, contact your specialty pharmacy provider for a replacement

Problem	Possible causes	Corrective actions
Low hydrogen (LH) 	• Device chamber is empty or distilled water level in the device chamber is too low 	• Unplug the device from its power source. Remove the dome assembly carefully, making sure not to spill its medicine. [illegible] distilled water and reconnect the dome assembly (see page 8)
	• The distilled water you are using [illegible]	• Empty device chamber [illegible] water up to level between the two arrow markings [illegible]
		• If device is properly powered but still does not function, contact your specialty pharmacy provider for a replacement

Troubleshooting the TYVASO Inhalation System (continued)

Problem	Possible causes	Corrective actions
No medicine comes out of the device during a treatment session	• No TYVASO® (treprostinil) Inhalation Solution in the medicine cup 	• Unplug the device from its power source. Fill medicine cup with 1 ampule of TYVASO.
	• Damaged medicine cup 	• Unplug the device from its power source. Replace the medicine cup.
	• Distilled water level in the device chamber is too high. 	• Unplug the device from its power source. Empty the device chamber then refill it with 45 mL of distilled water. 
	• Multiple medicine cups attached to the dome assembly 	• Unplug the device from its power source. Remove and dispose of all medicine cups in the device. Insert a single, new medicine cup into device chamber and fill with 1 ampule of TYVASO.

Problem	Possible causes	Corrective actions
Difficult to breathe in medicine through the mouthpiece	• Filter membrane is clogged.	• Unplug the device from its power source. Replace filter membrane (see page 11). 

Troubleshooting the TYVASO Inhalation System (continued)

Problem	Possible causes	Corrective actions
No "click" was heard when attaching the dome assembly	• No medicine cup at the chamber of the device	• Insert an empty medicine cup into the chamber of the device and fill it with one ampule of TYVASO
	• Multiple medicine cups attached to the dome assembly  	• Unplug the device from its power source. Remove and discard all medicine cups in the device. Insert a single new medicine cup into empty chamber and fill with 1 ampule of TYVASO.
	• Dome assembly is not securely in place 	• Unplug the device from its power source. Align the circle on the side of the dome assembly with the circle on the side of the device. Push down and screw the dome clockwise (right) until you hear a click, indicating the dome is securely in place connected to the medicine cup.

Problem	Possible causes	Corrective actions
Loss of power during treatment	• Device is disconnected from its power source	• Reconnect the device to a power source and press the OFF button to turn on the device. The display will show how many breaths are left in the treatment session. Press the START/STOP button to continue your treatment session.
	• Power source is temporarily disrupted (for example, electricity interrupted due to a storm)	• Reconnect the device to a power source and turn on the device (see page 18). The display will show how many breaths are left in that treatment session. Press the START/STOP button to continue your treatment session.

Specifications

Inhalation Device

Model	TD-100/A
Size	98 x 66 x 105 mm
Weight, inhalation device	280 g (9.9 ounces)
Types of power supply	AC wall plug 12V DC adapter 12V rechargeable battery
Power input	12V DC, 1.5A maximum
Operating power consumption	18 Watt maximum
Ultrasonic frequency	2.4 MHz (nominal)
Nebulization rate	0.50 - 0.55 mg/min (0.9% Saline)
Medicine cup capacity	6 mL, nominal
Compact fluid chamber capacity	45 mL, nominal
Electric protection class	II Type B
Storage temperature/humidity	-5 to 40°C/20-80% relative humidity
Operating temperature/humidity	15 to 25°C/40-75% relative humidity

Packaging Dimensions (Approximate Length x Width x Height)

Patient Starter Kit (PSK)	12.2" x 14.3" x 16.0"
Monthly Refill Kit (MRK)	9.9" x 6.1" x 16.1"

TYVASO Mass and Particle Specifications for 9 breaths

Mass Median Aerosol Diameter (MMAD)*	mean = 2.0 um SD = 0.3
Total Emitted Dose per Breath**	mean = 6.0 ug SD = 0.4
Total Aerosol Mass*	mean = 58 ug SD = 5.9
Total Respirable Dose*	mean = 44.6 ug SD = 3.5
Respirable Fraction*	mean = 73% SD = 5%
Geometric Standard Deviation (GSD)*	mean = 2.6 um SD = 0.4

* n=108 data points from n=3 inhalation devices. Each data point was 9 breaths.
** n=216 data points from n=6 inhalation devices. Each data point was 1 breath.

Accessories

ON-110H/A	Rechargeable battery
ON-100Z	12V DC adapter
ON-100N-US	AC wall plug
ON-102/TC	Medicine cup. Quantity-16
ON-108	Filter membranes
ON-120-C	Plugs
ON-101/C	Filter shell
TD-103/C	Dome assembly with baffle plate
ON-104/C	Inhalation piece
ON-105/C	Mouthpiece
TD-119	Water level cup
TD-153	Carrying case
TD-155	Distilled water carrier

Glossary

Accessories: Parts of the TYVASO Inhalation System. See pages 6 and 7.

Ampule: A sealed, lightweight clear plastic vial containing a 1-day supply of TYVASO (treprostinil) Inhalation Solution.

Control light for inhalation: A green LED on the top surface of the inhalation device that signals when you should inhale.

Baffle plate: A blue plastic piece that is inside the dome assembly. The baffle plate helps turn TYVASO into particles that are the correct size to inhale.

Black ring: A round seal that sits on the bottom of the dome externally. The seal helps ensure that TYVASO does not mix with the distilled water in the device chamber.

Display screen: A small screen on the inhalation device that displays number and letter prompts to guide you through your treatment sessions.

Distilled water: Water that is highly purified so that it contains only essential elements.

Dome assembly: The plastic accessory that contains the baffle plate and connects the mouthpiece, inhalation piece and filter shells to the base of the inhalation device.

Filter membrane: The white part that goes into the filter shells.

Filter shells: Plastic accessories that hold the filter membranes.

Inhalation piece: The plastic accessory that connects the mouthpiece with the dome assembly.

Inhalation device: The base of the TYVASO Inhalation System to which the accessories connect. The inhalation device contains the display screen and lights.

Inhale: How you will breathe in TYVASO with the TYVASO Inhalation System.

Medicine cup: The clear and plastic cone-shaped cup into which TYVASO is poured. The medicine cup fits inside the inhalation device chamber.

Mouthpiece: The plastic part that you will breathe through (using your mouth) to inhale TYVASO.

ON/OFF button: A manually activated control on the front of the device that switches between fully on and fully off power states.

Plugs: Plastic accessories that are inserted into the openings of the dome assembly between treatment sessions. Plugs help keep TYVASO from spilling if the inhalation device tips over.

Prompts: The audio and visual signals that help guide you through the treatment sessions.

Sensor: The silver object on the inside wall of the device chamber. The sensor must be covered with distilled water for the TYVASO Inhalation System to function properly.

Status light: A multicolored LED on the front of the inhalation device that indicates the device's operational status.

Start/Stop treatment button: A manually activated control on the front of the device that begins or pauses treatment.

Specialty pharmacy provider: A pharmacy that carries only specialized medicines and medical devices. Your specialty pharmacy provider is a good source of information about TYVASO and the TYVASO Inhalation System.

Treatment session: One of 4 daily sessions during which you will take TYVASO with a specific number of inhalations.

TYVASO: The prescription medicine that you will use with the TYVASO Inhalation System.

Water chamber: The white hollow portion in the center of the inhalation device into which distilled water and the medicine cup are placed.

Warranty Information

Your TYVASO Inhalation System is granted a full replacement or repair warranty good for two (2) years from your date of receipt of the TYVASO Inhalation System Starter Kit or five (5) years from the date of manufacture, whichever comes first. This warranty applies to the TYVASO Inhalation System device only. Accessory components are not covered under warranty.

Circumstances that may void your warranty include:

- Modification or disassembly of the TYVASO Inhalation System device by anyone other than a [illegible] technician
- Failure to comply with the written instructions for use that accompany the TYVASO Inhalation System
- Misapplication of the TYVASO Inhalation System

For all inquiries relating to service or warranty for your TYVASO Inhalation System, contact your specialty pharmacy provider. You should have the following information available:

- Device serial number, located on bottom of TYVASO Inhalation System
- Date TYVASO Inhalation System was activated
- Number of the lot that was [illegible]

TYVASO

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TYVASO®
INHALATION SYSTEM
Instructions for Use

TYVASO Inhalation Solution is for prescription use only.

For further questions and information, or to report an adverse reaction, please call 1-866-458-6479.

Emergency contact information

Clinician: _____

Nurse educator: _____

Specialty pharmacist: _____

United Therapeutics: _____

TYVASO®

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EXHIBIT 7

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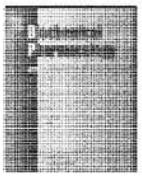

Binding and activity of the prostacyclin receptor (IP) agonists, treprostinil and iloprost, at human prostanoid receptors: Treprostinil is a potent DP_1 and EP_2 agonist

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ABSTRACT

The prostacyclin analogues, iloprost and treprostinil are extensively used in treating pulmonary hypertension. Their binding profile and corresponding biochemical cellular responses on human prostanoid receptors expressed in cell lines, have now been compared. Iloprost had high binding affinity for EP_1 and IP receptors (K_i 1.1 and 3.9 nM, respectively), low affinity for FP, EP_3 or EP_4 receptors, and very low affinity for EP_2, DP_1 or TP receptors. By contrast, treprostinil had high affinity for the DP_1, EP_2 and IP receptors (K_i 4.4, 3.6 and 32 nM, respectively), low affinity for EP_1 and EP_4 receptors and even lower affinity for EP_3, FP and TP receptors. In functional assays, iloprost had similar high activity in elevating cyclic AMP levels in cells expressing the human IP receptor and stimulating calcium influx in cells expressing EP_1 receptors (EC_{50} 0.37 and 0.3 nM, respectively) with the rank order of activity on the other receptors comparable to the binding assays. As with binding studies, treprostinil elevated cyclic AMP with a similar high potency in cells expressing DP_1, IP and EP_2 receptors (EC_{50} 0.6, 1.9 and 6.2 nM, respectively), but had low activity at the other receptors. Activation of IP, DP_1 and EP_2 receptors, as with treprostinil, can all result in vasodilatation of human pulmonary arteries. However, activation of EP_1 receptors can provoke vasoconstriction, and hence may offset the IP-receptor mediated vasodilator effects of iloprost. Treprostinil may therefore differ from iloprost in its overall beneficial pulmonary vasorelaxant profile and other pharmacological actions, especially in diseases where the IP receptor is down-regulated.

1. Introduction

The endogenous prostanoid, prostacyclin, is of substantial therapeutic benefit in the treatment of the highly debilitating disease, pulmonary hypertension [1–4]. Prostacyclin itself is however chemically unstable at physiological temperatures and pH, and rapidly decomposes to a relatively inactive breakdown product as reviewed by Whittle and colleagues [5,6]. Therefore, the early clinical use of prostacyclin, as the chemically synthesised material epoprostenol, necessitated the use of a high pH formulation and ice packs for its prolonged intravenous use. The development of chemically stable prostacyclin analogues such as iloprost, treprostinil and beraprost obviated the requirement for such a formulation [6]. These agents have been used clinically for different indications, including pulmonary hypertension, peripheral vascular disease as well as Raynaud's phenomenon and digital ulcers associated with scleroderma [7–13]. In particular, iloprost and treprostinil are currently used extensively in Europe and the US for the treatment of pulmonary arterial hypertension [14–18].

As with most other mediators, prostaglandins such as prostacyclin elicit their molecular, pharmacological and biochemical effects through binding and activation of specific receptor sites [19]. It was initially established by pharmacological techniques that there was a range of specific receptors for the naturally occurring prostanoids (see [20]) and these receptors have been subsequently cloned and expressed [19,21]. The original classification of the different prostanoid receptors [20,22,23] has remained essentially intact since the early proposals [24]. Thus, the receptors are identified as the IP, EP_1, EP_2, EP_3, EP_4, DP (now DP_1, see below), FP and TP receptor [23–25]. The IP, EP_2, EP_4 and DP_1 receptors are classically known to be G_s-coupled receptors linked to cyclic AMP (cAMP) generation, while EP_1, FP and TP receptors couple to calcium mobilisation pathways through G_q, G_i and as yet unidentified G proteins [19,25]. There are several splice variants of EP_3 which can couple negatively or positively to G_i or G_s, respectively [19].

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The natural ligand for the IP receptor is prostacyclin (PGI_2), with prostaglandin E_2 (PGE_2) for the EP receptors, $PGF_{2\alpha}$ for the FP receptors and thromboxane A_2 for the TP receptor [24]. A recent pharmacological study has suggested evidence for a second IP receptor on human airway epithelial cells that mediates the inhibition of cytokine release [26]. This is not thought to be a splice variant although its occurrence elsewhere has not been described. The original classification of the DP receptor with prostaglandin D_2 (PGD_2) as the natural ligand has now been designated as DP_1 [24]. This takes into account the more recently identified DP_2 receptor or $CRTh_2$ receptor, that while recognising PGD_2, is more closely associated with chemo-attractant molecules and has no significant homology with the other prostanoid receptors [24].

Despite their extensive clinical use over the past decade, there is relatively little direct comparative pharmacology of iloprost and treprostinil in experimental systems and models. It is generally assumed that both are potent agonists at the prostacyclin IP receptor and that such agonist activity predominantly underlies their respective responses, including their potent vasodilator effects in the pulmonary vasculature, at least under physiological conditions [27–29]. Indeed, based on this premise, novel agents that are highly selective agonists at the IP receptor such as the non-prostanoid moiety, selexipag, are being developed for clinical utilities including pulmonary hypertension [30,31]. However, the situation is more complex, since the prostacyclins appear to have functionally relevant effects at other prostanoid receptors as reviewed by Clapp and Patel [32].

Although the receptor binding profile of iloprost, including its high affinity for the IP as well as the EP_1, and EP_3 receptor, has been reported for both murine and human prostanoid receptors [21,33], there has been no reported comparable evaluation of treprostinil. Because of the multiple pathophysiological processes involved in pulmonary hypertension, there is a need to understand more about the respective pharmacology of these two extensively used prostacyclins. Thus, the current study investigates the binding profile of treprostinil on human prostanoid receptors, individually expressed in separate cell lines, and has directly compared this profile to that of iloprost in the same studies. In addition, the cellular responses of either an elevation of intracellular cyclic AMP or calcium levels as appropriate, as a consequence of activation of the individual human prostanoid receptors by either iloprost or treprostinil, have also been evaluated.

2. Methods and materials

2.1. In vitro radio-ligand binding assays

Evaluation of the affinities of treprostinil and iloprost for each prostanoid receptor was determined in radioligand binding assays using standard techniques. Cell lines, conditions and materials used are documented in Table 1 and broadly follow protocols previously described [21,34,35]. Briefly, cells from each cell line stably expressing the recombinant human prostanoid receptor were spun down at 4 °C and the cell pellet suspended in a 50 mM Tris/HCl (pH 7.4) buffer containing 5 mM EDTA, 20 mm NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 μg/ml trypsin inhibitor, 1 μg/ml leupeptin and 75 μg/ml phenylmethylsulphonyl fluoride.

Cell lysis was performed by ultra sonication (3 min at 4 °C) using a Vibro cell 72405, followed by centrifugation (Beckman Avanti J30I) of the resulting homogenate at 4 °C (50,000 × *g* for 15 min). The membrane pellet was resuspended in fresh Tris buffer containing 10% glycerol and stored as aliquots at −70 °C until used in the binding studies. Proteins levels were determined using the Bradford method and the optimised quantity of protein used in the binding studies was 16 μg for the TP receptor, 20 μg for the EP_2, EP_3, EP_4 and FP receptors, 40 μg for the IP receptor and 60 μg per sample for the EP_1 and DP_1 receptors. Incubations were carried out using nanomolar concentrations of the appropriate [^{3}H] radioligand (Table 1) in the absence or presence of various concentrations of the prostacyclin analogue (final solvent concentration was kept constant). Total binding was determined in the presence of vehicle. Non-specific binding was determined in the presence of 650–5000-fold excess of the corresponding non-labelled ligand. Following a 60–120 min incubation of ligands at room temperature (Table 1), samples were filtered rapidly under vacuum through glass fibre filters, dried, and then counted for radioactivity in a scintillation counter.

The specific ligand binding was calculated as the difference between total binding measured in the presence of radioligand alone and nonspecific binding determined in the presence of an excess of unlabelled ligand, as performed in the laboratory at Cerep (Le bois l'Evêque, France). Specific binding for ligands reached equilibrium after 30–40 min of incubation at room temperature, was stable for greater than 2 h and was determined to be saturable. Results are expressed as a percent of the control specific binding obtained.

Competition curves for each data-set were generated by non-linear regression analysis of the data (Prism 4.03; GraphPad, San Diego, USA) using a four parameter logistic (Hill) equation:

$$Y = D + \frac{(A - D)}{(1 + 10^{(X - \log IC_{50}) \times nH})} \quad (1)$$

where Y = specific binding, D = minimum specific binding, A = maximum specific binding, IC_{50} = the concentration that inhibits half of the control specific binding and nH = Hill factor. The inhibition constants (K_i) were calculated using the Cheng Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + (L/K_D)} \quad (2)$$

Table 1
Experimental conditions for prostanoid receptor radioligand binding assays. h = human; K_d = dissociation constant; RT = room temperature; HEK-293 = human embryonic kidney 293 cells; CHO = Chinese hamster ovary; 1321N1 = human glial brain astrocytoma.

Prostanoid receptor	Expression system/accession no.	Ligand	Concentration (nM)	K_d (nM)	Nonspecific (μM)	Incubation time @RT (min)
IP (h)	HEK-293/NM_000960	[^{3}H] iloprost	10	8	Iloprost (10)	60
EP_1 (h)	HEK-293/NM_000955	[^{3}H] PGE_2	1.5	1.5	PGE_2 (10)	120
EP_2 (h)	HEK-293/NM_000956	[^{3}H] PGE_2	3.0	3.0	PGE_2 (10)	120
EP_3 (h)	HEK-293/NM_198714	[^{3}H] PGE_2	0.5	0.8	PGE_2 (1)	120
EP_4 (h)	CHO/NM_000958	[^{3}H] PGE_2	0.5	0.3	PGE_2 (10)	120
DP_1 (h)	1321N1/NM_000953.1	[^{3}H] PGD_2	1.5	1.2	BW245C (1)	60
FP (h)	HEK-293/NM_000959	[^{3}H] $PGF_{2\alpha}$	2	3.8	Cloprostenol (10)	60
TP (h) (TXA_2)	HEK-293/U11271	[^{3}H] SQ 29548	5	4	U44069 (10)	60

where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor. Scatchard analysis was used to determine K_D from a plot of specific binding/free radioligand concentration *versus* specific binding giving a slope equivalent to $-1/K_D$ and are given in Table 1 (see Figure S1 of Supplementary Information for examples of Scatchard plots).

2.2. Receptor activation assays

2.2.1. Cyclic AMP assay

HEK 293 (expressing EP_2, EP_4) CHO (EP_3, IP) or 1321N1 (DP_1) cells were lifted with a non-enzymatic cell stripper and re-suspended in assay buffer at the desired cell density for each cell-line. Cyclic AMP was assayed in suspension of cells using a CisBio HTRF cAMPHiRange Kit (Cisbio US, Bedford, MA, USA) according to the manufacturer's protocol. Cells were incubated with the prostacyclin analogues for 20 min at 37 °C. The reaction was terminated by sequentially adding D2-labelled cyclic AMP and cryptate-labelled anti-cyclic AMP antibody contained in lysis buffer. The plate was incubated at room temperature for 60 min before reading of fluorescent emissions at 620 nm and 668 nm with excitation at 314 nm were made on a microplate reader (Molecular Devices, Sunnyvale, CA, USA). These experiments were performed in the laboratory at Multispan (Hayward, CA, USA). Data were converted from a cyclic AMP standard curve and expressed as cyclic AMP (nM).

2.2.2. Calcium mobilization

HEK293 cells expressing FP, TP or EP_1 receptors were seeded in 384-well plates at appropriate densities and cultured overnight. The calcium flux assay was conducted according to the manufacturer's protocol using the FLIPR Calcium 4 Assay Kit (R8142; Molecular Devices). Loading buffer, containing the calcium-sensitive dye, was added to the cells and incubated for 60 min at 37 °C. The plate was then transferred to a FlexStation® 3 benchtop multi-mode microplate reader (Molecular Devices), where compounds were automatically injected into each well. Intracellular calcium, monitored as changes in fluorescent, was recorded for 90 s with a single compound application occurring after 19 s. These experiments were performed in the laboratory at Multispan (Hayward, CA, USA). Assay results (5–10 determinations per analogue concentration) were plotted as relative fluorescence units (RFU).

2.3. Materials

Treprostinil was provided in powder form by United Therapeutics Corporation (Research Triangle Park, NC, USA). Iloprost (50:50 R/S isomer), BW245C, prostaglandin E_2 (PGE_2) and PGD_2 were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Cloprostenol, U-44069 and buffer reagents and materials were purchased from Sigma–Aldrich (Lyon, France). Treprostinil was dissolved in DMSO at a stock concentration of 10 mM and iloprost was dissolved in methylacetate at a concentration 13.9 mM. For concentration–response experiments, the highest agonist concentration used was 10 μM with serial 1:10 dilutions.

In binding assays, stable cells expressing respective human prostanoid receptors were used by Cerep (Table 1). The radioligands used in these studies (Table 1) were obtained from Perkin Elmer NEN (Courtaboeuf, Cedex 191945, France), or for iloprost, from Isobio (Fleurus, Belgium). Likewise for functional assays conducted in the laboratories of Multispan, stable cell lines expressing human receptors were: EP_1 (GenBank accession number NM_000955.2; Cat# C1201a) in HEK293T, EP_2 (GenBank Accession Number NM_000956.3; Cat# C1202) in HEK293T, EP_3 (GenBank Accession Number NM_000957; Cat# C1203-1a), in CHO-K1, EP_4 (GenBank Accession Number NM_000958; Cat# C1204) in HEK293T, FP (GenBank Accession Number NM_000959; Cat# C1205) in HEK293T, IP (GenBank Accession Number NM_000960; Cat# C1206-1) in CHO-K1, DP_1 (GenBank Accession Number NM_000953; Cat# C1200) in HEK293T and TP (TXA_2R; GenBank Accession Number NM_001060.4; Cat# C1365) in HEK293T were from Multispan.

2.4. Data analysis

In binding studies, IC_{50} values were obtained from each individual concentration–response curve for specific binding ($n = 6$) and used to determine the affinity constant, K_i.

Concentration-dependent relationships for each prostacyclin analogue stimulating elevations in either intracellular cyclic AMP or calcium (mean ± S.E.M. of n determinants per concentration as indicated) as appropriate, were constructed using a variable slope sigmoidal fitting routine in GraphPad Prism 4.03 (San Diego, CA, USA). The EC_{50} value, the concentration of agonist causing 50% of the maximal response (E_{max}), was determined from individual fits to each data-set and expressed as mean ± S.E.M. Statistical analysis was performed using GraphPad with significance assessed using a Student's t-test or ANOVA with correction for multiple comparisons. A P value <0.05 was considered significant.

3. Results

3.1. Radioligand binding data

The data obtained from the competition binding assays with the tritiated ligands in the presence of either iloprost (10^{-11} to 10^{-5} M) or treprostinil (10^{-11} to 10^{-5} M) for the eight recombinant human prostanoid receptors studied, the IP, EP_1, EP_2, EP_3, EP_4, DP_1, FP and TP receptor, are shown in Fig. 1. Both iloprost and treprostinil yielded concentration-dependent reductions in specific binding for each of the receptor types over the range of concentrations evaluated. However, neither prostacyclin analogue yielded a full specific binding curve for the TP receptor because of the high concentrations (>10 μM) that would have been required to reach full displacement of radioligand (Fig. 1). The derived affinity constant, the K_i value, for either iloprost or treprostinil at each prostanoid receptor, is given in Table 2. To aid comparison of this data to that obtained from earlier human prostanoid receptor assays, the K_i values reported for iloprost from the work of Abramovitz and colleagues [21], are also presented in Table 2.

The data from the current study shown in Table 2 indicate that iloprost has high binding affinities for the IP and EP_1 receptors, though this was significantly ($P = 0.002$) greater for the EP_1 receptor, as indicated by the lower K_i value. Its affinity for the FP, EP_3 and EP_4 receptors was some two log orders lower and was even lower for the DP_1, EP_2 and TP receptors (Table 2).

In general, the overall binding profile to the prostanoid receptors obtained in the current work with iloprost was similar to that previously reported for iloprost against human prostanoid receptors (see Table 2; data from Ref. [21]). Comparison of the K_i values in Table 2 indicates that the order of affinity for iloprost in the current work was $EP_1 > IP >> FP > EP_3 = EP_4 > DP_1 > EP_2 > TP$, while that reported previously by Abramovitz and colleagues [21] was $EP_1 = IP > EP_3 > EP_4 > FP > DP_1 > EP_2 > TP$. Thus, the main difference found between the two studies utilising iloprost was the ranking of the K_i for the FP receptor.

The prostanoid receptor binding profile for treprostinil differed from that observed with iloprost (Table 2). Treprostinil had a high and similar affinity for the DP_1 and EP_2 receptor, which was some 10-fold ($P < 0.01$, one way ANOVA) greater than that for the IP receptor. It had a much lower affinity for the EP_1 receptor, weaker

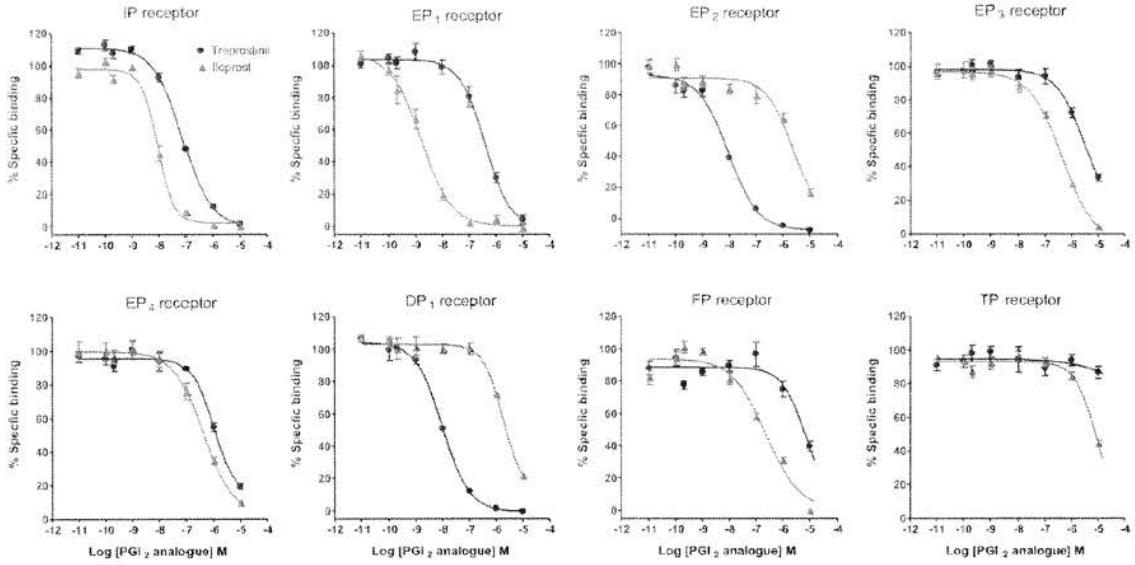

Fig. 1. Competition binding assays for different recombinant human prostanoid receptors. Receptors were stably expressed in HEK-293 (IP, EP_1, EP_2, EP_3, TP, FP), CHO (EP_4) or 1321N1 (DP_1) cell lines. The total specific and non-specific binding was determined for each [3H] ligand as per methods and equilibrium competition binding assays performed in the presence of 0.01–10,000 nM of either iloprost or treprostinil. Data are shown as mean ± S.E.M. of 6 individual determinations performed on two separate occasions. Statistical analysis using 2-way ANOVA indicated that differences in binding affinity curves existed between treprostinil and iloprost for the IP, EP_1, EP_2 EP_3 DP_1, FP ($P < 0.001$) but not EP_4 ($P = 0.08$) receptor.

affinity for the EP_4 receptor, and very weak affinity for EP_3, FP and TP receptors (Table 2). Thus, the rank order of affinity of treprostinil for the human prostanoid receptors based on the derived K_i values was $DP_1 = EP_2 > IP > EP_1 > EP_4 > EP_3 > FP > TP$.

In the current work, the K_i values at the IP receptor showed a 10-fold difference ($P < 0.001$, unpaired t-test) in affinity between iloprost and treprostinil. The major difference between the overall binding profile of iloprost and treprostinil for G_s-coupled receptors was the high affinity of treprostinil for the DP_1 and EP_2 receptor. This was reflected by the 230-fold and 325-fold lower K_i value obtained in the current study for the DP_1 and EP_2 receptor respectively with treprostinil compared with iloprost. Treprostinil had a higher K_i than iloprost at the EP_4 receptor, though overall the specific binding curves were not significantly different ($P = 0.08$, 2-way ANOVA). These binding studies also indicted that treprostinil had a 200-fold lower affinity for the EP_1 receptor than did iloprost, as well as a much lower affinity for the FP and TP receptor (Table 2).

Table 2
Prostanoid receptor binding profiles for treprostinil and iloprost. Specific binding was determined using displacement radioligand binding in cell membranes over expressing recombinant human prostanoid receptors. Values of the inhibition constant, K_i are shown as the mean ± S.E.M. of 6 individual determinations obtained on two separate occasions. The K_i for iloprost at the EP_1 receptor was significantly ($P < 0.002$) greater than that for the IP receptor, and its K_i for IP receptor was significantly ($P < 0.001$) greater than the K_i of treprostinil at this receptor. For comparison, the table also contains K_i values for iloprost obtained from historical binding data published by Abramovitz et al. for human prostanoid receptors expressed in HEK 293 (EBNA) cells [21]. NC = not calculable.

Receptor	Radioligand binding assay		Abramovitz et al. [21]
	Treprostinil K_i (nM)	Iloprost K_i (nM)	Iloprost K_i (nM)
IP	32.1 ± 0.2	3.9 ± 0.6	11 ± 1
EP_1	212 ± 56	1.1 ± 0.3	11 ± 1
EP_2	3.6 ± 0.3	1172 ± 159	1870 ± 176
EP_3	2505 ± 263	208 ± 26	56 ± 5
EP_4	826 ± 116	212 ± 27	284 ± 9
DP_1	4.4 ± 0.4	1016 ± 63	1035 ± 171
FP	4680 ± 927	131 ± 17	619 ± 159
TP	NC	3778 ± 375	6487 ± 29

3.2. Prostanoid receptor activation studies

Studies on the effect of iloprost or treprostinil over a wide concentration range (10^{-12} to 10^{-5} M) on functional responses in cells expressing each prostanoid receptor were conducted. The concentration–response curve for each prostacyclin analogue against each prostanoid receptor is shown in Fig. 2, the responses being determined, depending on the receptor under investigation, as an elevation of intracellular cyclic AMP or calcium influx (Fig. 2). Typical sigmoid curves were obtained for all but one of the prostanoid receptors with either analogue (Fig. 2). The exception was iloprost at the DP_1 receptor, which unlike in the binding study, showed an atypical sigmoidal relationship with a shallow slope, the response at 10 μM being comparable to the maximal response to treprostinil, achieved at 10 nM (Fig. 2). From the concentration–response data obtained for each prostanoid receptor, the EC_{50} was calculated and shown in Table 3.

The rank order of iloprost potency for evoking a response in cells expressing each particular prostanoid human receptor was $EP_1 = IP > EP_3 > FP > EP_4 > TP > DP_1 = EP_2$, which is broadly similar to the ranking observed in the binding studies. Thus, iloprost had high activity at both the IP and the EP_1 receptor in the expression system used and indeed had a similar EC_{50} value for activity (sub nanomolar) at either receptor. Furthermore, iloprost was 75-fold less active at the EP_3 receptor than at the IP receptor, 500–1000-fold less active at the FP and EP_4 receptor and had EC_{50} values in the micromolar range for activity at the EP_2, DP_1, and TP receptors (Table 3).

As with the radioligand binding studies, iloprost had higher activity in evoking a functional response in cells expressing the IP receptor than did treprostinil, having a 5-fold ($P < 0.01$, unpaired

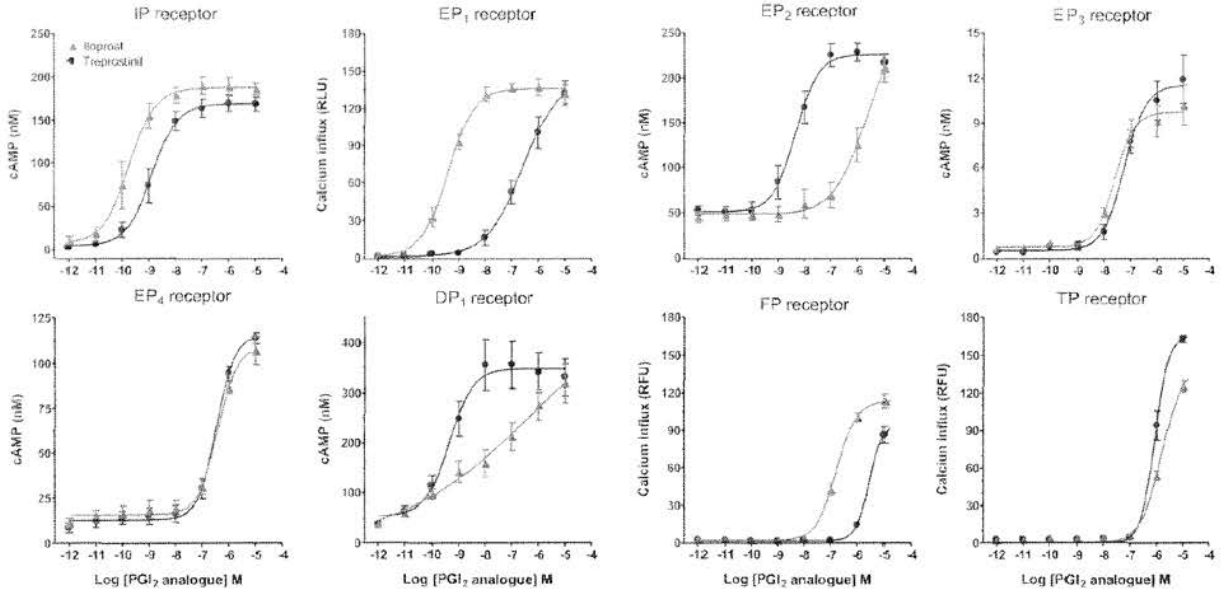

Fig. 2. Receptor activation assays in cells stably expressing human prostanoid receptors. Concentration-dependent increases in intracellular cyclic AMP (IP, EP_2, EP_3, EP_4, DP_1 receptors) or calcium (EP_1, FP, TP) were measured upon treatment with either treprostinil or iloprost (0.001–10,000 nM) for 1 h. Data are shown as mean ± S.E.M. of 5–10 determinations performed on 2–3 separate occasions. Curves have been generated from fitting data to a variable slope sigmoidal function. Statistical analysis using 2-way ANOVA indicated that differences in concentration response curves existed between treprostinil and iloprost for the IP, EP_1, EP_2 DP_1, FP, TP ($P < 0.001$) but not EP_3 and EP_4 ($P > 0.9$) receptor.

t-test) lower EC_{50} value (Table 3) and a concentration–response curve significantly shifted ($P < 0.001$, 2-way ANOVA) to the left (Fig. 2).

By contrast to the profile of iloprost, the rank order for evoking a response with treprostinil in cells expressing each separate receptor was $DP_1 \geq IP > EP_2 > EP_3 > EP_4 > EP_1 > TP > FP$, again in general agreement with the rank order for the radioligand binding studies. Thus, treprostinil had high potency in activating DP_1 and EP_2 receptors as well as the IP receptor. From comparison of the EC_{50} values, it was some 36-fold less active at the EP_3 receptor, 95-fold less active at the EP_4 and 150-fold less active at the EP_1 site than at the IP receptor. As can be seen from Table 3, treprostinil had little activity at the FP or TP receptor sites.

Table 3
Receptor activation assays in cells stably expressing human prostanoid receptors. For IP, EP_2, EP_3, EP_4 and DP_1 receptor activation assays, concentration-dependent intracellular cyclic AMP accumulation was measured upon treatment with either treprostinil or iloprost. For FP, TP and EP_1 receptor activation assays, concentration-dependent increases in intracellular calcium were measured upon prostacyclin analogue treatment. The concentration of agonist causing 50% of the maximal response, the EC_{50} value, were determined from the concentration–response curves (5–10 determinations per drug concentration performed on to 2–3 separate occasions) and shown as the mean ± S.E.M. The EC_{50} values for iloprost at the IP and EP_1 receptor were not significantly different ($P = 0.6$, unpaired *t*-test); the EC_{50} values at the DP_1 receptor for iloprost and treprostinil were significantly different ($P < 0.02$, unpaired *t*-test).

Receptor	Treprostinil EC_{50} (nM)	Iloprost EC_{50} (nM)
IP	1.9 ± 0.4	0.37 ± 0.10
EP_1	285 ± 143	0.3 ± 0.1
EP_2	6.2 ± 1.2	2094 ± 560
EP_3	68.9 ± 7	27.5 ± 0.5
EP_4	181 ± 37	389 ± 86
DP_1	0.6 ± 0.1	2059 ± 765
FP	>3500	191 ± 44
TP	919 ± 110	1417 ± 141

4. Discussion

The current study has compared the activity of two clinically used prostacyclin analogues, iloprost and treprostinil, in receptor binding assays and in biochemical functional responses using cells stably expressing individual human prostanoid receptors. The prostanoid receptors investigated were those classified as IP, EP_1, EP_2, EP_3, EP_4, DP_1, FP and TP [23,24]. Substantial differences in the profile of activity between these prostacylins have now been identified, the key findings being that unlike iloprost, treprostinil is a potent agonist at both the DP_1 and EP_2 receptor, while having little activity at the EP_1 receptor.

Previous work has reported on the binding of iloprost to these human prostanoid receptors [21], and it was reassuring that the K_i values and rank order of affinity derived from the current work is comparable. A K_i value of 11 nM for iloprost at the human IP receptor in that previous work, and 4 nM in the current study, are also similar to the K_i of 4 nM for iloprost at this receptor in another report [36]. Moreover, studies on the binding of iloprost to murine IP receptors gave a K_i value of 11 nM [33]. As described previously for both murine and human prostanoid receptors [21,33], iloprost also had high affinity for the human EP_1 receptor. Indeed, in the current work, the K_i value for the EP_1 receptor was even lower (1 nM) than for the IP receptor. Likewise, other radioligand binding studies have reported high affinity binding with iloprost for the human EP_1 receptor, with a K_i not significantly different from the natural ligand, PGE_2 [37].

Iloprost had a relatively low affinity for the human FP or EP_4 receptor, and even lower affinity for the EP_2, DP_1 or TP receptor in the current study, comparable to that found previously in radioligand binding studies on both murine and human prostanoid receptors [21,33]. In the former two studies however, iloprost did have significant affinity for the murine or human prostanoid EP_3 binding site, but this was less pronounced in the current work using the human EP_3 receptor. As the EP_3 receptor is known to exhibit a range of splice variants for both murine and human

receptors [19], this may have some bearing on differences in the K_i values obtained in these assays.

Findings on the relative affinities for the different prostanoid receptors in the binding assay were generally translated to activity in the biochemical functional assays utilised in the present work. Thus, iloprost had high activity in stimulating cyclic AMP levels in the cells expressing the human IP receptor or in stimulating calcium influx in cells expressing the EP_1 receptor; indeed the EC_{50} values for these responses were the same (~0.35 nM, Table 2). Earlier pharmacological studies using a range of isolated smooth muscle bioassay preparations also concluded that iloprost has potent activity at both the IP and EP_1 receptor [25,38,39].

In the present biochemical functional assays, iloprost also activated the human EP_3 receptor to elevate intracellular cyclic AMP levels, although the EC_{50} value was some 75-fold higher than that required to activate the response in cells expressing the IP receptor. Iloprost was less active on the cells expressing the FP or EP_4 receptor, and very much less active in eliciting a response in cells expressing the TP, EP_2 or DP_1 receptors. Earlier work in cells expressing either the human EP_2 or EP_4 receptor has also shown iloprost be a very weak agonist in terms of its ability to elevate cyclic AMP in such cells [40]. Recent studies in HEK-293 cells over-expressing EP_2 receptors also showed iloprost failing to elevate intracellular cyclic AMP [26]. However, iloprost had some activity in cells over-expressing the EP_4 receptor, and partial agonist activity in cells over-expressing the DP_1 receptor, with Wilson and colleagues [26] concluding that the latter receptor may be activated at high concentrations of iloprost. In the current study and in all previous work, iloprost likewise had very low activity on the DP_1 receptor expressed in a number of different cell systems including human platelets and COS-M6 cells [21,41,42].

In the present work, treprostinil exhibited a very different profile in the radioligand binding assays for the human prostanoid receptors when compared to iloprost. Thus, unlike iloprost, treprostinil had a high affinity for both EP_2 and DP_1 receptors in the binding assay, which was surprisingly, some 10-fold greater than that for the IP receptor. On the other hand, treprostinil had a 200-fold lower affinity for the EP_1 receptor compared with iloprost, and the affinity for the EP_3 and FP receptors was in the low to mid micromolar range as opposed to the nanomolar range for iloprost. Affinity for the EP_4 receptor was low for treprostinil and iloprost, and both had minimal affinity for the human TP receptor.

The rank order of activity of treprostinil in evoking changes in either cyclic AMP or intracellular calcium levels in the cells expressing the individual human prostanoid receptors was comparable to that found in the radioligand binding assays. Thus, treprostinil elevated cyclic AMP with a similar high potency in cells expressing either the IP or DP_1 receptor, and its activity on cells with the EP_2 receptor was also high. Other work assessing prostanoid receptor antagonists in murine alveolar macrophages has suggested that treprostinil acts on EP_2 receptors to inhibit phagocytosis and cytokine release [43]. In the current work, treprostinil was less active on cells expressing the human EP_3 or the EP_4 receptor, and poorly active on the EP_1 receptor, with very low activity on the TP and FP receptors.

As with the binding studies, the high activity of iloprost at the EP_1 receptor site along with the finding that treprostinil had high affinity and potent activity at the DP_1 and EP_2 sites, are the key differences in the profiles of these two prostacyclin analogues. Interestingly, from a phylogenic perspective, the EP_2, DP_1 and IP receptor are the most highly related receptors within one of two subgroups of prostanoid receptors [41,44]. Such potent activity of treprostinil at the DP_1 receptor provides a novel aspect to interpreting pharmacological activity of this prostacyclin analogue, as activation of the DP_1 receptor will lead to both vasodilatation and inhibition of human platelet aggregation, as does IP receptor activation [45,46].

In terms of pharmacological responses that could underlie the therapeutic benefit of these prostacyclin analogues in the clinical treatment of pulmonary hypertension, studies on human pulmonary vascular tissue are clearly important. It is known from studies utilising pharmacological agonists and antagonists that the prostanoid receptors involved in the relaxation of human pulmonary venous preparations *in vitro* are the DP_1 and IP receptors, and to a lesser extent the EP_4 receptor [47,48]. In human pulmonary artery preparations however, the IP receptor appears to be the predominant receptor involved in relaxation [47]. Additional studies have indicated that the prostanoid receptors involved in the contraction of human isolated pulmonary veins were the EP_1 and TP receptor [49]. Indeed, EP_1 receptors are expressed in human pulmonary veins, as demonstrated by immunohistochemistry [48]. Earlier pharmacological work had also suggested that EP_3 receptor agonists had potent contractile activity on the human isolated pulmonary artery [50].

It is not yet known whether the high affinity and potency of iloprost for the EP_1 receptor will lead to vasoconstriction and oppose the vasodilatation evoked through IP receptor activation in arteries or veins. This will depend on factors such as the relative density and distribution of the EP_1 and IP receptor in these tissues, especially human pulmonary vasculature. There is however, some evidence that activation of the EP_3 receptor, which like EP_1 receptor activation elicits vasoconstriction, can offset the vasodilator response to IP receptor activation by iloprost in rat small pulmonary arteries *in vitro* [51]. In other studies, EP_3 or EP_1 receptor activation has been suggested to limit the relaxant activity of prostacyclin analogues in guinea-pig aorta [52] or rabbit iliac artery [53]. Moreover, the vasorelaxant actions of both iloprost and treprostinil in rat tail artery was enhanced to a small but significant degree by an antagonist at the EP_3 receptor, suggesting a functional antagonism with IP receptors in this tissue [54].

Apart from the potential opposing functional interactions between the vasodilator and vasoconstrictor response following prostanoid receptor activation, there is the possibility of additive or synergistic effects through simultaneous activation of the different G_s-coupled prostanoid receptors, which theoretically could enhance the therapeutic efficacy of the prostacyclins. Iloprost has relatively poor affinity for the EP_4 receptor that can evoke vasodilatation in human vascular tissue [48,55], and even less affinity for the DP_1 and EP_2 receptors, that along with the IP receptor, are primarily involved in the pulmonary vasodilator response to prostanoids [56]. Therefore, additive or synergistic effects of iloprost at prostanoid receptors evoking vasodilatation, is unlikely. In contrast, the high affinity and activity of treprostinil at the human DP_1 and EP_2 receptors in addition to the IP receptor could synergise to potently evoke a vasodilator response, while the minimal activity of treprostinil at EP_1 receptors would not be expected to produce an opposing vasoconstriction. This profile suggests that treprostinil could have a comparatively preferential vasodilator profile in vascular tissue, particularly in the human pulmonary circulation.

The difference in the pharmacological profile between iloprost and treprostinil in some models may hence reflect activity at multiple prostanoid receptor sites. Thus in human pulmonary arterial smooth muscle cells, treprostinil evoked a full dose-dependent elevation of intracellular cyclic AMP, whereas iloprost was less potent and reached a far lower maximal response [57]. Whether this reflected (a) activation by treprostinil of multiple prostanoid receptors coupled to G_s compared with iloprost (b) that iloprost was only a partial agonist at these sites, (c) that the

response to iloprost at the IP receptor was limited by concurrent EP_1 and EP_3 receptor activation or (d) a combination of the above, is not known.

The disparity of the profile between iloprost and treprostinil at the various prostanoid receptors will have importance when determining the overall pharmacological events that they initiate, especially when used to treat disease. This could also contribute to any differences in the degree of side-effects of these prostacyclins in clinical use, including those exerted on the gastro-intestinal tract. Under physiological conditions, both analogues are potent agonists at the IP receptor, which may dominate the nature of the overall pharmacological responses in vascular tissue. However, it has been demonstrated clearly in two studies using human pulmonary tissue, that in idiopathic pulmonary arterial hypertension, the expression of the IP receptor is down-regulated when compared to control tissue, as detected by both immunoblotting and immunohistochemical techniques [29,58]. Under such conditions of low IP receptor density or stimulus-coupling activity, the pharmacological responses of either iloprost or treprostinil through IP receptors could potentially be compromised. Indeed, in a rat model of pulmonary hypertension where almost complete down-regulation of the IP receptor was observed, it was suggested that iloprost may act through another vasodilator receptor, the EP_4 receptor, as this was not similarly down-regulated [58]. The expression of the EP_4 receptor has been detected in human pulmonary vein using immunohistochemical techniques [48]. However, the relatively poor affinity and activity of iloprost at the human EP_4 receptor suggests that activation of this receptor is unlikely to occur in the therapeutic dosing range of iloprost, the upper plasma concentrations achieved with intravenous administration in humans for example, being less than 1 nM [59].

Should expression of IP receptors be sufficiently down-regulated in pulmonary vascular disease to reduce efficacy at the IP receptor, treprostinil could have the capacity to act on the other key vasodilator prostanoid receptors in the lung, namely the DP_1 receptor and the EP_2 receptor. As treprostinil has high affinity and activity at these latter prostanoid receptors, such positive interactions should be achieved within the same clinical dose range that affects IP receptors, with plasma concentrations of treprostinil in patients treated by intravenous or subcutaneous routes ranging from 2.5 to 25 nM [60]. This would require that unlike the IP receptor, the DP_1 and EP_2 prostanoid receptors were not similarly down-regulated in human pulmonary vascular disease. Interestingly, EP_2 receptor expression in pulmonary arterial smooth muscle cells did not appear to be affected by monocrotaline treatment that produced experimental pulmonary hypertension in rats [58], though its effects on DP_1 expression were not monitored.

The importance of the differential prostanoid receptor agonist profile of iloprost and treprostinil will therefore become clearer with further knowledge of the pathology of this disease, particularly as regards to changes in IP and other prostanoid receptor expression or desensitisation and their coupled functional activity in the pulmonary vasculature. Moreover, consideration of pharmacological actions other than the vasoactive properties of the prostacyclins is warranted. Thus, the degree of involvement of IP receptor or other receptor activation in the processes limiting the characteristic exaggerated vascular smooth muscle proliferation in pulmonary hypertension requires careful evaluation [28,29]. All such information may guide the eventual selection, based on its pharmacological profile, of a particular prostacyclin analogue or IP agonist for the various aetiologies that comprise the spectrum seen in pulmonary hypertensive patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bcp.2012.03.012.

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