Pediatr Cardiol (2010) 31:598–606
DOI 10.1007/s00246-010-9645-5

ORIGINAL ARTICLE

Vasodilator Testing with Nitric Oxide and/or Oxygen in Pediatric Pulmonary Hypertension

Robyn J. Barst · Gabriella Agnoletti · Alain Fraisse · James Baldassarre · David L. Wessel · For the NO Diagnostic Study Group

Received: 14 September 2009 / Accepted: 19 January 2010 / Published online: 20 April 2010

Abstract The objective of this study was to determine whether a combination of inhaled nitric oxide (iNO) and O_2 is more effective than 100% O_2 or iNO alone for acute vasodilator testing in children. An open, prospective, randomized, controlled trial was conducted at 16 centers. Subjects were children 4 weeks to 18 years of age with pulmonary hypertension (PH) and increased pulmonary vascular resistance (PVR) undergoing right heart catheterization for acute vasodilator testing. All patients were tested with each of three agents (80 ppm iNO, 100% O_2, combination of 80 ppm iNO/100% O_2) in three 10-min treatment periods, and hemodynamic measurements obtained. Primary outcome measures were percentages of acute responders with O_2 alone vs. iNO/O_2 and iNO alone vs. iNO/O_2. More patients on the combination were acute responders compared with O_2 or iNO alone (26% vs. 14%, $P = 0.019$, and 27% vs. 24%, $P = 0.602$, respectively). Changes in PVR index and mean pulmonary arterial pressure vs. baseline were greater with iNO/O_2 vs. either O_2 or iNO alone ($P < 0.001$). Survival at 1-year follow-up included (1) 90.9% of acute responders to the combination, compared with 77.8% of nonresponders to the combination, and (2) 85.7% of acute responders to O_2 alone, compared with 80.6% of nonresponders to O_2. Key conclusions are as follows. In children with PH and increased PVR, more acute responders were identified with the iNO/O_2 combination vs. O_2 alone. While there was no significant difference in acute responder rate with iNO alone vs. iNO/O_2, the combination improved pulmonary hemodynamics acutely better than iNO alone. One-year survival data show similar rates between the iNO/O_2 and the O_2 alone groups; however, the combination may be more effective than O_2 alone in discriminating survivors versus nonsurvivors at long-term follow-up.

Keywords (MeSH headings) Nitric oxide · Diagnostic techniques · Cardiovascular · Hypertension · Pulmonary

Clinical Trials Identifier: NCT00626028. Institutions where work was completed are as follows. ***France*:** Hôpital Necker Enfants Malades, Paris; CHU la Timone–Hôpital d'Enfants, Marseille; Hôpital d'Enfants, Nancy. ***Spain*:** Hospital Materno-Infantil XII de Octubre, Madrid; Hospital Gregorio Maranon, Madrid; Hospital Sant Joan de Deu, Barcelona; Hospital Infantil Vall d'Hebron, Barcelona. ***The Netherlands*:** Beatrix Children's Hospital, University Hospital, Groningen. ***United Kingdom*:** The Royal Brompton Hospital, London. ***United States*:** Columbia University, New York; The Children's Hospital, Denver, CO; Washington University, St. Louis, MO; Children's Hospital Medical University of South Carolina, Charleston; Children's Hospital—Stanford, Palo Alto, CA; Ohio State University Medical Center & Columbus Children's Hospital, Columbus; Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

R. J. Barst (✉)
Division of Pediatric Cardiology, Columbia University, 31 Murray Hill Road, Scarsdale, New York, NY 10583, USA
e-mail: robyn.barst@barstconsulting.com

G. Agnoletti
Service de Cardiologie Pediatrique Groupe, Hôpital Necker Enfants Malades, Paris, France

A. Fraisse
CHU la Timone-Hôpital d'Enfants, Marseille, France

J. Baldassarre
INO Therapeutics/Ikaria, 6 Route 173, Clinton, NJ 08809, USA

D. L. Wessel
Children's National Medical Center, Washington, DC, USA

Pulmonary hypertension (PH) is defined by a mean pulmonary artery pressure (PAPm) of ≥25 mm Hg at rest. When the PH is associated with increased pulmonary vascular resistance (PVR), the PH is, by consensus, pulmonary arterial hypertension (PAH) and is defined by (1) PAPm ≥25 mm Hg at rest, (2) pulmonary capillary wedge pressure (PCWP) ≤15 mm Hg, and (3) PVR index (PVRI) >3 U · m^2 [2, 20]. For PAH patients, i.e., PH patients with increased PVR, an acute response with acute vasodilator testing is useful in predicting efficacy with chronic calcium channel blockade in medically treated patients [5, 6, 18, 21–23] and in predicting prognosis following surgical interventions (e.g., congenital heart surgery and/or heart or heart–lung transplantation) [3, 11, 16]. True PAH acute responders, i.e., idiopathic PAH acute responders treated with calcium channel blockers, have an excellent prognosis, with a 95% 5-year survival rate [23, 25].

Oxygen (O_2) is a pulmonary vasodilator and systemic vasoconstrictor. Previously, 100% O_2 was considered standard for acute vasodilator testing, especially in pediatric PH patients with unrepaired congenital heart disease (CHD), to determine surgical operability [13, 19]. However, some patients with a reactive pulmonary vasculature fail to respond to acute vasodilator testing with O_2 alone [1, 10]. In PAH, inhaled nitric oxide (iNO) can acutely decrease PAPm and PVRI selectively, with minimal systemic effects [8, 12, 15, 17]; iNO has a short half-life, rapidly binding to hemoglobin to form methemoglobin. In a pilot study, iNO in combination with O_2 (iNO/O_2) appeared to decrease PVRI and PAPm (more than either agent alone) in pediatric PH patients [1].

The objective of this study was to determine if the iNO/O_2 combination is better for acute vasodilator testing than either 100% O_2 alone or iNO alone. Furthermore, we were interested in evaluating whether these data would be useful in predicting long-term outcomes in children with PH and increased PVR. The study design included prospectively following all patients for up to 3 years after acute vasodilator testing to address this question.

Materials and Methods

Study Participants

Consecutive children undergoing cardiac catheterization were prospectively enrolled at 16 centers in the United States and Europe (September 2004–October 2006). The study was conducted according to ICH Good Clinical Practice; prior to study initiation, the study was approved both by national health authorities of individual countries and by local ethics committees. Inclusion criteria included an age of 4 weeks to 18 years and a diagnosis of PH with PVRI >3 U · m^2. Patients were studied under general anesthesia or with conscious sedation, according to the practice of the investigator. Exclusion criteria included PCWP >20 mm Hg, focal infiltrates on chest X-ray, history of intrinsic lung disease, and treatment with a phosphodiesterase inhibitor, prostacyclin analogue, or sodium nitroprusside. Written informed consent (and assent as appropriate) was obtained from all parents and/or patients.

Interventions

Patients were studied during five periods: Baseline 1, Treatment 1, Treatment 2, Baseline 2, and Treatment 3. All patients were to receive three study treatments, with treatment sequence randomized by center in blocks of four; these sequences were generated centrally and provided to the investigators in sealed envelopes for each patient individually. Treatment was not blinded. In Treatment 1, patients received either 80 ppm iNO alone or 100% O_2 alone, and in Treatment 3, they received the alternative. All patients received the 80 ppm iNO/100% O_2 combination in Treatment 2. A 10-min washout period occurred after Treatment 2, prior to the second set of baseline measurements. No washout was necessary after Treatment 1, as all patients received 80 ppm iNO/100% O_2 in Treatment 2.

Patients under general anesthesia were intubated and received NO for inhalation via an INOvent delivery system through a t-connector downstream of the inspiratory limb of a mechanical ventilator. Patients under conscious sedation received NO for inhalation via an INOvent delivery system through a properly fitted, sealed face mask. A total of 52% of patients received study drug via endotracheal tube, while the remaining 48% did so via face mask.

Once the sequence was assigned, treatment was unblinded. Each treatment was for 10 min prior to obtaining hemodynamics, and Baseline 2 was ≥10 min. Serious adverse events were recorded until hospital discharge or for 24 h, whichever occurred first. Cardiac index was obtained by thermodilution (if no shunts) or by Fick (with shunts). For Fick, O_2 consumption was determined by each investigator according to local practice. INO/Ikaria Therapeutics (Clinton, NJ, USA) provided iNO study drug and delivery devices.

Outcome Measures

The primary outcome measures were the acute response rate with O_2 alone vs. the iNO/O_2 combination and the acute response rate with iNO alone vs. the iNO/O_2 combination. Patients were classified as "acute responders" as follows [5, 6, 18, 21, 22, 25].

1. Patients without an unrestricted shunt at the ventricular or ductal level: PAPm decrease ≥20% *and* no cardiac index decrease (within 5%).
2. Patients with an unrestricted shunt at the ventricular or ductal level: PAPm decrease ≥20% *and* no cardiac index decrease (within 5%) *or* PVRI decrease ≥25% *and* no cardiac index decrease (within 5%).

Secondary outcome measures included changes vs. baseline in PVRI, PAPm, cardiac index, and vital signs.

Sample Size

A sample size of 100 patients (with ≥25 CHD patients and ≥25 idiopathic PAH patients) was calculated at 80% power to detect a 24% response rate difference between iNO/O_2 and O_2 alone, with a type 1 error of 5%.

Statistical Methods

All analyses were performed at INO Therapeutics by Instat LLC (Mendham, NJ, USA) using SAS Proprietary Software Release 9.1 (SAS Institute Inc., Cary, NC, USA). There was no imputation of missing data. For demographics and efficacy tabulation statistics, patients with missing data were not included in the denominator for the frequency percentage calculations. Response rate differences were tested using the McNemar test for paired proportions. Since all patients were to receive all three treatments, a random effects ANCOVA was used to test continuous variables, with patient and baseline value in the model. Carryover effects were not tested.

Intent-to-treat patients were all patients randomized regardless of actual receipt of treatment, treatment received, or appropriateness of enrollment after baseline measurements. Efficacy analyses were also performed on the per-protocol population. The per-protocol population included all patients who took study medication and met all enrollment criteria.

Results

Patient Disposition

One hundred thirty-six patients were randomized and 124 received at least one treatment; of these, 78 met all enrollment criteria. One hundred nine patients had data collected with both iNO/O_2 and with O_2 alone, 108 had data collected with both iNO/O_2 and with iNO alone, and 106 had data collected with both iNO alone and with O_2 alone (Table 1, Fig. 1). Forty-three percent of randomized patients had a baseline PVRI ≤3 U · m^2 (due to PVRI calculated after study completion), i.e., did not meet all inclusion criteria. All patients were included in the intent-to-treat population (Fig. 1).

Table 1 Baseline demographics

Parameter	Value
Age, yr	$N = 136$
Mean ± SD	6 ± 6
Min, max	0.1, 18.7
Weight (kg)	$N = 136$
Mean ± SD	20 (17)
Min, max	2.7, 70.0
Gender, *n*	$N = 136$
Male	68 (50%)
Female	68 (50%)
Race, *n*	$N = 136$
White	81 (60%)
Black	55 (40%)
Diagnosis, *n*	
Idiopathic PAH	30 (22%)
Cardiomyopathy	6 (4%)
CHD	100 (74%)
Shunt	75 (75%)
No shunt	25 (25%)
PVRI (mean baseline)	10.4 U · m^2
PAPm (mean baseline)	45 mm Hg
Intubated for procedure, *n*	
Yes	65 (48%)
No	61 (45%)
Unknown	10 (7%)
Supplemental oxygen at baseline, *n*	
Yes	30 (22%)
No	106 (78%)
Cardiac output method, *n*	
Fick	103 (76%)
Thermodilution	29 (21%)
Unknown	4 (3%)

Note: *CHD* congenital heart disease, *PAH* pulmonary arterial hypertension, *PAPm* mean pulmonary artery pressure, *PVRI* pulmonary vascular resistance index

Baseline Characteristics

Patients ranged in age from 4 weeks to 18 years. The predominant associated condition was CHD (100 patients; 74%); of the 100 patients, 75 (75%) had an unrestricted shunt at the ventricular or ductal level and 6 (6%) also had pulmonary venous hypertension with PCWP >15 mm Hg). Baseline PVRI was well matched across the three treatments (mean ± SD U · m^2) with O_2 (10.0 ± 9.7), NO and O_2 (10.8 ± 10.3), and NO (10.3 ± 10.3). Across patients,

Fig. 1 Patient disposition

baseline PVRI was not significantly different between Baseline 1 and Baseline 2. In the intent-to-treat population, the mean baseline PVRI was 10.3 vs. 14.2 U · m^2 in the per-protocol population.

Efficacy

Due to the crossover design of the study, we were able to compare the percentage of acute responders in each of the three treatment groups (Fig. 2). Of the 109 patients tested

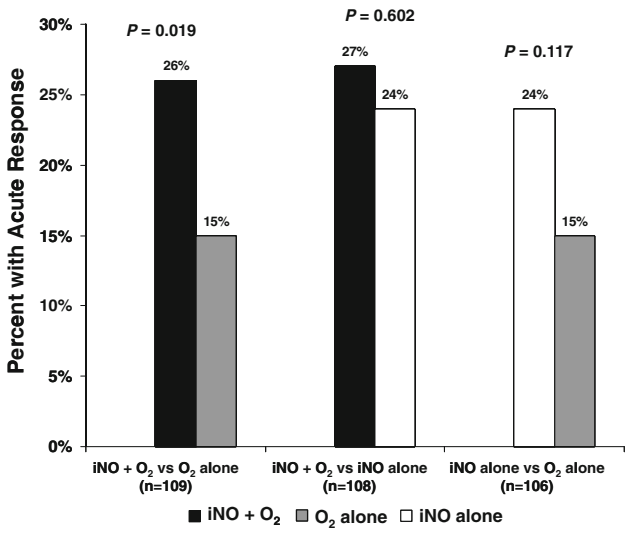

Fig. 2 Acute responders to the evaluated treatments

both with O_2 alone and with iNO/O_2, 16 (14%) and 28 (26%) patients, respectively, were acute responders (Table 2a; $P = 0.019$ McNemar test). Of the 108 patients tested both with iNO alone and with iNO/O_2, 26 (24%) and 29 (27%) patients, respectively, were acute responders (Table 2b; $P = 0.602$). Of the 106 patients tested both with O_2 alone and with iNO alone, 16 (15%) and 25 (24%) patients, respectively, were acute responders (Table 2c; $P = 0.117$). The results showed no differences between (1) the intent-to-treat and the per-protocol populations, (2) patients with and those without shunts, or (3) patients who were intubated and those who were not. Seven patients met the criteria for acute response to O_2 alone but not to the combination. These patients either had a drop in cardiac index of >5% or had a significant difference between the first and the second baseline such that the change in PAPm or PVRI in the final treatment period did not meet the criteria.

All three treatments decreased PVRI and PAPm vs. baseline (Table 3). The iNO/O_2 combination decreased PVRI and PAPm more than either O_2 or iNO alone ($P < 0.001$). Similar changes were seen in the per-protocol population and in patients with vs. without shunts. Across all treatments, the idiopathic PAH patients and the PH patients with pulmonary venous hypertension, i.e., PCWP >15 mm Hg, were more likely to respond to acute vasodilator testing than associated PAH patients with CHD ($P = 0.034$). There were no significant changes in systemic hemodynamic parameters (Table 3).

One-year follow-up data were available for 85 of the 136 randomized patients, of whom 70 were alive and 15 had died (Table 4). In the primary end-point cohort of 109 patients, data were available for 76 patients at 1 year. Two of the 22 acute responders to the iNO/O_2 combination (9.1%) and 2 of the 14 acute responders to O_2 alone (14.3%) had died. The survival rate of the acute responders to the iNO/O_2 combination appeared to be higher than that of either O_2 alone nonresponders or iNO/O_2 combination nonresponders (90.9% vs. 80.6% and 77.8%, respectively). The survival rate of iNO/O_2 acute responders also appeared to be higher than that of O_2 alone acute responders. These data support the hypothesis that the 1-year prognosis for acute responders to the combination of iNO/O_2 (90.9%) is comparable to that for acute responders to O_2 alone (85.7%). Whether survival at 2 or 3 years remains similar will require further study.

Adverse Events

Of the 124 patients who received at least one treatment, 7 reported a serious adverse event; 4 were considered treatment related by the investigator. Serious adverse events were reported for four of the seven patients during the study, with two of the patients stopping study treatment due to the

Table 2 Outcomes: Intention-to-treat population

	Treatment with combination iNO and O_2 ($n = 109$)		
(A) Treatment with combination iNO and O_2 vs. O_2 alone[a]			
Treatment with O_2	Nonresponder	Responder	Total
Nonresponder	74 (68%)	19 (18%)	93
Responder	7 (6%)	9 (8%)	16
Total	81	28	109
	Treatment with combination iNO and O_2 ($n = 108$)		
(B) Treatment with combination iNO and O_2 vs. iNO alone[b]			
Treatment with iNO	Nonresponder	Responder	Total
Nonresponder	64 (59%)	18 (17%)	82
Responder	15 (14%)	11 (10%)	26
Total	79	29	108
	Treatment with iNO ($n = 106$)		
(C) Treatment with iNO alone vs. O_2 alone[c]			
Treatment with O_2	Nonresponder	Responder	Total
Nonresponder	69 (65%)	21 (20%)	90
Responder	12 (11%)	4 (4%)	16
Total	81	25	106

Note: iNO, inhaled nitric oxide

[a] $P = 0.019$, McNemar test

[b] $P = 0.602$, McNemar test

[c] $P = 0.117$, McNemar test

Table 3 Summary of hemodynamic changes (intention-to-treat population)

	Treatment		
	iNO and O_2	O_2 alone	iNO alone
Cardiac output, l/min (SD) Baseline	2.26 (1.43)	2.23 (1.37)	2.33 (1.35)
Δ from baseline	−0.03 (1.01)	−0.03 (0.70)	0.03 (0.88)
% Δ from baseline	−1.3%	−1.3%	1.3%
PVRI, $U \cdot m^2$ (SD)	($n = 117$)	($n = 113$)	($n = 113$)
Baseline	10.8 (10.30)	10.0 (9.65)	10.3 (10.33)
Posttreatment	7.8 (8.75)	8.5 (8.63)	9.2 (10.45)
Δ from baseline	−2.9 (4.75)	−1.5 (3.13)	−1.1 (3.04)
% Δ from baseline	−29.6%	−15.2%	−15.9%
SVRI, $U \cdot m^2$ (SD) Baseline	17.2 (8.86)	17.6 (9.22)	18.0 (8.44)
Posttreatment	18.7 (9.04)	18.9 (8.78)	17.8 (9.40)
Δ from baseline	1.4 (5.94)	1.3 (5.16)	−0.2 (4.65)
% Δ from baseline	8.1%	7.4%	−1.1%
PVRI/SVRI ratio Baseline	0.628	0.568	0.572
Posttreatment	0.417	0.450	0.517
% Δ from baseline	−32.6%	−20.8%	−9.7%
	($n = 124$)	($n = 121$)	($n = 120$)
PAPm, mm Hg (SD) Baseline	45.3 (16.78)	44.2 (16.30)	45.0 (17.57)
Posttreatment	38.3 (16.38)	40.7 (14.57)	41.0 (17.94)
Δ from baseline	−7.1	−3.5	−4.1

Note: *iNO* inhaled nitric oxide, *PAPm* mean pulmonary artery pressure, *PVRI* pulmonary vascular resistance index, *SVRI* systemic vascular resistance index. Pairwise comparisons (change in PVRI): iNO + O_2 vs. O_2, $P < 0.001$; iNO + O_2 vs. iNO, $P < 0.001$; O_2 vs. iNO, $P = 0.171$. Pairwise comparisons (change in PAPm): NO + O_2 vs. O_2, $P < 0.001$; NO + O_2 vs. iNO, $P < 0.001$; O_2 vs. iNO, $P = 0.637$

Table 4 One-year survival rates for children with PH and increased PVR according to AVT responses

	Subjects with 1-yr follow-up information ($n = 76$)			
	O_2 responder	O_2 nonresponder	iNO + O_2 responder	iNO + O_2 nonresponder
N	14	62	22	54
Died	2	12	2	12
Alive	12	50	20	42
% survival	85.7	80.6	90.9	77.8

Note: *AVT* acute vasodilator testing, *iNO* inhaled nitric oxide, *PH* pulmonary hypertension, *PVR* pulmonary vascular resistance

Table 5 Serious adverse events

Baseline diagnosis	Baseline PCWPm (mm Hg)	Event[a]	Outcome	Relationship
CHD	18	Hypotension/cardiac arrest	Fatal	Not related
CHD	10	Hypotension/bradycardia	Fatal	Related
CHD	9	Hypoxia/bradycardia	Fatal	Related
CHD	20	Pulmonary edema	Recovered	Unrelated
IPAH	10	Cardiac arrest	Recovered	Related
IPAH	7	Hypoxia	Recovered	Unrelated
CHD	30	Hypoxia/ECG changes	Recovered	Related

Note: *CHD* congenital heart disease, *ECG* electrocardiogram, *IPAH* idiopathic pulmonary arterial hypertension, *PCWPm* mean pulmonary capillary wedge pressure

[a] Event, outcome, and relationship are as per investigator's assessment

serious adverse event; one patient died. Serious adverse events were also reported in three other patients in the immediate poststudy period; two patients died (Table 5). Given the short duration of each treatment, it was problematic to assign a relationship to one specific intervention.

Discussion

As PAH treatments increase, and our ability to successfully repair CHD or perform heart or heart–lung transplantation improves, the importance of adequately evaluating these patients with acute vasodilator testing becomes more important than in prior decades, when medical treatments were more limited and surgical techniques less advanced. Based on the results of this study, the iNO/O_2 combination identified more acute responders than O_2 alone, although the acute response rate with the combination was not significantly different than with iNO alone. However, iNO/O_2 decreased PVRI and PAPm more than either O_2 alone or iNO alone. All three treatments were pulmonary selective, i.e., neither systemic vascular resistance (SVR) nor cardiac index changed; however, the PVR-to-SVR ratio decreased the most with iNO/O_2. In addition, at 1-year follow-up, children who responded to the combination appeared to be just as likely to survive as those who responded to O_2 alone. Whether this will remain at longer follow-up requires further study.

The rationale for acute vasodilator testing in the diagnostic evaluation of PH patients with increased PVR is based on two factors: (1) acute responders appear to have a better prognosis (whether treated medically or surgically), and (2) acute responders treated medically are more likely to have a sustained beneficial response with calcium channel blockers than nonresponders [5, 6, 18, 21–23, 25]. Furthermore, treating nonresponders with calcium channel blockers is contraindicated. The ideal acute vasodilator testing agent is pulmonary selective with a short half-life. Current acute vasodilator testing recommendations in adult PAH patients are to use either iNO or intravenous epoprostenol [4, 9, 14]. Although there are no evidence-based guidelines for selection of vasodilators, the consensus is that iNO is the preferred vasodilator, while intravenous epoprostenol is an acceptable alternative. Based on the data from this study, at least in children, the iNO/O_2 combination appears to be similar to iNO alone in identifying acute responders, but better than O_2 alone (although the magnitude of the changes is greatest with the combination vs. either agent alone).

The definition of a "positive" response remains controversial. For adult PAH patients, the criteria for an acute

responder had been a ≥20% PAPm decrease without a cardiac index decrease for ≥25 years [5, 6, 18, 21, 22]. Based on a long-term idiopathic PAH adult follow-up study [23], however, the current consensus for acute response was recently changed to a decrease in PAPm of ≥10 mm Hg, leading to an absolute PAPm of ≤40 mm Hg, with a normal or high cardiac index. Unfortunately, this new definition may not identify all children who are acute responders with demonstrated long-term calcium channel blocker efficacy [25]. In the present study, the response criteria utilized appeared to effectively identify those children with a variety of PH conditions who were likely to do well at follow-up.

The current standard of care includes acute vasodilator testing before considering chronic vasodilator therapy. Inclusion of children with PH associated with cardiomyopathy allowed us to examine whether vasodilator therapy could have a potential role as a palliative bridge to transplantation. Baseline PCWP was limited to a maximum of 20 mm Hg in the interest of safety. However, based on this study, it remains unclear whether vasodilator therapy can sufficiently lower PVRI to a level considered acceptable to perform a heart transplant alone, without the need for combined heart–lung transplantation.

Serious adverse events were reported in 5.7% of the patients, comparable with an approximate 6% rate of serious adverse events in published surveys [7, 24]. Inhaled NO can induce acute pulmonary edema in patients with pulmonary venous hypertension. Three of the seven serious adverse events occurred in patients with PCWPs >15 mm Hg, including one CHD patient in whom acute vasodilator testing with iNO further increased the PCWP, precipitating acute pulmonary edema. We suggest that clinicians remain mindful of left ventricular function during acute vasodilator testing.

Study limitations include the following. (1) Patients who had a large decrease in PAPm and PVRI but a small decrease in cardiac index (>5% change) were not considered acute responders based on prespecified criteria. However, these strict criteria for identifying acute responders requiring "no" decrease in cardiac index may have underestimated this population, i.e., a 5–10% decrease in cardiac index may represent an acceptable variability when cardiac index remains within the normal range with acute vasodilator testing. (2) Discrepancies between the intent-to-treat and the per-protocol populations occurred because patients were enrolled prior to calculation of baseline PVRI, with precatheterization PVRI estimates higher than PVRI calculated from cardiac catheterization data. In patients with shunts, echocardiographic PVRI estimates are often inaccurate despite reasonably accurate right ventricular systolic pressure estimates. Because of inaccurate prestudy PVRI estimates, some patients completed the study before baseline PVRI was calculated, i.e., the calculated baseline PVRI had not exceeded 3.0 U · m^2 as required in the inclusion criteria. Nonetheless, our findings remain consistent for both intent-to-treat and per-protocol analyses. Patients with PVRI just below 3.0 U · m^2 demonstrated acute vasoreactivity during the study treatments in a manner similar to patients with higher PVRI. Our study is consistent with the pilot study by Atz et al. [1], who also found an apparent advantage with the iNO/O_2 combination than with either agent alone. Additionally, Balzer et al. [3] reported that iNO/O_2 vs. O_2 alone increased the number of CHD patients with PH meeting the surgical operability criteria. (3) Although the first and last treatments were randomly assigned, the second treatment was always the iNO/O_2 combination. Without randomization of all three treatments, we cannot exclude an interaction of time with treatment or investigator bias. Nevertheless, the results appear to be robust and consistent and are further strengthened by the results at 1-year follow-up.

This study confirms the pulmonary selectivity with iNO; furthermore, acute vasodilator testing with the iNO/O_2 combination identified more acute responders than with O_2 alone. Although there were no significant differences noted in the acute response rate with the iNO/O_2 combination vs. iNO alone, the combination decreased PAPm, PVRI, and the PVR/SVR ratio more than either agent alone. Therefore, we recommend acute vasodilator testing with the iNO/O_2 combination.

The 1-year survival data in this study may provide some insight as to the benefit of acute vasodilator testing utilizing an iNO/O_2 combination vs. O_2 alone. While overall survival was similar between the combination and the O_2 alone groups (91% vs. 86%), an appreciable difference in 1-year survival between acute responders and nonresponders in each respective group was observed (91% vs. 78% and 86% vs. 81% in the combination and O_2 alone groups, respectively). Thus, the combination of iNO with O_2 may be more effective than O_2 alone in discriminating survivors versus nonsurvivors long-term. This, however, can only be elucidated by future trials that examine patients who are similarly treated based on acute vasodilator testing response. In addition, whether these differences between acute responders and nonresponders will continue to increase over longer follow-up remains to be determined and will provide another focus of future research in this area.

Further follow-up should also help us determine the "best" definition for an acute responder and the utility of acute vasodilator testing with the iNO/O_2 combination vs. iNO alone, both in predicting the prognosis in medically treated pediatric PH patients with increased PVRI and in predicting surgical/interventional suitability in CHD patients with PH and increased PVRI.

Acknowledgments This study was sponsored by INO Therapeutics/Ikaria, Clinton, New Jersey, USA. Investigators are as follows. France: Prof. D. Sidi, Dr. G. Agnoletti, Hôpital Necker Enfants Malades, Paris; Dr. A. Fraisse, Dr. A. Dragulescu, CHU la Timone–Hôpital d'Enfants, Marseille; Dr. Marçon, Hôpital d'Enfants, Nancy. Spain: Dr. F. Gutierrez Larraya, Dr. A. Mendoza, Hospital Materno-Infantil XII de Octubre, Madrid; Dr. J. L. Zunzunegui Martinez, Hospital Gregorio Maranon, Madrid; Dr. J. J. Bartrons Casas, Dr. F. Prada Hermógenes, Hospital Sant Joan de Deu, Barcelona; Dr. J. Girona Comas, Hospital Infantil Vall d'Hebron, Barcelona. The Netherlands: Prof. Dr. R. Berger, Beatrix Children's Hospital, University Hospital, Groningen. United Kingdom: Dr. A. Magee, L. Davies Shaughnessy, RN, The Royal Brompton Hospital, London. United States: Dr. R. Barst, Columbia University, New York; Dr. S. Abman, Donna Parker, RRT, The Children's Hospital, Denver, CO; Dr. D. Balzer, Washington University, St. Louis, MO; Dr. Andrew Atz, Teresa Atz, RN, Children's Hospital Medical University of South Carolina, Charleston; Dr. J. A. Feinstein, Val Scott, RN, Lucile Salter Packard Children's Hospital—Stanford, Palo Alto, CA; Dr. C. Daniels, Ohio State University Medical Center & Columbus Children's Hospital, Columbus; Dr. R. Hirsch, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Competing Interests Dr. Robyn J. Barst has served as a consultant to, and received research support from, Actelion, Eli Lilly, Gilead, GSK, INO/Ikaria, Novartis, and Pfizer. Dr. Gabriella Agnoletti was reimbursed for attending an investigator meeting (Edinburgh) and was paid to include 10 more patients in the study. Dr. Alain Fraisse was reimbursed for attending two investigator meetings for this study (Miami and Edinburgh) and is a consultant to Sanofi-Aventis. Dr. James Baldassarre is an employee of Ikaria. Dr. David L. Wessel serves as a consultant to Ikaria and has received research funds from Pfizer.

References

1. Atz AM, Adatia I, Lock JE, Wessel DL (1999) Combined effects of nitric oxide and oxygen during acute pulmonary vasodilator testing. J Am Coll Cardiol 33:813–819
2. Badesch DB, Champion HC, Gomez-Sanchez MA, Hoeper MM, Loyd JE, Manes A, McGoon M, Naeije R, Olschewski H, Oudiz RJ, Torbicki A (2009) Diagnosis and assessment of pulmonary arterial hypertension. J Am Coll Cardiol 54:S55–S66
3. Balzer DT, Kort HW, Day RW, Corneli HM, Kovalchin JP, Cannon BC, Kaine SF, Ivy DD, Webber SA, Rothman A, Ross RD, Aggarwal S, Takahashi M, Waldman JD (2002) Inhaled nitric oxide as a preoperative test (INOP Test I): the INOP Test Study Group. Circulation 106(12; Suppl 1):I76–I81
4. Barst RJ, Gibbs JS, Ghofrani HA, Hoeper MM, McLaughlin VV, Rubin LJ, Sitbon O, Tapson VF, Galie N (2009) Updated evidence-based treatment algorithm in pulmonary arterial hypertension. J Am Coll Cardiol 54:S78–S84
5. Barst RJ, Maislin G, Fishman AP (1999) Vasodilator therapy for primary pulmonary hypertension in children. Circulation 99:1197–1208
6. Barst RJ (1986) Pharmacologically induced pulmonary vasodilation in children and young adults with primary pulmonary hypertension. Chest 89:497–503
7. Bennett D, Marcus R, Stokes M (2005) Incidents and complications during pediatric cardiac catheterization. Paediatr Anaesth 15:1083–1088
8. Cockrill BA, Kacmarek RM, Fifer MA, Bigatello LM, Ginns LC, Zapol WM, Semigran MJ (2001) Comparison of the effects of nitric oxide, nitroprusside, and nifedipine on hemodynamics and right ventricular contractility in patients with chronic pulmonary hypertension. Chest 119:128–136
9. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G, Vahanian A, Auricchio A, Bax J, Ceconi C, Dean V, Filippatos G, Funck-Brentano C, Hobbs R, Kearney P, McDonagh T, McGregor K, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Vardas P, Widimsky P, Sechtem U, Al Attar N, Andreotti F, Aschermann M, Asteggiano R, Benza R, Berger R, Bonnet D, Delcroix M, Howard L, Kitsiou AN, Lang I, Maggioni A, Nielsen-Kudsk JE, Park M, Perrone-Filardi P, Price S, Subirana Domenech MT, Vonk-Noordegraaf A, Zamorano JL (2009) Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J 30:2493–2537
10. Houde C, Bohn DJ, Freedom RM, Rabinovitch M (1993) Profile of paediatric patients with pulmonary hypertension judged by responsiveness to vasodilators. Br Heart J 70:461–468
11. Kieler-Jensen N, Ricksten SE, Stenqvist O, Bergh CH, Lindelov B, Wennmalm A, Waagstein F, Lundin S (1994) Inhaled nitric oxide in the evaluation of heart transplant candidates with elevated pulmonary vascular resistance. J Heart Lung Transplant 13:366–375
12. Krasuski RA, Warner JJ, Wang A, Harrison JK, Tapson VF, Bashore TM (2000) Inhaled nitric oxide selectively dilates pulmonary vasculature in adult patients with pulmonary hypertension, irrespective of etiology. J Am Coll Cardiol 36:2204–2211
13. Marshall HW, Swan HJ, Burchell HB, Wood EH (1961) Effect of breathing oxygen on pulmonary artery pressure and pulmonary vascular resistance in patients with ventricular septal defect. Circulation 23:241–252
14. McLaughlin VM, Archer SA, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, Varga J, Harrington RA, Anderson JL, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Grines CL, Hlatky MA, Jacobs AK, Kaul S, Lichtenberg RC, Lindner JR, Moliterno DJ, Mukherjee D, Pohost GM, Rosenson RS, Schofield RS, Shubrooks SJ, Stein JH, Tracy CM, Weitz HH, Wesley DJ, ACCF/AHA (2009) ACCF/AHA clinical expert consensus document on pulmonary hypertension. Circulation 119:2250–2294
15. Morales-Blanhir J, Santos S, de Jover L, Sala E, Pare C, Roca J, Rodriguez-Roisin R, Barbera JA (2004) Clinical value of vasodilator test with inhaled nitric oxide for predicting long-term response to oral vasodilators in pulmonary hypertension. Respir Med 98:225–234
16. Pagano D, Townend JN, Horton R, Smith C, Clutton-Brock T, Bonser RS (1996) A comparison of inhaled nitric oxide with intravenous vasodilators in the assessment of pulmonary haemodynamics prior to cardiac transplantation. Eur J Cardiothorac Surg 10:1120–1126
17. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J (1991) Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. Lancet 338:1173–1174
18. Rich S, Kaufmann E, Levy PS (1992) The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. N Engl J Med 327:76–81
19. Roberts JD Jr, Lang P, Bigatello LM, Vlahakes GJ, Zapol WM (1993) Inhaled nitric oxide in congenital heart disease. Circulation 87:447–453
20. Rubin LJ (2004) Diagnosis and management of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 126(Suppl 1):7S–10S
21. Sitbon O, Brenot F, Denjean A, Bergeron A, Parent F, Azarian R, Herve P, Raffestin B, Simonneau G (1995) Inhaled nitric oxide as

a screening vasodilator agent in primary pulmonary hypertension. A dose-response study and comparison with prostacyclin. Am J Respir Crit Care Med 151(2; Pt 1):384–389
22. Sitbon O, Humbert M, Jagot JL, Taravella O, Fartoukh M, Parent F, Herve P, Simonneau G (1998) Inhaled nitric oxide as a screening agent for safely identifying responders to oral calcium-channel blockers in primary pulmonary hypertension. Eur Respir J 12:265–270
23. Sitbon O, Humbert M, Jais X, Ioos V, Hamid AM, Provencher S, Garcia G, Parent F, Herve P, Simonneau G (2005) Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. Circulation 111:3105–3111
24. Taylor CJ, Derrick G, McEwan A, Haworth SG, Sury MR (2007) Risk of cardiac catheterization under anaesthesia in children with pulmonary hypertension. Br J Anaesth 98:657–661
25. Yung D, Widlitz AC, Rosenzweig EB, Kerstein D, Maislin G, Barst RJ (2004) Outcomes in children with idiopathic pulmonary arterial hypertension. Circulation 110:660–665