A Randomized, Controlled Trial to Investigate the Efficacy of Nebulized Poractant Alfa in Premature Babies with Respiratory Distress Syndrome

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Objective To investigate the efficacy and safety of nebulized poractant alfa (at 200 and 400 mg/kg doses) delivered in combination with nasal continuous positive airway pressure compared with nasal continuous positive airway pressure alone in premature infants with diagnosed respiratory distress syndrome.

Study design This randomized, controlled, multinational study was conducted in infants at $28^{0/7}$ to $32^{6/7}$ weeks of gestation. The primary outcome was the incidence of respiratory failure in the first 72 hours of life, defined as needing endotracheal surfactant and/or mechanical ventilation owing to prespecified criteria. Secondary outcomes included the time to respiratory failure in the first 72 hours, duration of ventilation, mortality, incidence of bronchopulmonary dysplasia, and major associated neonatal comorbidities. In addition, the safety and tolerability of the treatments were assessed reporting the number and percentage of infants with treatment-emergent adverse events and adverse drug reactions during nebulization.

Results In total, 129 infants were randomized. No significant differences were observed for the primary outcome: 24 (57%), 20 (49%), and 25 (58%) infants received endotracheal surfactant and/or mechanical ventilation within 72 hours in the poractant alfa 200 mg/kg, poractant alfa 400 mg/kg, and nasal continuous positive airway pressure groups, respectively. Similarly, secondary respiratory outcomes did not differ among groups. Enrollment was halted early owing to a change in the benefit-risk balance of the intervention. Nebulized poractant alfa was well-tolerated and safe, and no serious adverse events were related to the study treatment.

Conclusions The intervention did not decrease the likelihood of respiratory failure within the first 72 hours of life. *(J Pediatr 2022;246:40-7)*.

Trial Registration ClinicalTrials.gov: NCT03235986

Although many premature infants with mild to moderate respiratory distress syndrome (RDS) can be supported adequately with nasal continuous positive airway pressure (nCPAP), continuous positive airway pressure (CPAP) failure in the first week of life occurs in approximately 40%-50% of premature infants with a gestational age of less than 29 weeks, according to the Australian and New Zealand Neonatal Network.[1,2] A retrospective, single-center study conducted in Europe has reported a CPAP failure rate of 45% in premature infants with a gestational age between $24^{0/7}$ to $31^{6/7}$ weeks.[3]

The efficient delivery of nebulized surfactant could reduce CPAP failure and improve outcomes of premature infants. Compared with intubate-surfactant-extubate and less invasive surfactant administration techniques, nebulized surfactant represents a truly noninvasive surfactant delivery method, involving no requirement for laryngoscopy or the peridosing events associated with liquid surfactant instillation in the airway (eg, transient airway obstructions, desaturations, and hypercarbia).[4]

AUC	Area under the curve
FiO_2	Fraction of inspired oxygen
CPAP	continuous positive airway pressure
nCPAP	Nasal continuous positive airway pressure
RDS	Respiratory distress syndrome
SpO_2	Oxygen saturation
TEAE	Treatment-emergent adverse events

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Animal studies have shown equivalent pulmonary efficacy of nebulized surfactant in terms of gas exchange and lung mechanics, a better pulmonary distribution, and less abrupt hemodynamic changes compared with intratracheal surfactant.[5-9] Nevertheless, translating these findings into clinical practice has proven exceptionally challenging owing to the extremely small airways, low lung volumes, and rapid respiratory rates of premature infants, which reduce deposition of the aerosol particles in the lungs.[10] An awareness of the particular physiological features of premature infants has enabled the development of tailored nebulization strategies aimed at improving the lung deposition and the overall efficacy of nebulized surfactant in these patients.[11-14] This randomized, controlled study was designed to compare the efficacy of 2 doses of nebulized surfactant (200 and 400 mg/kg) with nCPAP alone in spontaneously breathing premature infants with diagnosed mild to moderate RDS.

Methods

The trial (NCT03235986) was conducted in 34 centers from 6 European countries (**Table I**; available at www.jpeds.com). It was approved by the Institutional Review Board in compliance with the regulations of each country. Written informed consent was obtained from parents or legal guardian of each infant. The study was monitored by an Independent Safety Monitoring Board that examined the progress of the trial. Study monitoring was performed by a contract research organization (IQVIA). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (the Declaration of Helsinki).

Eligibility and Recruitment

The study was conducted on spontaneously breathing preterm neonates aged $28^{0/7}$-$32^{6/7}$ weeks gestational age with mild to moderate RDS, confirmed by either chest radiograph or lung ultrasound assessment before randomization, receiving nCPAP 5-8 cm H_2O and fraction of inspired oxygen (FiO_2) between 0.25 and 0.40 to maintain an oxygen saturation (SpO_2) between 88% and 95% for at least 30 minutes. Randomization was to occur between 60 minutes and 12 hours after birth.

Exclusion criteria included the early need for intubation within 1 hour after birth, the use of surfactant before study entry, RDS not secondary to surfactant deficiency, evidence of severe asphyxia, major congenital abnormalities, prolonged rupture of membranes (>21 days duration), presence of air leaks, and intraventricular hemorrhage grade III or higher identified by echocardiography and known before study entry. Hypotension and hemodynamic instability were judged by the attending clinicians and represented exclusion criteria if a pharmacological intervention was required.

Study Design

The study was a multicenter, randomized, open-label, controlled trial. Infants were randomized to one of the 3-arm parallel-groups: poractant alfa (Curosurf, Chiesi Farmaceutici) at 200 and 400 mg/kg doses or to remain on nCPAP alone. These doses were selected based on the advice of the Independent Safety Monitoring Board after analyzing the data from the pilot, dose-escalating tolerability study (**Figure 1** and **Table II**; available at www.jpeds.com), in which no clear efficacy signs were observed with a dose of 600 mg/kg. Moreover, preclinical studies showed maximum effects on gas exchange and lung mechanics with the 200 mg/kg and 400 mg/kg doses.[5,12] Apart from requiring an extended nebulization time, the 600 mg/kg dose was associated with lower carbon dioxide exchange and surfactant accumulation in the upper airways of some animals.[12]

A balanced block randomization scheme was prepared via a computerized system. One redosing was allowed in both active treatment groups, with the administration of an additional dose of nebulized poractant alfa at 200 mg/kg to occur between 3 and 12 hours after the start of the first dose if (1) no peridosing adverse event started during the administration of the first dose, and (2) the inclusion criteria need for FiO_2 persisted.

Poractant alfa was delivered continuously by a vibrating-membrane nebulizer (investigational eFlow Neos, PARI Pharma GmbH). The device was placed as close to the patient as possible, between the nasal prongs and the connection of the ventilator circuit to avoid the aerosol dilution by the bias flow (**Figure 2**; available at www.jpeds.com). Preclinical studies have demonstrated that this nebulizer can deliver high doses of undiluted poractant alfa (>1000 mg), producing consistent surfactant aerosols of a mass median aerodynamic diameter of 2.8 μm that retain their biophysical properties.[12,15,16]

The reference therapy was nCPAP alone delivered with a continuous flow CPAP driver from a list that had been previously tested for compatibility with the nebulizer (**Figure 2**). Other noninvasive respiratory support strategies were not permitted.

Study Outcomes

The primary efficacy outcome was respiratory failure in the first 72 hours of life, defined as infants needing endotracheal surfactant administration and/or mechanical ventilation owing to an FiO_2 of more than 0.40 to maintain SpO_2 between 88 and 95% for at least 30 minutes, apnea (>4 episodes per hour or >2 episodes per hour that required positive pressure ventilation), or persistent respiratory acidosis (arterial partial pressure of carbon dioxide, a $PaCO_2$ of >65 mm Hg and a pH of <7.20 on blood gas).[17]

Infants with at least 1 of these 3 items met the criterion for respiratory failure. Secondary outcomes included the time to respiratory failure in the first 72 hours, respiratory failure during the whole study (until bronchopulmonary dysplasia assessment), SpO_2, FiO_2 and SpO_2/FiO_2 after

treatment, duration of invasive ventilation in the first 72 hours of life, duration of invasive and noninvasive respiratory support during hospital stay, mortality, incidence of bronchopulmonary dysplasia, and major associated neonatal comorbidities.[18]

The safety and tolerability of the treatments were assessed reporting the number and percentage of infants with treatment-emergent adverse events (TEAE) and adverse drug reactions during nebulization (peridosing TEAEs) and for the whole study.

Statistical Analyses

A sample size of 84 infants per group would be required to detect a 50% relative decrease for the primary outcome, with a power of 80%, at a 2-sided significance level of 5% and assuming a 5% non-evaluable rate. The respiratory failure in the control group was assumed to be 40%.[19-21] Respiratory failure in the first 72 hours of life and during the whole study, and redosing requirement were compared using a chi-square test. The relative risk (RR) ratios with their 95% CIs were also calculated. Fisher exact test was used to compare the percentage of infants with respiratory failure in the subgroup analysis (post hoc analyses).

The time to respiratory failure in the first 72 hours was compared using a log-rank test. The SpO_2, FiO_2, and SpO_2/FiO_2 ratio values and their changes from baseline were summarized at each postbaseline time point. Comparisons vs control group were performed using a linear mixed model for repeated measures. The area under the curve (AUC) of the FiO_2 and SpO_2/FiO_2 ratio $AUC_{0\text{-}3h}$ and $AUC_{0\text{-}72h}$ was compared with ANOVA model. Duration variables were compared using a Mann-Whitney *U* test. Efficacy analyses were conducted with the intention-to-treat population, defined as all randomized neonates with a gestational age of $28^{0/7}$ to $32^{6/7}$ weeks who received nCPAP and then started nebulized poractant alfa (active-treatment group only) with at least 1 available evaluation of efficacy after baseline. Safety data were summarized by treatment group using descriptive statistics.

Results

The study was conducted from August 28, 2017, to May 5, 2020. Figure 3 shows the CONSORT diagram of the study. From the 307 infants enrolled, 129 were randomized to 1 of the 3 arms (43 in each arm). One infant in the poractant alfa 200 mg/kg group and 2 in the poractant alfa 400 mg/kg group were randomized but did not receive treatment and were excluded by the intention-to-treat population including 42, 41, and 43 infants in the poractant alfa 200 mg/kg, poractant alfa 400 mg/kg, and nCPAP groups, respectively. In total, 42, 40, and 43 infants completed the study; 1 infant in the poractant alfa 400 mg/kg group died.

Nebulization of the first dose was completed successfully in all neonates. One case of surfactant leak from the prongs was reported during nebulization with nebulized poractant alfa 200 mg/kg, but was managed without major impact. Most neonates had the mouth closed during nebulization and were calm throughout.

On April 23, 2020, the sponsor, in agreement with the Independent Safety Monitoring Board's opinion, issued a notification of permanent recruitment interruption to all participants after the evaluation of the safety and efficacy profiles of the first 120 randomized neonates based on a

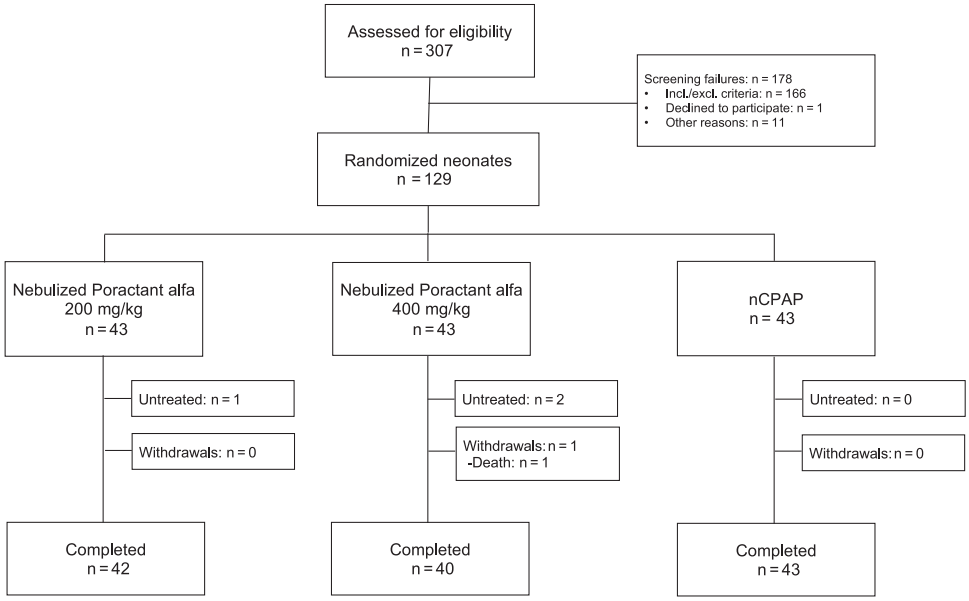

Figure 3. CONSORT diagram for study recruitment and treatment. *Incl.*, inclusion; *excl.*, exclusion.

change in the benefit-risk balance driven by a negligible efficacy for the whole study population.

Baseline Demographics and Clinical Characteristics

The demographics and baseline clinical characteristics are shown in Table III. There was a higher percentage of male infants in the 400 mg/kg group and a lower birthweight in the 200 mg/kg group.

Primary Outcome

No significant differences between poractant alfa treatments vs nCPAP were observed (Table IV; poractant alfa 200 mg/kg vs nCPAP: RR ratio, 0.98 [95% CI, 0.68-1.42]; poractant alfa 400 mg/kg vs nCPAP: RR ratio, 0.84 [95% CI, 0.56-1.26]).

Most infants with respiratory failure were given rescue endotracheal surfactant, with the intubate-surfactant-extubate technique most commonly applied (in 12 [30%], 6 [15%], and 9 [21%] with poractant alfa 200 mg/kg, poractant alfa 400 mg/kg, and nCPAP, respectively), followed by less invasive surfactant administration (in 6 [14%], 3 [7%], and 12 [28%] neonates, respectively) and then the standard surfactant administration method with mechanical ventilation (in 6 [14%], 10 [24%], and 2 [5%] neonates, respectively).

The use of surfactant outside respiratory failure criteria occurred in a similar proportion of neonates with nebulized poractant alfa 400 mg/kg and nCPAP (4 [9.8%] neonates and 4 [9.3%] neonates, respectively) and was the most commonly reported major protocol deviation, followed by the use of noninvasive ventilation other than nCPAP (5 [12.2%] neonates and 2 [4.7%] neonates, respectively). There were no major protocol violations in the poractant alfa 200 mg/kg group.

Secondary Outcomes

Post hoc analyses showed a difference in avoiding respiratory failure within 72 hours in the intervention groups for the gestational age of 31 weeks or more subgroup, in particular for infants receiving poractant alfa at a 400 mg/kg dose (poractant alfa 200 mg/kg vs nCPAP: RR ratio, 0.64 [95% CI, 0.30-1.37]; poractant alfa 400 mg/kg vs nCPAP: RR ratio, 0.46 [95% CI, 0.22-0.99], Table V). The Kaplan-Meier estimate of the first quartile time to respiratory failure was 9.42 (95% CI, 5.17-17.55), 12.92 (95% CI, 3.33-18.02), and 1.45 (95% CI, 0.50-8.48) hours with poractant alfa 200 mg/kg, poractant alfa 400 mg/kg, and nCPAP, respectively; this finding indicated that the time to respiratory failure was later with poractant alfa treatments (Figure 4, A, available at www.jpeds.com); however, the log-rank test analyses indicated no statistically significant differences in these delays (poractant alfa 200 mg/kg vs nCPAP: $P = .471$; poractant alfa 400 mg/kg vs nCPAP: $P = .207$). Few infants were redosed (4 [9.5%] poractant alfa 200 mg/kg vs 3 [7.3%] with poractant alfa 400 mg/kg), with no statistically significant difference between groups.

A decrease in the FiO_2 was observed in the poractant alfa 200 mg/kg and poractant alfa 400 mg/kg groups up to at least 2 hours after treatment (Figure 5, A, available at www.jpeds.com). The SpO_2 was similar with all 3 treatments up to 1.5 hours after treatment, after which a numerical increase in the nCPAP group was observed up to 24 hours (Figure 5, B). The SpO_2/FiO_2 ratio was numerically higher with nebulized poractant alfa up to at least 1 hour after treatment (Figure 4, B). Subsequently, oxygenation was similar between the treatments. The $AUC_{0\text{-}3h}$ of the SpO_2/FiO_2 ratio was significantly higher with poractant alfa 200 mg/kg vs nCPAP ($P = .010$), but only numerically higher with poractant alfa 400 mg/kg vs nCPAP ($P = .062$). No statistically significant differences were observed in the $AUC_{0\text{-}72h}$ of the SpO_2/FiO_2 ratio. Similarly, there were no significant differences in the duration of invasive

Table III. Demographics and baseline clinical characteristics

Variables	Nebulized poractant alfa 200 mg/kg (n = 42)	Nebulized poractant alfa 400 mg/kg (n = 41)	Control nCPAP (n = 43)
Patient characteristics			
Gestational age (wk)	30.4 ± 1.4	30.90 ± 1.16	30.6 ± 1.4
Birth weight (g)	1330 ± 422	1469 ± 328	1450 ± 346
Male sex	22 (52)	28 (68)	20 (46)
Antenatal steroids	38 (90)	38 (93)	40 (93)
Cesarean delivery	35 (83)	36 (88)	32 (74)
Apgar score			
1 min, median (range)	7 (1-10)	7 (3-9)*	8 (3-10)
5 min, median (range)	8 (6-10)	8 (5-10)	9 (7-10)
Respiratory stimulants	38 (90)	40 (98)	42 (98)
Time to randomization from birth (h), mean (range)	4.6 (1.2-11.9)	4.5 (1.2-11.5)	3.9 (1.0-10.1)
Time to start of first dose from birth (h), mean (range)	5.5 (1.7-12.5)	5.6 (1.7-12.7)	–
Respiratory status at baseline			
FiO_2 (%)	32.5 ± 6.6	32.1 ± 5.3	30.0 ± 4.6
SpO_2 (%)	92.5 ± 2.2	92 ± 2.3	93 ± 2.7
$PaCO_2$ (mm Hg)	47 ± 9	52.5 ± 8.0	48.0 ± 8.5

Values are mean ± SD or number (%) unless otherwise noted.
*n = 40.

Table IV. Primary outcome, reasons for respiratory failure, and procedures applied after respiratory failure

Variables	Nebulized poractant alfa 200 mg/kg (n = 42)	Nebulized poractant alfa 400 mg/kg (n = 41)	Control nCPAP (n = 43)
Primary outcome			
Respiratory failure within 72 hours	24 (57)	20 (49)	25 (58)
P-value vs nCPAP	.926	.390	–
Reasons for respiratory failure*			
FiO_2 > 0.4 for > 30 minutes	22 (52)	18 (44)	20 (46)
Significant apnea	2 (5)	3 (7)	7 (16)
$PaCO_2$ > 65 mm Hg and pH < 7.2	1 (2)	3 (7)	1 (2)
Procedure applied after respiratory failure			
Endotracheal surfactant	24 (57)	19 (46)	23 (53.5)
Standard administration method	6 (14)	10 (24)	2 (5)
Intubate-surfactant-extubate	12 (29)	6 (15)	9 (21)
Less invasive surfactant administration	6 (14)	3 (7)	12 (28)
Mechanical ventilation†	7 (17)	11 (27)	4 (9)

$PaCO_2$, partial pressure of carbon dioxide.
Values are number (%).
*Infants could have had more than 1 reason for respiratory failure.
†One infant in the poractant alfa 200 mg/kg group received surfactant with intubate-surfactant-extubate and then also mechanical ventilation. One infant in the poractant alfa 400 mg/kg group received only mechanical ventilation. Two infants in the nCPAP group received only mechanical ventilation.

ventilation, noninvasive respiratory support, and oxygen supplementation during the first 72 hours of life (Table V).

A total of 173, 162, and 152 TEAEs were reported in 38 (90.5%), 33 (80.5%), and 40 (93.0%) neonates with poractant alfa 200 mg/kg, nebulized poractant alfa 400 mg/kg, and nCPAP, respectively. Two peridosing TEAEs were reported in 1 neonate with poractant alfa 200 mg/kg, and 4 peridosing TEAEs in 3 neonates with nebulized poractant alfa 400 mg/kg (Table VI). The only peridosing TEAE reported in more than 1 neonate was infantile apnea. No adverse drug reactions were reported. One infant in the nebulized poractant alfa 400 mg/kg group died owing to cardiopulmonary failure, which was considered not related to the study treatment. The incidence of neonatal morbidities was not significantly different among the groups.

Table V. Secondary outcomes

Outcomes	Nebulized poractant alfa 200 mg/kg (n = 42)	Nebulized poractant alfa 400 mg/kg (n = 41)	Control nCPAP (n = 43)
Respiratory failure at 72 h by gestational age*			
Gestational age < 31 weeks, n (%)	19 (63)	14 (67)	14 (54)
RR (95% CI)	1.18 (0.75-1.84)	1.24 (0.78-1.98)	
P value vs nCPAP	0.588	0.551	-
Gestational age of ≥31 weeks, n (%)	5 (42)	6 (30)	11 (65)
RR (95% CI)	0.64 (0.30-1.37)	0.46 (0.22-0.99)	-
P value vs nCPAP	0.274	0.050	-
Respiratory failure in the whole study, n (%)	24 (57)	21 (51)	25 (58)
P value vs nCPAP	0.926	0.524	-
IMV in the first 72 h			
n (%)	13 (31)	14 (34)	9 (21)
Duration (h), mean (range)	25.9 (0.2-62.9)	37.22 (3.7-68.4)	31.5 (0.2-64.8)
P value vs nCPAP	0.482	0.369	-
IMV duration during hospital stay			
n (%)	14 (33)	15 (36.5)	10 (23)
Duration (d), mean (range)	6.1 (0.0-35.2)	5.23 (0.2-27.4)	3.28 (0.3-10.3)
P value vs nCPAP	0.852	0.576	-
Noninvasive respiratory support duration during hospital stay			
n (%)	42 (100)	41 (100)	43 (100)
Duration (d), mean (range)	14.2 (1.4-54.7)	9.31 (0.5-34.0)	8.63 (0.1-38.7)
P value vs nCPAP	0.052	0.537	-
Oxygen supplementation alone			
n (%)	16 (38)	9 (22)	13 (30)
Duration (d), mean (range)	4.2 (0.1-19.0)	3.84 (0.0-11.1)	5.5 (0.0-27.1)
P value vs nCPAP	0.852	0.987	-
BPD by 36 weeks PCA, n (%)	7 (16)	5 (12)	4 (9)
P value vs nCPAP	0.312	0.640	-

BPD, bronchopulmonary dysplasia; *IMV*, invasive mechanical ventilation; *PCA*, post-conceptional age.
*Post hoc analysis.

Table VI. Safety outcomes and major comorbidities

Outcomes	Nebulized poractant alfa 200 mg/kg (n = 42)	Nebulized poractant alfa 400 mg/kg (n = 41)	Control nCPAP (n = 43)
Safety of surfactant nebulization			
Duration of nebulization (first dose, min), mean (range)	13.46 (5.0-37.0)	28.92 (10.0-60.0)	-
Adverse drug reactions	0 (0)	0 (0)	-
TEAE			
Infants with any TEAE	38 (90)	33 (80)	40 (93)
Total number of events	173	162	152
Peridosing TEAEs	1 (2)	3 (7)	-
Total number of events	2	4	-
Serious TEAEs	8 (19)	3 (7)	5 (11)
Total number of events	9	4	5
Infants with any major comorbidities			
NEC	0 (0)	1 (2)	2 (5)
IVH	2 (5)	1 (2)	0 (0)
PDA	5 (12)	6 (15)	8 (19)
ROP	1 (2)	0 (0)	1 (2)
Deaths	0 (0)	1 (2)	0 (0)

NEC, necrotizing enterocolitis; *IVH*, intraventricular hemorrhage; *PDA*, patent ductus arteriosus; *ROP*, retinopathy of prematurity.
Values are number (%) unless otherwise noted.

Discussion

We report the results from a randomized, controlled trial designed to assess the efficacy of different doses of nebulized poractant alfa in preterm neonates aged $28^{0/7}$ to $32^{6/7}$ weeks with confirmed RDS. There were no statistically significant differences with either dose of nebulized poractant alfa vs nCPAP in the incidence of respiratory failure in the first of 72 hours of life. However, post hoc analyses showed a difference favoring the intervention for the more mature population subgroup (gestational age of ≥31 weeks). Nebulized poractant alfa was well-tolerated and safe; there were no serious adverse events related to the study treatment. Enrollment was halted early owing to a change in the benefit-risk balance of the intervention.

Three clinical studies have reported efficacy of nebulized surfactant in premature infants.[13,14,22] In a single-center, randomized, controlled trial, Minocchieri et al reported a significant decrease of intubation within 72 hours of life with nebulized poractant alfa delivered with an investigational eFlow Neos nebulizer compared with nCPAP alone in infants born at $29^{0/7}$-$33^{6/7}$ weeks of gestation.[13] Nonetheless, the benefits of nebulized surfactant were limited to the $32^{0/7}$-$33^{6/7}$ weeks of gestation stratum. In a multicenter, controlled trial, Cummings et al randomized 457 infants born at a mean gestational age of 33 weeks (range, 23-41 weeks of gestation) to either receive aerosolized calfactant via the mouth, using a modified nebulizer with a pacifier adapter, or usual care.[14] The authors reported a significant reduction of the primary outcome, which was the rate of intubation for surfactant instillation.[14] Nevertheless, this study had a pragmatic design and the decision to intubate and give surfactant was not based on objective criteria but was left up to the attending neonatologist, which is a significant source of bias in this study.[23] The subgroup analysis revealed that the significant improvement of the primary outcome was only observed for the 31-32 and 35-36 weeks of gestation strata. In a single-center, randomized, phase II study, Sood et al reported a significant reduction of the need for intubation within 72 hours in infants born at $24^{0/7}$-$36^{6/7}$ weeks of gestation treated with various doses of nebulized beractant compared with historical controls; however, non-randomized retrospective controls received caffeine at a significantly later age, which the authors acknowledged as a factor that could have influenced the primary outcome.[22]

Compared with the trial by Cummings et al, the present study used a different vibrating-membrane nebulizer and surfactant, had more stringent entry criteria in terms of gestational age ($28^{0/7}$-$32^{6/7}$), set predetermined objective criteria to define the primary outcome, and had a clearly defined oxygen threshold for study entry.[14] Cummings et al set a FiO_2 threshold of 0.25 to 0.40 as study entry requirement but, owing to the variability in the application of noninvasive respiratory support pressure, which was higher in some centers, the minimum oxygen requirement was removed in the fifth month of the trial.[14] In the present study, only infants with a confirmed RDS diagnosis were enrolled, whereas Cummings et al enrolled infants with suspected or confirmed RDS.[14] Last, any kind of noninvasive respiratory support was allowed in their study, whereas nCPAP was the only permitted non-invasive respiratory support modality in this trial.

The present study shares features with the Minocchieri et al trial, including a similar nebulizer and the same surfactant preparation, the exclusive use of CPAP as noninvasive respiratory support, and the primary efficacy outcome.[13] However, they set lower supplemental oxygen requirements for study entry (0.22-0.30), used a face mask as the CPAP interface during aerosol delivery, and the attending clinician could intubate infants with perceived significant respiratory distress in the absence of elevated FiO_2 or $PaCO_2$, which might have introduced a bias toward the primary outcome.

The lack of statistically significant differences in the primary and secondary outcomes is in line with the findings

observed in the Minocchieri et al study for the lower gestational age stratum ($29^{0/7}$ to $31^{6/7}$ weeks of gestation).[13] However, the longer time to first respiratory failure and the significant improvement in oxygenation in the first hour after initiating the therapy with nebulized poractant alfa denotes a physiological effect. Nonetheless, the positive effect of nebulized poractant alfa may have been restricted to the infants in the highest gestational age stratum (gestational age ≥31 weeks), who showed a trend toward a lower rate of intubation compared with infants treated with nCPAP alone, especially after treatment with poractant alfa 400 mg/kg. Nevertheless, we can speculate that the actual poractant alfa aerosol deposition in the infants' lungs was lower than expected compared with in vitro and animal studies (>10% of the nominal dose).[12,24,25] The difference in lung deposition may be explained by the fact that preclinical studies were conducted using tightly sealed neonatal ventilation circuits to minimize the air leaks around the nCPAP interface, which are common in clinical practice and might have contributed to a significant surfactant aerosol loss in the present study. A recent mathematical modelling study investigating the convective aerosol transport during neonatal noninvasive respiratory support estimated that up to 70% of the aerosol delivered by the nebulizer could be lost owing to leaks at the patient interface.[26] In-vitro studies also highlighted that the size and geometry of different commercially available nasal prongs influenced the aerosol delivery and significantly affected the lung dose.[27] The same type of nasal prongs was distributed across all centers; however, different sizes were applied according to the infant's characteristics and may have accounted for certain variability. It is also worth noting that the study included 34 recruiting centers, with many of them treating relatively few infants; this factor may have limited the improvement of the learning curve of the attending clinicians in implementing the new surfactant administration technique. Nebulized poractant alfa was safe and well-tolerated. However, 7 infants on active treatment developed a pneumothorax and 3 were diagnosed intraventricular hemorrhage, which were not observed in the control group. None of these events were assessed as related to the study drug, and the relevance of this finding should be addressed carefully in future trials.

This study has several strengths. It was designed based on a comprehensive preclinical development aimed at finding the best methodology to deliver surfactant to premature infants efficiently.[12,15,16,27] The trial used a miniaturized vibrating-membrane nebulizer optimized to deliver large doses of undiluted and functional surfactant without clogging the membrane. The meticulous trial design provided a controlled framework to investigate the safety and efficacy of nebulized surfactant, setting rigorous criteria at study entry of infants with confirmed RDS. Respiratory failure was defined by objective clinical indicators to minimize bias. nCPAP was the only permitted noninvasive respiratory support type, and the same type of nasal prongs and humidifier were used in all sites to diminish the center-to-center variability.

The study also has some limitations. The protocol did not mandate sealing of the nasal prongs with straps. We speculate that the leaks at the patient interface may have represented a significant source of surfactant aerosol loss. Blinding the intervention was not feasible in the present study, because it would have required doubling the attending staff in all centers (ie, unblinded staff to set up the device and administer treatment, and blinded for deciding interventions and data collection), including weekends and night shifts. In addition, using a sham procedure such as saline nebulization would neither be scientifically or ethically acceptable. Therefore, respiratory failure may have been affected by bias because clinicians could observe which infants had not received treatment and might have had a lower threshold for intervening with endotracheal surfactant. The aerosol particle size as determined in vitro was 2.8 μm with the drug-device combination of the present trial, which slightly increased to 3 μm in subsequent measurements under high relative humidity conditions.[12,15,27] Particles with a diameter of less than 5 μm are well-known to achieve deep lung deposition in adults and other pediatric patients; however, the ideal particle size for premature neonates has not been defined yet.[10] Particle size measurements at the outlet of a neonatal endotracheal tube with an internal diameter of 3.0 mm showed that the mass median aerodynamic diameter of the particles exiting the tracheal tube was 1.4 μm, suggesting that smaller particle diameters may be required to cross the narrow airways of human neonates.[28] Hence, the particle size of approximately 3 μm may have resulted in less direct surfactant delivery to the alveoli in the present study. Further studies should try to decrease the air leaks around the patient interface by either holding the interface in place during the whole nebulization process or using alternative patient interfaces (eg, face or nasal masks). Further studies powered to evaluate the efficacy in the more mature infants should also be considered together with the use of other noninvasive respiratory support types, such as nasal intermittent positive pressure ventilation and synchronized nasal intermittent positive pressure ventilation.

In preterm infants born at $28^{0/7}$-$32^{6/7}$ weeks of gestation with RDS, the administration of nebulized poractant alfa at 200 mg/kg or 400 mg/kg doses alongside nCPAP did not decrease the likelihood of respiratory failure within the first 72 hours of life compared with nCPAP alone. No particular safety concerns were raised with active treatment. ■

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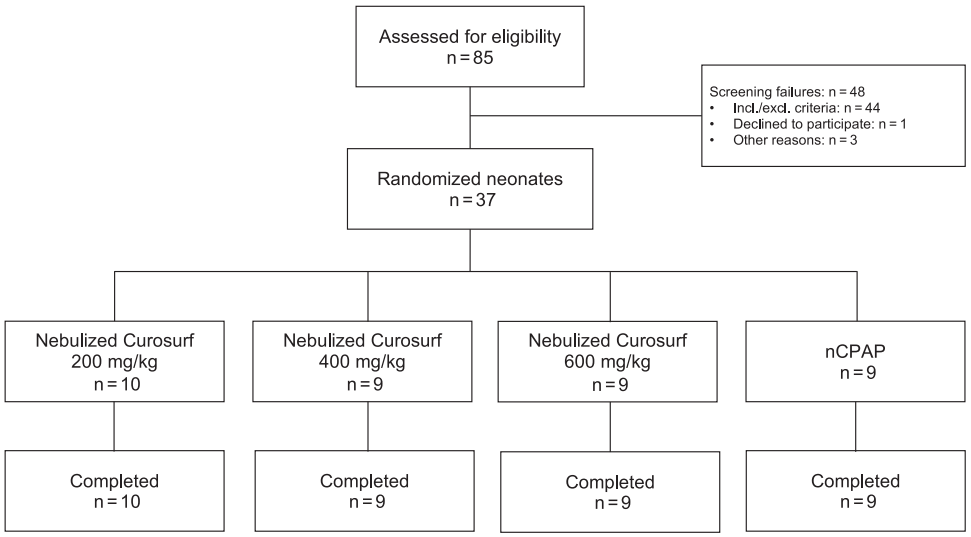

Figure 1. CONSORT diagram for study recruitment and treatment of the pilot tolerability study. The pilot tolerability study was conducted between 28 August 2017 and 21 June 2018. One infant with a lower gestational age (<28 weeks) was randomly allocated to the nebulized poractant alfa 200 mg/kg but was excluded from the analysis because they were outside population gestational age requirement. *Incl.*, inclusion; *excl.*, exclusion.

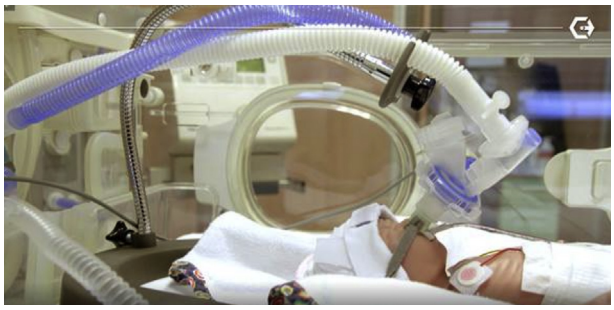

Figure 2. Nebulization setup used in the clinical trial mounted on a manikin. Poractant alfa was administered during nasal CPAP using a customized eFlow Neos vibrating membrane nebulizer. The device was placed between the nasal prongs and the connection of the ventilator circuit to optimize the lung dose. The CPAP tubing and the nebulizer were kept in place using a holding frame with a specific indication of its inclination, as shown in the figure. To avoid the variability of the nebulization process across centers, only a few continuous nCPAP drivers were permitted. The compatibility of the investigational nebulizer was tested with the most commonly used ventilators to deliver nCPAP in Europe (Acutronic Fabian HFO, Acutronic Fabian, Draeger Babylog 8000, Draeger Babylog VN500, SLE 5000, and Leoni Plus), which were, in turn, the allowed nCPAP drivers in the clinical trial. Moreover, all sites were provided with the same type of nasal prongs (Inspire, Inspiration health) and humidifier (MR850, Fisher &Paykel).

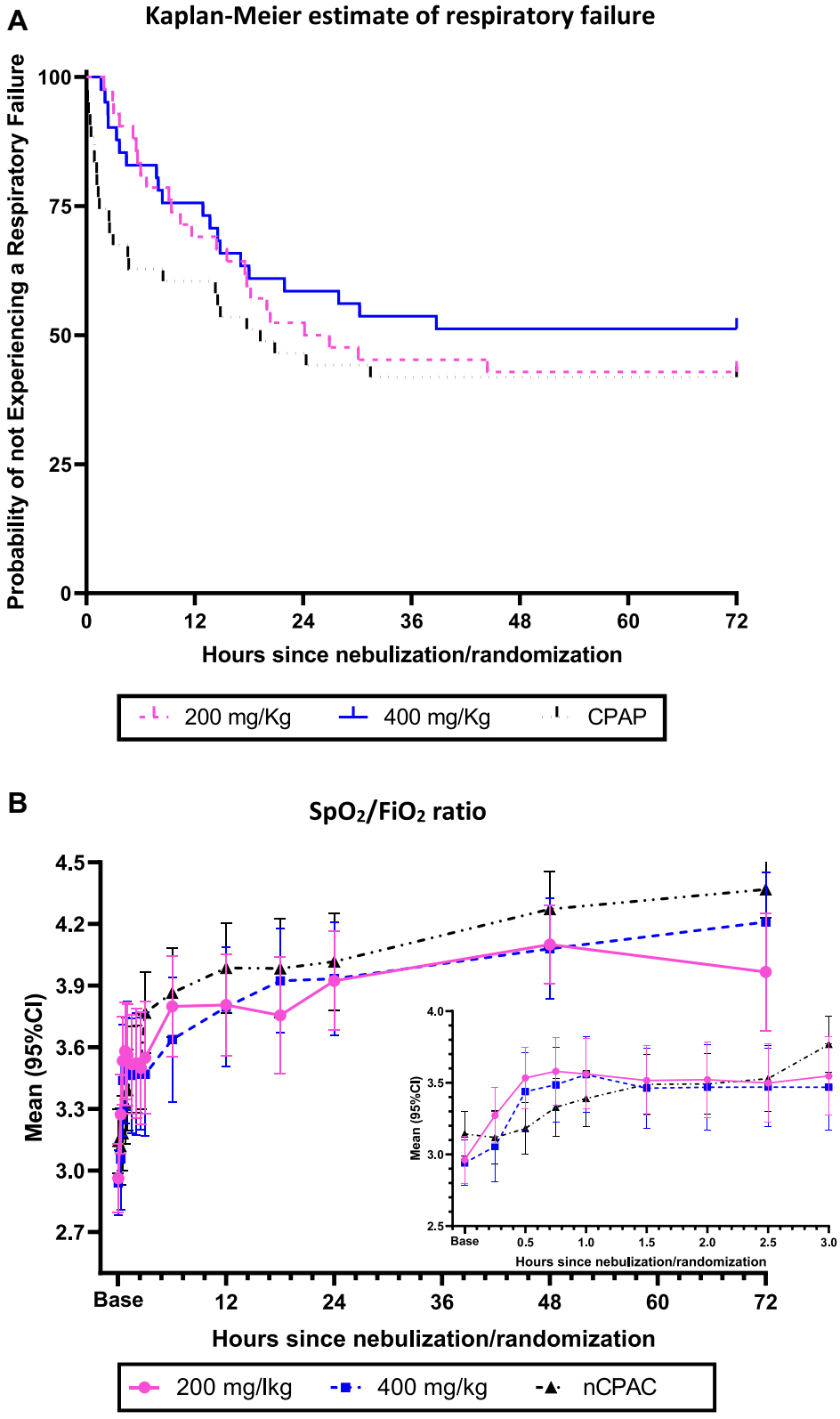

Figure 4. **A,** Kaplan-Meier estimate of respiratory failure and **B,** evolution of the SpO_2/FiO_2 ratio in the different groups in the first 72 hours. In **B,** the inset shows the first 3 hours after nebulization/randomization; the mean ± the 95% CI is shown. For the control group, the baseline (Base) was the measurement taken at randomization. For the surfactant nebulization groups, the baseline was the measurement taken at first nebulization start or just before (within 10 minutes before the start of nebulization).

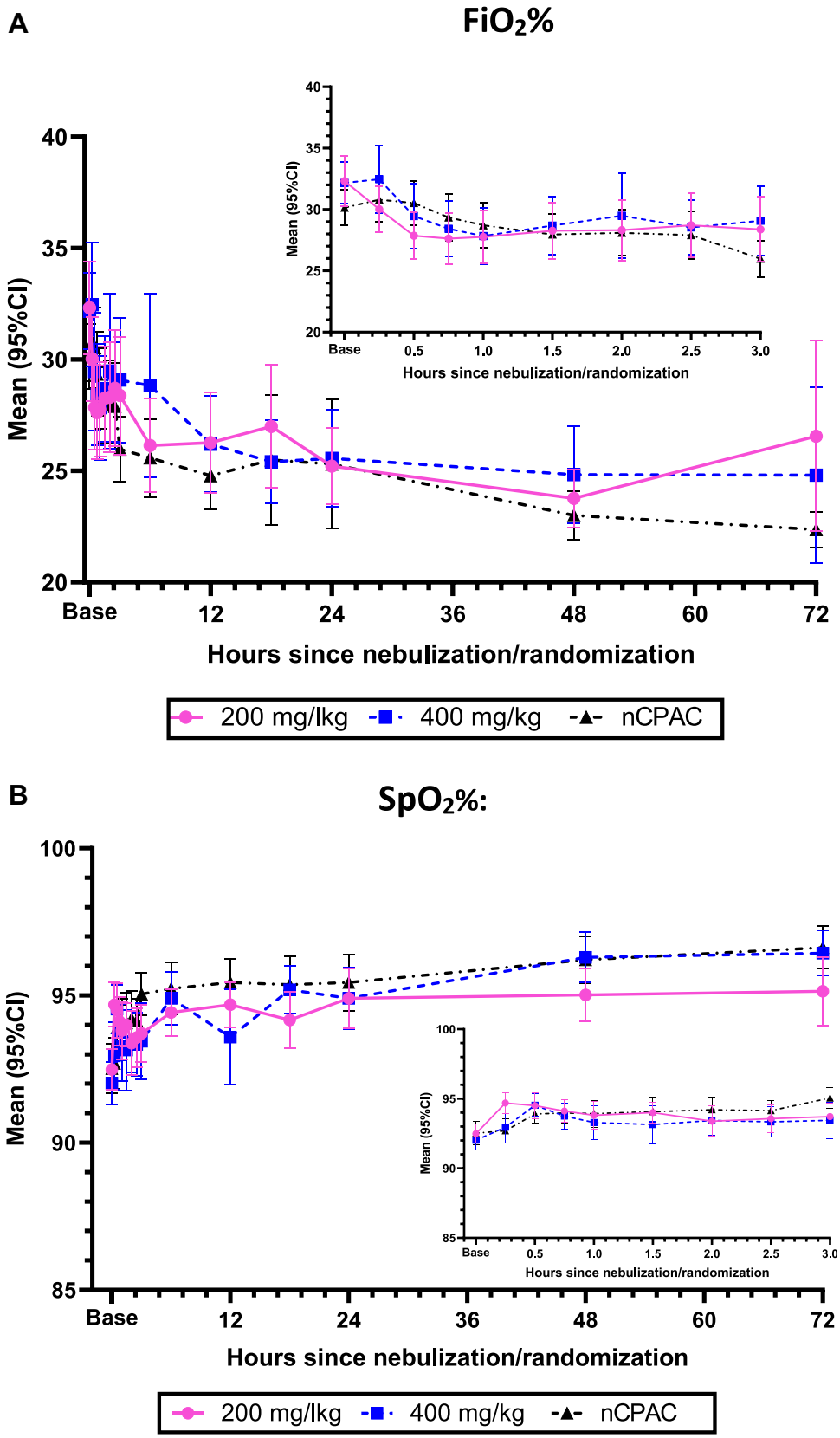

Figure 5. **A,** FiO_2 and **B,** SpO_2 evolution in the different groups in the first 72 hours. In **B,** the inset shows the first 3 hours after nebulization/randomization; the mean ± the 95% CI is shown. For the control group, the baseline (Base) was the measurement taken at randomization. For the surfactant nebulization groups, the baseline was the measurement taken at first nebulization start or just before (within 10 minutes before the start of nebulization).

Table I. Patient disposition by country

	Nebulized poractant alfa 200 mg/kg (n = 43)		Nebulized poractant alfa 400 mg/kg (n = 43)		nCPAP (n = 43)		Overall* (n = 307)
Countries	Randomized (n)	Completed (n)	Randomized (n)	Completed (n)	Randomized (n)	Completed (n)	Screened n
Austria	2	2	0	0	3	3	21
Czech Republic	9	9	9	8	2	2	56
Hungary	11	11	6	6	14	14	66
Italy	13	12	14	13	15	15	97
Poland	4	4	5	4	1	1	23
UK	4	4	9	9	8	8	44

The study was conducted in 34 recruiting centers (43 centers initiated) in 7 countries.
*Number of screened neonates.

Table II. Safety and exploratory efficacy outcomes of the dose-escalating tolerability study

Outcomes	Nebulized poractant alfa 200 mg/kg (n = 9*)	Nebulized poractant alfa 400 mg/kg (n = 9)	Nebulized poractant alfa 600 mg/kg (n = 9)	Control nCPAP (n = 9)
Patient characteristics				
Gestational age (wk)	30.6 ± 1.9	30.3 ± 1.2	30.9 ± 1.4	30.4 ± 1.6
Birth weight (g)	1263 ± 308	1373 ± 287	1428 ± 225	1490 ± 399
Male sex	5 (56)	7 (78)	6 (67)	6 (67)
Antenatal steroids	7 (78)	7 (78)†	9 (100)	8 (89)
Cesarean delivery	8 (89)	6 (75)†	9 (100)	7 (78)
Apgar score				
1 min, median (range)	8.0 (5.0-9.0)	8.0 (5.0-9.0)	6.0 (5.0-8.0)	7.0 (4.0-10.0)
5 min, median (range)	9.0 (7.0-10.0)	9.0 (7.0-10.0)	8.0 (7.9-9.0)	9.0 (7.0-10.0)
Time to randomization from birth (h), mean (range)	3.7 (2.3-5.7)	4.0 (1.8-5.9)	6.5 (2.7-11.0)	2.6 (1.1-4.1)
Respiratory status at baseline				
FiO_2 (%)	33.3 ± 7.9	30.1 ± 5.1	30.6 ± 4.2	29.8 ± 3.5
SpO_2 (%)	91.3 ± 2.5	93.2 ± 1.5	91.1 ± 6.9	94.1 ± 1.8
$PaCO_2$ (mm Hg)	53.2 ± 7.9	53.1 ± 8.6	49.7 ± 7.4	59.1 ± 13.3
Safety of surfactant nebulization				
Duration of nebulization (min), first dose, mean (range)	11.9 (9.0-15.0)	28.8 (17.0-45.0)	39.5 (20.0-55.0)	-
Adverse drug reactions	0 (0)	0 (0)	0 (0)	0 (0)
TEAE				
Infants with any TEAE	9 (100)	9 (100)	9 (100)	9 (100)
Total number of events	34	51	52	59
Peridosing TEAEs	0 (0)	0 (0)	0 (0)	-
Serious TEAEs	0 (0)	0 (0)	0 (0)	2 (22)
Outcomes				
Respiratory failure 72 h	4 (44)	6 (67)	5 (56)	6 (67)
IMV in the first 72 h	3 (33)	4 (44)	2 (22)	2 (22)
IMV duration during hospital stay				
n (%)	5 (56)	4 (44)	2 (22)	3 (33)
Duration (d), mean (range)	2.42 (0.3-6.1)	3.28 (0.2-8.1)	1.10 (0.8-1.4)	4.9 (0.5-13.4)
Noninvasive respiratory support duration during hospital stay (d), mean (range)	13.9 (1.0-42.5)	11.4 (2.0-39.3)	13.1 (0.5-55.1)	11.6 (1.1-37.4)
Pneumothorax	0 (0)	0 (0)	0 (0)	0 (0)
BPD	0 (0)	1 (11)	1 (11)	0 (0)
Deaths	0 (0)	0 (0)	0 (0)	0 (0)

IMV, invasive mechanical ventilation; *d*, days; *BPD*, Bronchopulmonary dysplasia; $PaCO_2$, partial pressure of carbon dioxide.
Values are mean ± SD or number (%) unless otherwise noted. There was no statistically significant difference in the incidence of respiratory failure with any dose of nebulized poractant alfa vs nCPAP (Fisher exact test). No statistically significant differences between nebulized poractant alfa treatments vs nCPAP were observed for the duration of invasive and noninvasive respiratory support (Mann-Whitney *U* test).
All infants were reported with at least 1 TEAE in this part of the study. A total of 34, 51, 52, and 59 TEAEs were reported with nebulized poractant alfa 200 mg/kg, poractant alfa 400 mg/kg, poractant alfa 600 mg/kg, and nCPAP alone, respectively. TEAEs reported in more than 2 neonates with any treatment were hyperbilirubinemia, anemia, and neonatal sepsis. The majority of TEAEs were mild or moderate in intensity and resolved by the end of this part of the study. A single serious TEAE was reported in 2 neonates (22%) on nCPAP (necrotizing enterocolitis and intraventricular hemorrhage, respectively). No surfactant-related peridosing TEAEs, adverse drug reactions (ADR), or TEAEs leading to death were reported.
*One neonate with a lower gestational age (<28 weeks) was randomly allocated to the nebulized poractant alfa 200 mg/kg, who was not included in the analysis populations.
†n = 8.