CODE: CCD-5633AA1-02

ClinicalTrials.gov ID: NCT02452476

CLINICAL STUDY PROTOCOL

IND No : 122619

A MULTICENTER, DOUBLE BLIND, RANDOMIZED, SINGLE DOSE, ACTIVE-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF SYNTHETIC SURFACTANT (CHF 5633) IN COMPARISON TO PORCINE SURFACTANT (PORACTANT ALFA, CUROSURF®) IN THE TREATMENT OF PRETERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME.

Version No.: 4.0

Date: 31st January 2018

Chiesi Farmaceutici S.p.A.
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GENERAL INFORMATION

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Central Laboratory for Immunogenicity Evaluation:	[redacted]
Central Laboratory for Biomarker Evaluation:	[redacted]

VERSION HISTORY

Version	Date	Change History
1.0	*25 May 2015*	*Original version*
2.0	*07 August 2015*	• *Administrative change of the Sponsor's name from Chiesi USA, Inc to Chiesi Farmaceutici S.p.A. (Italy);* • *Clarification of the procedures for re-dosing with study drug;* • *Incorporation of a dynamic randomization method to assure the balance of the treatment groups by investigational site and gestational age group;* • *Clarification of the pharmacovigilance procedure for adverse events reporting/follow-up and CRO contact details;* • *Broadening of the safety assessment window for performance of cranial ultrasound from within 48 hours to within 7 days of life;* • *Delineation of the allowed time deviations from post-dose times;* • *Correction of minor typographical errors.*
3.0	*08 February 2017*	• *Administrative change of the SPONSOR MEDICAL EXPERT (Clinical Research Physician);* • *Clarification of the timings in hours for Phases 3 (Section 7.1.3), 4 (Section 7.1.4), 5 (Section 7.1.5), 6 (Section 7.1.6);* • *Correction of bronchopulmonary dysplasia (BPD) diagnosis in Section 7.1.9 to align it to the one contained in Appendix II;* • *Update and correction of the list of Morbidities or Complications of prematurity at study entry;* • *Correction of minor typographical errors and further clarifications on some protocol definitions and procedures (i.e. Mortality/BPD rate, data collection at planned time points and for tracheal aspirates).*
4.0	*31 January 2018*	• *To replace the Coordinating Investigator;* • *To update the protocol template according to ICH Topic E6 (R2), CPMP/ICH/135/95, 'Note for Guidance on Good Clinical Practice', Step 4, November 2016.*

PROTOCOL OUTLINE

Study Title	A multicenter, double blind, randomized, single dose, active-controlled study to investigate the efficacy and safety of synthetic surfactant (CHF 5633) in comparison to porcine surfactant (Poractant alfa, Curosurf ®) in the treatment of preterm neonates with respiratory distress syndrome.
Sponsor	Chiesi Farmaceutici S.p.A., Via Palermo 26/A, 43122 Parma (Italy)
Name of the Product	CHF 5633 Synthetic surfactant
Centers	Approximately 20 investigational sites will be involved.
Indication	Treatment of Respiratory Distress Syndrome (RDS).
Study design	Double blind, randomized, single dose, active-controlled study.
Study phase	Proof of Concept (POC)/Phase II
Objectives	Main objectives of this study are: • to investigate the short term efficacy profile of CHF 5633 vs. porcine surfactant (Poractant Alfa, Curosurf®) in terms of reduced oxygen requirement and ventilatory support • to evaluate the mid-term efficacy profile in terms of reduced incidence of bronchopulmonary dysplasia (BPD) and mortality/BPD rate at 36 weeks post menstrual age (PMA), mortality rate at 28 days post-natal age (PNA) and 36 weeks PMA, RDS-associated mortality through 14 days of age and other major co-morbidities of prematurity. Moreover, other objectives of the present study are: • to evaluate the need for re-dosing in the 2 treatment groups according the pre-defined criteria; • to evaluate the inflammatory status through the measurements of specific biomarkers of inflammation in tracheal aspirates (in a subgroup of babies who require endotracheal intubation for mechanical ventilation when feasible); • to assess immunogenicity through the measurement of antibodies to SP-B analogue (CHF 5736.03) and SP-C analogue (CHF 4902.03) contained in CHF 5633. • to perform the evaluation of oxygenation status through invasive measurements [in a subgroup of babies who have

	an arterial (umbilical, peripheral) access, when feasible]; • to evaluate the safety profile in the two treatment groups in terms of adverse events (AEs) and adverse drug reactions (ADRs), vital signs, hematology and biochemistry values.
Treatment duration	One intratracheal administration of CHF5633 200mg/kg or Poractant alfa (Curosurf®) 200 mg/kg within 24 hours from birth. If needed, up to two repeat doses of 100 mg/kg each may be administered at approximately 12-hour intervals with the same surfactant as the first dose, according to the double blind design and the below pre-defined criteria: • lack of reduction in FiO_2 >0.10 to keep arterial oxygen saturation (SpO_2) between 88–95% or other clinical evidence of lack of efficacy within 12 hours after first/previous dosing; • babies mechanically ventilated with FiO_2 ≥0.30 for babies 24 to 26^{+6} weeks and FiO_2 ≥0.35 for babies 27 to 29^{+6} weeks within 12 hours after first/previous dosing; • babies not mechanically ventilated with FiO_2 ≥0.35 for babies 24 to 26^{+6} weeks and FiO_2 ≥0.40 for babies 27 to 29^{+6} weeks within 12 hours after first/previous dosing. However, if a neonate still fails to show a clinical improvement after further re-dosing with the same surfactant as the first dose (e.g. FiO_2>0.40 to maintain $SatO_2$ 88-95%, respiratory acidosis defined as pCO_2 >65 mmHg/8.5 kPa and pH<7.20 on an arterial or capillary blood gas), he/she could receive an alternative surfactant treatment according to Investigator's discretion.
Test product dose/route/regimen	CHF5633 200mg/kg synthetic surfactant sterile suspension in 3.0 ml glass vials with a total concentration of 80 mg/ml for intratracheal administration, containing the synthetic phospholipids: dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and sodium salt, synthetic surfactant protein C analogue and synthetic surfactant protein B analogue [SP-C 1.5%, SP-B 0.2%, total phospholipids 98.3% (w/w)]. CHF5633 100 mg/kg will be used for further re-dosing when needed.
Reference product dose/route/regimen	Poractant alfa (Curosurf®) 200mg/kg sterile suspension in 3.0 ml glass vials with a total concentration of 80 mg/ml for intratracheal administration, standard natural surfactant prepared from porcine

	lungs and containing almost exclusively polar lipids, in particular phosphatidylcholine (about 70% of the total phospholipid content), and about 1% of specific low molecular weight hydrophobic proteins SP-B and SP-C. Poractant alfa 100mg/kg will be used for further re-dosing when needed.
Number of patients	126 patients (63 in each treatment group) will be randomized in the study.
Study population	Preterm neonates with a gestational age of 24^{+0} weeks up to 29^{+6} weeks with RDS
Inclusion/exclusion criteria	<u>Inclusion Criteria</u> Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study: 1. Written informed consent obtained by parents/legal representative (according to local regulation) prior to any study-related procedures 2. Inborn preterm neonates of either sex with a gestational age of 24^{+0} weeks up to 29^{+6} weeks 3. Clinical course consistent with RDS 4. Requirement of endotracheal surfactant administration within 24 hours from birth 5. Fraction of inspired oxygen (FiO_2) $\geq$0.30 for babies 24^{+0} to 26^{+6} weeks and $FiO_2 \geq$0.35 for babies 27^{+0} to 29^{+6} weeks to maintain SpO_2 between 88–95% <u>Exclusion Criteria</u> The presence of any of the following will exclude a subject from study enrolment: 1. Use of surfactant prior to study entry and need for intratracheal administration of any other treatment (e.g. nitric oxide) 2. Known genetic or chromosomal disorders, major congenital anomalies (cardiac malformations, myelomeningocele etc) 3. Mothers with prolonged rupture of the membranes (>21 days duration) 4. Strong suspicion of congenital pneumonia/infection, sepsis 5. Presence of air leaks if identified and known prior to study

	entry 6. Evidence of severe birth asphyxia 7. Neonatal seizures prior to study entry 8. Any condition that, in the opinion of the Investigator, would place the neonate at undue risk 9. Participation in another clinical trial of any placebo, drug or biological substance conducted under the provisions of a protocol.
Most relevant allowed concomitant treatments	Any concomitant medication required for the normal care of preterm neonates will be permitted during the study.
Most relevant forbidden concomitant treatments	• Surfactant treatment prior to study entry • Intratracheal administration of any other treatment (e.g. nitric oxide) prior to study entry • Other investigational drugs.
Efficacy variables (and/or pharmacokinetics variables)	• Ratio of arterial oxygen saturation determined by pulse oximetry and fraction of inspired oxygen (SpO_2/FiO_2) in the first 7 days post-treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5 and 7), at Day 28±2 PNA, at discharge home and at 36 weeks PMA. • Fraction of inspired oxygen (FiO_2) in the first 7 days post-treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5 and 7), at Day 28±2 PNA, at discharge home and at 36 weeks PMA. • Respiratory Severity Score (RSS) [FiO_2 corrected by mean airway pressure (MAP): FiO_2 x MAP] in the 2 days post-treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours and on Day 2); • Oxygen Saturation Index (OSI) [FiO_2 x MAP x100/SpO_2] in the 2 days post-treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours and on Day 2); • SpO_2 in the first 7 days post-treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5 and 7), at Day 28±2 PNA, at discharge home and at 36 weeks PMA. • Mean airway pressure (MAP), peak inspiratory pressure (PIP) and positive end-expiratory pressure (PEEP) in the first 7 days of post-treatment • Mortality/BPD rate defined as the incidence of patients who are dead or alive with a diagnosis of BPD at 36-week PMA; • BPD incidence at 36-week PMA; • Mortality rate at Day 28 PNA and at 36-week PMA;

	• RDS-associated mortality rate through 14 days of age; • Percentage of patients requiring at least one rescue surfactant dose; • Percentage of patients with at least one reading of FiO_2=0.21 (i.e. room air) within 24 hours from first dosing with test treatment; • Time (hours) to reach FiO_2=0.21 (i.e. room air); • Duration of invasive mechanical ventilation (days), defined as the time until the first successful extubation lasting more than 24 hours; • Duration of non-invasive ventilation (days), defined by nasal continuous positive airway pressure (nCPAP), Bi-level positive airway pressure (BiPAP), nasal intermittent positive pressure ventilation (NIPPV), high flow nasal cannula (HFNC); • Duration of oxygen alone, defined as receiving supplemental oxygen without invasive or non-invasive ventilator support; • Biomarkers of inflammation: CXCL8, Interleukin 1β (IL1β), Interleukin 6 (IL6), Tumor Necrosis Factor-alpha (TNF-α), myeloperoxidase (MPO), prior to study drug administration, at 24 hours and on Day 2 (48 hours) (in a subgroup of babies who require endotracheal intubation for mechanical ventilation when feasible). • Alveolar–arterial (A-a) Oxygen gradient at 3, 6, 12, 18, 24 hours and day 2 post treatment (in a subgroup of babies who have an arterial access, when feasible) • Oxygenation Index (OI) at 3, 6, 12, 18, 24 hours and day 2 post treatment (in a subgroup of babies who have an arterial access, when feasible)
Safety variables	• AEs and ADRs • Vital signs (systolic and diastolic blood pressure, heart rate) and SpO2 during administration • Incidence of major neonatal morbidities (for the list of neonatal morbidities and complications of prematurity see section 10.8) • Hematology and blood chemistry: full blood count (FBC), urea, creatinine and electrolytes (sodium, potassium, calcium, phosphorus), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, C-reactive protein • Immunogenicity assessment will be carried out in one blood sample collected approximately 5 weeks after administration (with a window from 3 to 6 weeks)

	• Bayley Scales of Infant Development at 24 months (±3 months) corrected age. • Health status questionnaire (health problems, illnesses, injuries, well-being, diet, respiratory assessment) at 24 months (±3 months) corrected age.
Sample size calculation	According to the exploratory nature of this study, no formal sample size calculation was performed. A maximum number of sixty-three randomized neonates per treatment group (126 in total) is deemed sufficient to describe the efficacy and safety of CHF5633 compared to Poractant Alfa.
Statistical methods	Efficacy variables • SpO_2/FiO_2 will be analyzed using a linear mixed model for repeated measures (MMRM) including treatment, time point, treatment by time point interaction, investigational site and gestational age group as fixed effects and pre-dose ratio as covariate. The adjusted means in each treatment group, the adjusted mean difference between treatments and their 95% confidence intervals (CIs) at each time point and averaged over the first 24 hours will be estimated by the model. SpO_2/FiO_2 will be compared between treatments at the remaining post-treatment time points (i.e., Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA) using mixed model including treatment, investigational site and gestational age group as fixed effects and pre-dose ratio as covariate. SpO_2/FiO_2 will be analyzed by subgroups based on ventilator support used. • FIO_2 will be analyzed at each time point and averaged over the first 24 hours post dose using the same model used for the SpO_2/FiO_2. Pre-dose FIO_2 will be used as covariate. FiO_2 will be compared between treatments at additional post-treatment time points (i.e., Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA) using linear mixed model including, treatment, investigational site and gestational age group as fixed effects and pre-dose FIO_2 as covariate. • RSS will be analyzed at each time point and averaged over the first 24 hours post study drug intake using the same model used for the SpO_2/FiO_2. RSS at day 2 will be analyzed using linear mixed model including, treatment, investigational site and gestational age group as fixed effects. • OSI will be analyzed at each time point and averaged over the first 24 hours post study drug intake using the same model used for the SpO_2/FiO_2.

	OSI at day 2 will be analyzed using linear mixed model including, treatment, investigational site and gestational age group as fixed effects. • SpO_2, MAP, PIP, PEEP values as well as changes from baseline will be summarized by treatment group by means of descriptive statistics at each post-treatment timepoints. • Mortality/BPD rate at 36-week PMA will be compared by treatment by means of Cochran-Mantel-Haenszel (CMH), adjusting for stratification factors (i.e. GA group and investigational site). Relative risk (RR) and related 95% confidence interval will be provided. • The incidence of BPD at 36-week PMA will be compared by treatment as Mortality/BPD rate. • The mortality rate at 36-week PMA and at Day 28 will be compared by treatment as Mortality/BPD rate. • RDS-associated mortality rate through 14 days of age will be compared by treatment as for Mortality/BPD rate. • The percentage of patients requiring at least one rescue surfactant dose will be compared by treatment group by means of a Fisher's exact test at 5% significance interval. Odds ratio (OR) and related exact 95% CI will be also provided. Patients with pulmonary hemorrhage will be excluded from this summary. • The median duration time of invasive mechanical ventilation, oxygenation and non-invasive ventilation will be compared between groups by the Mann-Whitney U-test. • Percentage of patients with at least one reading of FiO_2 equal to 0.21 (i.e. at room air) within 24 hours from first intake will be compared by treatment groups by means of a Fisher's exact test at 5% significance interval. Odds ratio (OR) and related exact 95% CI will be also provided. • The median duration time to reach FiO_2 equal to 0.21 will be compared between groups by the Mann-Whitney U-test. • Mean values of biomarkers of inflammation (i.e., CXCL8, IL1β, IL6, TNF-α, MPO) in the tracheal aspirates as well as change from baseline to 24 hours and on Day 2 (48 hours) will be summarized by treatment by means of descriptive statistics. • A–a gradient and OI values as well as change from baseline in the first 48 hours after the first surfactant intake (i.e., at 3, 6, 12, 18, 24 hours and on day 2) will be summarized by treatment by means of descriptive statistics. <u>Safety variables</u> • Incidence of AEs will be summarized by treatment group both

	in term of frequency of neonates with at least one AE and in term of frequency of AEs (number of events). All AEs, related AEs, serious AEs, AEs leading to death will be summarized by SOC and PT. • Major neonatal morbidities (see section 10.8) will be summarized by treatment group. • At each time point (at 0 -for HR only-, 30 minutes, at 1, 3, 6, 12, 24 hours, Days 2, 3, 5, 7 and 28, discharge home and at 36 weeks PMA), the absolute value and the change from baseline in vital signs (SBP, DBP and HR) will be summarized by treatment group using descriptive statistics. SpO2 during administration will be summarized as well. • Hematology and chemistry parameters will be listed. • Immunogenicity titers will be listed. • Bayley Scales of Infant Development assessed at 24 months (±3 months) corrected age will be summarized by means of descriptive statistics. • Health status questionnaire assessed at 24 months (±3 months) corrected age will be summarized by means of descriptive statistics.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

A-a	Alveolar-arterial
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
BiPAP	Bi-level Positive Airway Pressure
BPD	Bronchopulmonary Dysplasia
BW	Birth Weight
CPAP	Continuous Positive Airway Pressure
CRA	Clinical Research Associate
eCRF	electronic Case Report Form
CRO	Clinical Research Organization
DBP	Diastolic Blood Pressure
DPPC	Dipalmitoylphosphatidylcholine
FBC	Full Blood Count
FiO_2	Fraction of inspired Oxygen
GA	Gestational Age
GCP	Good Clinical Practice
HFOV	High Frequency Oscillatory Ventilation
HFNC	High-Flow Nasal Cannula
HR	Heart Rate
ICH	International Conference on Harmonization
EC	Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISMB	Independent Safety Monitoring Board
ITT	Intention to Treat
IVH	Intraventricular Hemorrhage
LSMs	Least Square Means
MAA	Marketing Authorization Application
MAP	Mean Airway Pressure
MedDRA	Medical Dictionary for Regulatory Activities
NEC	Necrotizing enterocolitis
NICU	Neonatal Intensive Care Unit
NIPPV	Nasal Intermittent Positive Pressure Ventilation
NO	Inhaled nitric oxide
OI	Oxygenation Index
OSI	Oxygen Saturation Index
PCO_2	Arterial Carbon Dioxide Tension
PDA	Patent Ductus Arteriosus
PEEP	Positive End-Expiratory Pressure
PIE	Pulmonary Interstitial Emphysema
PIP	Peak Inspiratory Pressure
PMA	Post-Menstrual Age
PNA	Post-Natal Age
PP	Per-Protocol
PVL	Periventricular Leukomalacia
RDS	Respiratory Distress Syndrome
ROP	Retinopathy of Prematurity

RSS	Respiratory Severity Score
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SpO_2	Arterial Oxygen Saturation by Pulse Oximetry
SUSAR	Suspected Unexpected Serious Adverse Reaction

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1. BACKGROUND INFORMATION AND STUDY RATIONALE

Neonatal respiratory distress syndrome (RDS) is the most common respiratory disease of premature neonates and its incidence is directly proportional to the degree of prematurity. It occurs especially in extremely preterm infants, as illustrated in a study of the National Institute of Child Health and Human Development Neonatal Research Network, that showed an incidence of RDS of 93% in a cohort of 9575 extreme premature infants with gestational age (GA) of 28 weeks or below, born between 2003 and 2007 [(1)]. Despite a constant improvement of neonatal care, RDS still represents one of the major causes of mortality in preterm infants, as confirmed by a very recent American review (64 deaths per 1000 live births) [(2)].

The clinical features of RDS start appearing soon after birth. RDS is characterized by tachypnea (rapid breathing), expiratory grunting, subcostal and intercostal retractions, cyanosis, nasal flaring, apnea and/or hypothermia in extremely immature neonates. In the pre-surfactant era, the disease led rapidly to progressive respiratory failure in the first 2-3 days of life [(Error! Reference source not found.)] and, in the most severe cases, to the death of premature babies. Moreover, RDS can be followed by respiratory complications, such as bronchopulmonary dysplasia (BPD), most commonly in those infants who required ventilatory support and/or oxygen therapy for RDS treatment [(8)].

The pathophysiology of RDS has been correlated to lung immaturity and surfactant deficiency. Surfactant, a macro-aggregate composed by highly organized lipids and specific proteins, is produced by type 2 cells in the alveoli and covers the air liquid interface. It mainly reduces surface tension and prevents alveolar collapse, particularly at end-expiration, so that adequate gas exchange is maintained throughout the ventilatory cycle [(5,8)]. In preterm babies, RDS develops mostly for the deficiency of surfactant production, but different kinds of insults may deteriorate surfactant function (inhibition, inactivation, dysfunction, degradation).

Surfactant therapy has become the standard of care in management of preterm neonates with RDS and is commonly accepted as being crucial in management of RDS [(8)]. Surfactant treatment of babies with or at risk of RDS reduces mortality and the incidence of pulmonary air leaks such as pneumothorax by about 50% [(7)].

Natural surfactants (containing SP-B and SP-C) derived from animal sources (porcine or bovine) and synthetic surfactants have been evaluated extensively in preterm neonates. The natural surfactants commonly available worldwide include Survanta® (beractant), InfasurfTM (calfactant; licensed in the US and Israel but not in the EU), Curosurf® (poractant alfa), Alveofact® (bovactant; national European authorization). They produce better results than synthetic, protein-free preparations [(8)] and overall poractant alfa (Curosurf®) has been regarded as the gold standard for the treatment of RDS [(6)]. There have been several attempts over the years to replace animal-derived surfactants with more effective synthetic surfactants [(9-10)]. The first-generation synthetic surfactants, no longer commercially available, contained only phospholipids and no surfactant proteins. The superiority of animal surfactants over these first protein-free synthetic preparations was due to the presence of the hydrophobic proteins SP-B and SP-C [(11)]. The functional importance of the surfactant proteins SP-B and SP-C in the phospholipid monolayer encouraged attempts to improve the function of synthetic surfactants by the addition of human analogues of both surfactant proteins SP-B and SP-C. An effective synthetic surfactant should comprise both these peptides. Synthetic new-generation surfactants consisting of peptide and lipids may have several advantages over the

current natural surfactants: less infectious risk, less batch-to batch variability and no ethnographic issues.

Among the new-generation synthetic surfactants (containing SP-B and/or SP-C analogues) only lucinactant (proposed name Surfaxin®) reached the stage of being used in comparative clinical trials in preterm neonates [12-13]. Surfaxin was approved by FDA in March 2012 for the prevention of RDS in high risk, premature infants and it was launched in the US in November 2013. Unlike all available animal-derived surfactants, Surfaxin contains only one surfactant synthetic peptide (sinapultide/KL4). Since CHF 5633 contains two synthetic peptides and has a proposed indication for treatment of RDS, surfaxin would not represent a good comparator for CHF 5633 both in terms of chemical characteristics and therapeutic indication. Surfaxin also requires high volume of administration (5.8 mL per kg) and a cumbersome preparation procedure in the neonatal intensive-care unit (NICU) setting, which makes it a less-than-ideal candidate for comparison in a CHF 5633 trial.

The new synthetic surfactant CHF 5633 is the first fully synthetic surfactant in which the lipid phase is enriched by both peptide analogues to human SP-B and SP-C, proteins that are of crucial importance for lung surface activity. Moreover, CHF 5633 contains a suitable phospholipid composition of phosphatidylglycerol and phosphatidylcholine, optimized to maintain integrity over a large range of surface tension, with enhanced adsorption and spreading characteristics. It is presented as a sterile suspension for intratracheal administration as a single dose and the intended indication is treatment of RDS in premature neonates.

The in vitro and in vivo non-clinical studies showed that CHF 5633 displays at least similar surfactant activity to animal derived surfactant.

The in vitro experiments showed that CHF 5633 reduces surface tension at the air/liquid interface as efficiently as animal derived surfactant. The dynamic surface response/spreading data illustrated that CHF 5633 results in reduction of surface tension very close to that achieved by Curosurf®. Moreover it was as fast as Curosurf® in promoting spreading at the interface [14].

The in vivo efficacy of CHF 5633 as surfactant replacement was evaluated in preterm newborn rabbits. This model is regarded as an appropriate and sensitive experimental benchmark for neonatal respiratory distress syndrome [15]. It closely resembles the conditions of premature babies affected by RDS in the sense that the lungs of these animals are not yet able to produce their own surfactant but can undertake gas exchange and they can expand in response to exogenous surfactant administration. Curosurf® was used as reference standard. Intratracheal administration of CHF 5633 resulted in a marked improvement of lung expansion and the response was not statistically different from that of the animal-derived surfactant Curosurf® [16]. As CHF 5633 contains the essential surface active components of natural human pulmonary surfactant required for treatment of RDS, it is anticipated that this new surfactant has the potential to offer an increased benefit to risk balance over the existing therapies for the life threatening and potentially debilitating consequences of RDS in preterm neonates. This concept is supported by the current experience with natural and artificial surfactants, and by the activity of the product itself, that has been demonstrated, as described above, both in in vitro models and in a relevant in vivo disease model.

Based on these results it is anticipated that CHF 5633 will restore the functional surfactant film in the lungs of newborns with RDS. The anticipated improved therapeutic potential of this product is related to an improved safety profile. Most importantly the modified amino acid sequence of the

SP-B and SP-C peptide analogues improves resistance to degradation. This might result in overall enhanced efficacy with potentially fewer patients requiring repeated administrations.

In a first-in-human Phase I study [17] conducted in European Countries (Germany, UK and Czech Republic) mainly aimed at investigating the safety and tolerability of intratracheal administration of two different escalating single doses of CHF 5633 (100 and 200 mg/kg) in preterm neonates with RDS from 27^{+0} to 33^{+6} weeks gestational age.

The primary aim of this study was the evaluation of the safety and tolerability of intratracheal administration of two doses of CHF5633, 100 and 200 mg/kg, in preterm neonates with RDS, in terms of adverse events (AE) and adverse drug reactions (ADR), haematology and biochemistry values, incidence of major neonatal morbidities and mortality. The secondary objectives of this study were to explore the effect of the drug on oxygenation and ventilatory requirements and need for rescue surfactant treatment. Moreover, the immunogenicity through the measurement of antibodies to SP-B analogue (CHF 5736.03) and SP-C analogue (CHF 4902.03) contained in CHF 5633 as well as the extent of systemic exposure were evaluated.

CHF 5633 demonstrated a good safety profile and also an encouraging preliminary efficacy profile at both 100mg/kg and 200 mg/kg. Only one adverse drug reaction was reported following drug administration at 200 mg/kg: obstruction of the endotracheal tube) with no persistent effects on oxygen requirement or ventilatory support. The main objective of the present Phase 2 proof-of-concept (POC) study is to provide a proof-of-efficacy profile of CHF 5633 in comparison with Poractant Alfa (Curosurf®) in the same clinical procedural setting to sustain (or not) further clinical development.

Inflammation seen during RDS may be associated with the course of RDS and the promotion of bronchopulmonary dysplasia (BPD) [18]. A synthetic surfactant may provide a less potent immunological stimulus than an animal product. An exploratory examination of whether the nature and extent of the inflammatory response during RDS is related to the administered surfactant will be performed in a subgroup of centers and babies in this study. The good safety profile of CHF 5633 obtained in the European FIH study will be further evaluated in the present study in comparison to Curosurf® administered to extremely preterm and very preterm neonates, the potential target population of a surfactant replacement therapy nowadays. This trial will be conducted in compliance with the Declaration of Helsinki (1964 and amendments), the current Good Clinical Practices and all other applicable laws and regulations.

2. STUDY OBJECTIVES

2.1 Primary Objectives

Main objectives of this study are:

- to investigate the short term profile of CHF 5633 vs. porcine surfactant (Poractant Alfa, Curosurf®) in terms of reduced oxygen requirement and ventilatory support
- to evaluate the mid-term profile in terms of incidence of BPD and mortality/BPD rate at 36-week post menstrual age (PMA), mortality rate at 28 days PNA and at 36 weeks PMA, RDS-associated mortality through 14 days of age and other major co-morbidities of prematurity.

2.2 Secondary Objectives

- To evaluate the need for re-dosing in the two treatment groups;
- to evaluate the inflammatory status through the measurements of specific biomarkers of inflammation in tracheal aspirates (in a subgroup of babies who require endotracheal intubation for mechanical ventilation, when feasible);
- to assess immunogenicity through the measurement of antibodies to SP-B analogue (CHF 5736.03) and SP-C analogue (CHF 4902.03) contained in CHF 5633
- to perform the evaluation of invasive measurements of ventilator support (in a subgroup of babies when feasible);
- to evaluate the safety profile in the two treatment groups in terms of adverse events (AEs) and adverse drug reactions (ADRs), vital signs, hematology and biochemistry values.

3. STUDY DESIGN

This is a multicenter, double blind, randomized, single dose, active-controlled study. Efficacy and safety assessments will be performed in the 24 hours following study treatment administration (at minutes 30, at hours 1, 3, 6, 12, 18 and 24), in the next 6 days (at days 2, 3, 5 and 7) and in the follow-up periods [(at days 28±2 postnatal age (PNA), discharge home and at 36 weeks postmenstrual age (PMA)]. Moreover, a further clinical assessment will be performed at 24 months (±3 months) corrected age to check the neurological and general health status related to the condition of prematurity (Table 1).

TABLE 1: TIMETABLE

	TREATMENT A CHF5633 (synthetic surfactant) 200 mg/kg (re-dosing if needed with CHF5633 100mg/kg)
On admission prior to study treatment	
	TREATMENT B Poractant Alfa (porcine surfactant) 200 mg/kg (re-dosing if needed with Poractant Alfa 100mg/kg)

Time	Within 24 hours	0	30'	1h	3h	6h	12h	18h	24h	2 days	3 days	5 days	7 days	28 days PNA	Discharge home	36 wks PMA	24 - month clinical assess.
Phase	1	2								3	4	5	6	7	8	9	10

Randomisation and Test treatment administration

End of the trial

The end of the trial will correspond to the date of discharge home of the last baby from the approved investigational sites (recruiting sites) or from another hospital where the treated baby could have been transferred from the original recruiting site for continuation of clinical care.

In case the last baby is discharged home prior to 36-week PMA, the end of the trial will correspond to a clinical assessment at 36-week PMA (on-site visit or phone call).

The approved investigational site is responsible for the proper follow-up, data collection and documentation of the primary and secondary outcomes until the baby is discharged home. The investigator of the recruiting site will have to keep contacts and provide the continuing care site with written instruction about management of the above as well as for the reporting of serious AE, if any.

4. SUBJECT SELECTION CRITERIA

4.1 Subject Recruitment

The study population consists of premature neonates with RDS. Up to one hundred and twenty-six (126) patients of either sex with clinical finding of RDS (63 in each treatment arm) are expected to be randomized in the study. In this trial the eligible neonates will be drawn from those born in the collaborating centers (inborn neonates), who meet all the inclusion and none of the exclusion criteria. No limitation by ethnic origin, gender or social status will be used. RDS is defined in the study population by the presence of respiratory symptoms and signs in the first 24 hours of life, associated to the need for increased inspired oxygen requirements.

4.2 Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Written informed consent obtained by parents/legal representative (according to local regulation) prior to any study-related procedures
2. Inborn preterm neonates of either sex with a gestational age of 24^{+0} weeks up to 29^{+6} weeks
3. Clinical course consistent with RDS
4. Requirement of endotracheal surfactant administration within 24 hours from birth
5. Fraction of inspired oxygen (FiO_2) ≥0.30 for babies 24^{+0} to 26^{+6} weeks and FiO2 ≥0.35 for babies 27^{+0} to 29^{+6} weeks to maintain SpO_2 between 88–95%.

4.3 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment:

1. Use of surfactant prior to study entry and need for intratracheal administration of any other treatment (e.g. nitric oxide)
2. Known genetic or chromosomal disorders, major congenital anomalies (cardiac malformations, myelomeningocele etc)
3. Mothers with prolonged rupture of the membranes (>21 days duration)
4. Strong suspicion of congenital pneumonia/infection, sepsis
5. Presence of air leaks if identified and known prior to study entry
6. Evidence of severe birth asphyxia
7. Neonatal seizures prior to study entry
8. Any condition that, in the opinion of the Investigator, would place the neonate at undue risk
9. Participation in another clinical trial of any placebo, drug or biological substance conducted under the provisions of a protocol.

4.4 Subject Withdrawals

Parents/legal representative have the right to withdraw the subject from the study at any time for any reason.
The Investigator has also the right to withdraw the subjects from the study in the event of:

- An adverse event which is considered intolerable by the Investigator prior to or after surfactant administration
- An abnormal laboratory test result that the Investigator considers clinically significant and warranting the withdrawal of the subject prior to surfactant administration.
- An intercurrent clinical condition/disorder/anomaly that necessitates pharmacological treatment with a drug which interacts in any way with the test treatment prior to surfactant administration
- The development of any exclusion criterion prior to surfactant administration.

The Investigator is responsible for the optimal individual treatment for the subject. The Investigator must fill in the "Study Termination" page in the electronic case report form (eCRF) explaining the main reason for withdrawal.

5. CONCOMITANT MEDICATIONS

Subjects may be treated with other medications as needed in the critical care situation. The treating physician will manage neonates as he/she deems to be in their best interests. Concomitant medications will be recorded in the CRF.

5.1 Permitted concomitant Medications

Any concomitant medication required for the normal care of preterm neonates will be permitted during the study.

5.2 Non-permitted previous and concomitant Medications

1. Surfactant treatment prior to study entry
2. Intratracheal administration of any other treatment (e.g. nitric oxide) prior to study entry
3. Other investigational drugs.

6. TREATMENT(S)

6.1 Appearance and Content

The drug product CHF 5633 is a sterile suspension, with a pH of 6.0 compatible with the physiologic one in the lungs' and designed specifically for endotracheopulmonary instillation. The suspension contains a combination of two phospholipids and two peptides, in a sterile saline solution at the final concentration of 80 mg/ml. The drug product is filled in sealed 3.0 ml glass vials under nitrogen atmosphere. The composition is reported in the table below.

Table 2.4/5: Drug Product composition

Ingredients	Quantity per mL (mg)	Function
CHF 4902.03 (SP-C analogue)	1.2	Surfactant's co-adjuvant agent
CHF 5736.03 (SP-B analogue)	0.16	Surfactant's co-adjuvant agent
CHF 5300.00 (DPPC)	39.32	Surfactant
CHF 5301.02 (POPG, sodium salt)	39.32	Surfactant
0.9% sodium chloride sterile solution for injection	q.s. to1.0 mL	Suspending agent
1% sterile acetic acid to pH	q.s to pH	pH adjustment agent
1.4% sodium bicarbonate sterile solution qs to pH	q.s to pH	pH adjustment agent

Sterile nitrogen flow is used to flush bottle's head space.

Poractant Alfa (Curosurf®) is a standard natural surfactant treatment prepared from porcine lungs for intratracheal administration and containing almost exclusively polar lipids, in particular phosphatidylcholine (about 70% of the total phospholipid content), and about 1% of specific low molecular weight hydrophobic proteins SP-B and SP-C, with a total concentration of 80 mg/ml. The drug product is filled in sealed 3.0 ml glass vials.

Chiesi Farmaceutici S.p.A. will supply the study medication and also provide the Pharmacist/Investigator with appropriate certificates of analytical conformity.

6.2 Dosage and Administration

6.2.1 Selection of doses in the study

Surfactant doses used in the study follow the recommendations approved in the USA SmPC for the administration of Poractant Alfa (Curosurf®), determined as 200 mg/kg for the first dose and 100 mg/kg for additional doses. For this reason, CHF 5633 will be initially administered at the dosage of 200 mg/kg (total drug substance components).
Pharmacodynamic studies, both in vitro and in vivo in premature newborn rabbits [16], showed that 200 mg/kg of CHF 5633 has a profile comparable to that of approved naturally derived surfactants (Survanta® and Poractant Alfa, Curosurf®).
In the FIH trial [17] a lower dose of 100 mg/kg was included for safety reasons and showed to be safe and effective. The same positive profile was demonstrated for the upper dose of 200 mg/kg.
Extreme preterm neonates' lungs present very immature features, such as simplified saccular airways, no true alveoli and wide interstitial spaces, making them very susceptible to lung injury [18]. Given the vulnerability of this population, the higher dose of 200 mg/kg has been chosen to achieve a clear efficacy signal and the possible greatest beneficial effect.

6.2.2 Dosage

CHF 5633 2.5 ml/kg (200 mg/kg) sterile suspension in one single intratracheal administration within 24 hours from birth.
CHF 5633 1.25 ml/kg (100 mg/kg) will be used for further re-dosing when needed (up to two repeat doses).

Poractant Alfa (Curosurf®) 2.5 ml/kg (200 mg/kg) sterile suspension in one single intratracheal administration within 24 hours from birth. .
Poractant Alfa 1.25 ml/kg (100 mg/kg) will be used for further re-dosing when needed (up to two repeat doses).

6.2.3 Administration

Before the administration of CHF 5633 or Poractant Alfa, the condition of the neonate should be stabilized, correcting any acidosis, hypotension, anaemia or hypothermia. The following modes and techniques for surfactant administration are the ones to be used in the present study:

a. Disconnecting endotracheally intubated neonates from the ventilator, leaving the endotracheal tube
   Disconnect the infant momentarily from the ventilator and administer the suspension, as a single bolus, directly into the lower trachea via the endotracheal tube. Perform approximately one minute of hand-bagging and then reconnect the infant to the ventilator at the same settings as before administration.

b. Without disconnecting endotracheally intubated neonates from the ventilator
   Administer the suspension, as a single bolus, directly into the lower trachea by passing a catheter through the suction port into the endotracheal tube or using an endotracheal tube with a side port.

c. Infants stabilized on nasal continuous positive airway pressure (nCPAP) may also receive surfactant via an endotracheal tube solely introduced for the purpose of surfactant administration or with brief ventilation and rapid extubation. The tube is withdrawn after surfactant administration, and the infant is put on CPAP. If the condition of the baby worsens, standard of care measures could be applied at the discretion of the investigator

The CHF 5633 suspension will be withdrawn from the vial using a sterile syringe through a large-gauge needle (e.g., at least 20 gauge) and the effective dose calculated on the basis of the weight of the neonate could be administered as a single bolus dose or as two half bolus doses into the lower part of the trachea, positioning the neonate on each side as each bolus is being administered.

Since both test treatments will be refrigerated, each vial must be warmed up slowly to room temperature e.g. by placing it in an incubator for about 1 hour or keeping it in hands for 5-10 minutes and gently turned upside down, without shaking, avoiding the formation of foam. Artificial warming methods should not be used.

Re-treatment will be undertaken according to the criteria reported in Section 7.1 The exact time of the rescue treatment will be noted in the appropriate section of the eCRF.
No suctioning of the airways should be performed for two hours after each surfactant instillation unless the treating physician deems this appropriate, in which case this will be noted in the appropriate section of the eCRF.
All used vials will be retained at the site until the conclusion of the trial. Each vial is for single use only. Any leftover medication is to be retained for drug accountability. And any need for re-dosing will require a new vial to be issued via IRT (***see Section 6.6***).
After surfactant administration, the specific method of respiratory support (mechanical ventilation or non-invasive support) will be provided at the discretion of the investigator

6.2.4 Subject Training

Not Applicable.

6.3 Packaging

Chiesi Farmaceutici S.p.A. will supply both test treatments. A central depot will be in charge of clinical trial supplies' distribution to the Investigational sites.
Glass vials containing the study treatment will be packed in double blind condition. An initial supply containing single vials of 3 ml each of CHF5633/Poractant Alfa for both initial dosing and possible re-dosing will be provided to the investigational sites and will be packed in an outer box. Each vial is for single use only. Any need for re-dosing will require a new vial to be issued via IRT (***see Section 6.6***).

6.3.1 Primary packaging

Glass vial of 3 ml volume at a concentration of 80 mg/ml for both initial dosing and possible re-dosing. The actual volume of instilled test treatment will be reported in the relevant section of the eCRF.

6.3.2 Secondary packaging

A holder containing one glass vial of 3 ml volume.

6.4 Labeling

All labeling will be in English and according to US law and FDA regulatory requirements in compliance with Annex 13 and GMPs.

6.5 Treatment allocation

A dynamic randomization method will be used to balance the treatment groups by investigational site and gestational age group (i.e. from 24^{+0} to 26^{+6} weeks and from 27^{+0} to 29^{+6} weeks). Patients will be centrally assigned to one of the two treatment arms on admission through an IRT system (Interactive Response Technology, combination of voice and web response system and also referred as IVRS/IWRS).

The IRT will allocate the patient to a certain treatment group using the Pocock and Simon minimization algorithm [19] and assign the study medication kit number corresponding to the patients' treatment group. IRT specifications will be fully described in a specific document. The IRT will also generate a confirmation after every IRT transaction is performed.

The Investigator will call the IRT on admission to receive the patient number, and before treatment administration to randomize the patient and to obtain the medication kit number. Detailed instructions for use of IRT will be provided to the site.

The patient will be identified by a unique number of six digits: the country will be the first digit, the investigational site number will be the second two digits and the following three digits will be the progressive numbering of the patient within each site (e.g. 101001, 101002 etc.).

6.6 Treatment Code

The medication list will be provided to the labeling facility but will not be available to patients, Investigators, monitors or employees of the center involved in the management of the trial before unblinding of the data, unless in case of emergency.
The Sponsor's clinical team will also be blinded during the study as they will not have direct access to the randomization list nor to the medication list.
In case of emergency, unblinding of the treatment code will be done through IRT. The treatment group will be disclosed and confirmation will follow (by fax and/or notification email). The IRT will be designed to send a confirmation (by fax and/or notification email) to the site for every transaction performed by the Investigators. The Investigator will be provided with usernames and passwords for randomization purposes and to unblind the study treatment in case of emergency situation, where he/she considers essential to know what treatment the patient was taking. The IRT will promptly notify the Sponsor and the Clinical Monitor whenever a treatment code is unblinded.
Users from Chiesi Global Pharmacovigilance will have their own passwords to unblind patients in case of SUSARs to be reported to the competent Regulatory Authorities and Ethic Committees/IRB.

6.7 Treatment compliance

Each CHF 5633 or Poractant Alfa vial will be designated by code number and medication number according to the medication list and the actual volume of instilled test treatment will be noted in the hospital chart and on the eCRF of the study. The residual volume in each vial will have to be checked by the study monitor with the support of the Investigator or the hospital pharmacist during the monitoring visits, and all the residual vials will be maintained until the end of the study.
Moreover, also the code and medication number of the surfactant (CHF 5633 or Poractant Alfa) vials used as rescue treatment as well as the actual volume instilled will be recorded in the hospital chart, in the eCRF of the study and in the relevant surfactant accountability form and will be checked by the clinical monitor at the study visits. The completely or partially used vials will be kept in a sealed labelled plastic box/envelop with patient's screening number/initials until the end of the study for final accountability.

6.8 Drug Storage

The Pharmacist/Investigator will be responsible for the safe storage of all medications assigned to this study, in a secure place with restricted access, and maintained within the appropriate ranges of temperature.
CHF 5633 and Poractant Alfa will have to be stored between 2° and 8°C, protected from light (see CTS instruction leaflet)

6.9 Drug Accountability

The Investigator or pharmacist is responsible for the management of all the test treatments to be used for the study. Test treatments should be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense the study drugs.
An inventory will be maintained by the Investigator or pharmacist (or other nominated individual), to include a signed account of all the test treatment(s) received, dispensed and returned by each subject at the planned visits.
At the conclusion or termination of the study, the Investigator or the pharmacist shall conduct and document a final drug supply (used and unused) inventory. An explanation will be given for any discrepancies.
No surfactant material supplied for this trial is to be used for any other purpose.
In addition a signed account shall be kept of all test treatments administered to each patient.
All the test treatments supplied, used or unused, will be returned to the Sponsor or to the designated CRO under Sponsor's responsibility or destroyed directly by the Pharmacy of the involved investigational site upon provision of destruction certificate and documentation assuring the occurred destruction according to standard procedures and filed both at investigator's site file and at Sponsor/CRO trial master file.

6.10 Provision of additional care

At study completion, it is the Investigator's responsibility to prescribe appropriate treatment for the patient.

7. STUDY PLAN

7.1 Study Schedule

As described in Section 3. Study Design, this study will be conducted in phases and follow-up periods: on admission to the study (within the first 24 hours of life), study treatment administration and up to 24 hours after receiving it (at 30 min., 1, 3, 6, 12, 18, 24 hours), 2, 3, 5 and 7 days after initial treatment, and follow-up periods (28±2 days PNA, discharge home and 36 weeks PMA). Moreover, a further clinical assessment will be performed at 24 months (±3 months) corrected age to check the neurological and general health status related to the condition of prematurity.

If needed, up to two repeat doses of 100 mg/kg each may be administered at approximately 12-hour intervals with the same surfactant as the first dose, according to the double blind design and the below pre-defined criteria:

- lack of reduction in FiO_2 ≥0.10 to keep arterial oxygen saturation (SpO_2) between 88–95% or other clinical evidence of lack of efficacy within 12 hours after first/previous dosing;
- babies mechanically ventilated with FiO_2 ≥0.30 for babies 24^{+0} to 26^{+6} weeks and FiO_2 ≥0.35 for babies 27^{+0} to 29^{+6} weeks within 12 hours after first/previous dosing;
- babies not mechanically ventilated with FiO_2 ≥0.35 for babies 24^{+0} to 26^{+6} weeks and FiO_2 ≥0.40 for babies 27^{+0} to 29^{+6} weeks within 12 hours after first/previous dosing.

However, if a neonate still fails to show a clinical improvement after further re-dosing with the same surfactant as the first dose (e.g. FiO_2>0.40 to maintain $SatO_2$ 88-95%, respiratory acidosis defined as pCO_2 >65 mmHg/8.5 kPa and pH<7.20 on an arterial or capillary blood gas), he/she could receive an alternative surfactant treatment according to Investigator's discretion. Such treatment would need to be appropriately documented as a concomitant medication in the CRF.
The study phases and follow-up periods are detailed in the following sections and summarized in the following flow chart (Table 2):

TABLE 2 - FLOW-CHART

ASSESSMENT	ON ADMISSION prior to study treatment within first 24 hours of life (Day -1)	MINUTES/HOURS/DAYS								DAYS/WEEKS						
		0	30' (±5')	1-h (±10')	3-h (±30')	6-h (±30')	12-h (±30')	18-h (±30')	24-h (±30')	Day 2	Day 3	Day 5	Day 7	Day 28±2 PNA	Discharge home	36-WEEK PMA	24-month clinical assess.
Randomization/Study treatment administration		X															
Parental Informed Consent (1)	X																
Inclusion/Exclusion Criteria	X																
Complications during pregnancy	X																
Demographic data	X																X
Apgar Score	X																
Neonatal complications	X																
Vital signs	X	X(+)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chest x-ray	X(*)								X(*)								
Cranial sonography	X(*)												X (**)	(X)*			
Haematology and Biochemistry									X								
Arterial Oxygen Saturation (SpO_2)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Ventilator settings (FiO_2, PIP, MAP, PEEP) (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
A-a Oxygen gradient and OI (3)	X				X	X	X	X	X	X							
Duration of oxygen alone									X	X	X	X	X	X	X	X	
Neonatal Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Immunogenicity assessment in serum	X													(X)***			
Tracheal aspirates (3)	X								X	X							
Assessment of BPD																X	
Adverse Events/Adverse Drug Reactions	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Bayley Scales																	X
Health status questionnaire																	X

1. Signature of Parental Informed Consent can be obtained prior to birth or after birth; 2. PIP, MAP, PEEP to be monitored and collected until Day 7; 3. In a subgroup of babies when feasible; (X)+ Only heart rate at time 0 within 5 min from study drug administration; (X)* When applicable, at discretion of the Investigator ; (X)** At least one cranial ultrasound within the first 7 days of life; (X)*** The blood sampling has to be taken with a window of 3 to 6 weeks from administration.

7.1.1 Phase 1 - On admission prior to study treatment administration (within first 24 hours of life)

The study will be explained to the parents of a potentially eligible baby prior to birth, if possible, giving the parents the maximum time to make an informed choice. Once their written informed consent has been obtained either prior to or after birth, the neonate's eligibility to take part in the study will be assessed according to the post-birth inclusion and exclusion criteria. If the baby becomes eligible, then the medical history of the mother, the complications during pregnancy and the prenatal use of corticosteroids will be collected.

If the baby becomes eligible, then the following information will be collected:

- birth weight;
- Apgar score;
- neonatal complications and concomitant medications;
- clinical signs of RDS (Chest X-ray when applicable, at discretion of the Investigator);
- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- Respiratory support (invasive or non-invasive), ventilatory settings (PIP, FiO_2, PEEP, MAP), OSI and RSS;
- Invasive oxygen measurements (A-a oxygen gradient, OI), when feasible, in a subgroup of infants
- 400 µL blood sample for immunogenicity assessments in serum ;
- Cranial ultrasound when applicable, at discretion of the Investigator;
- Tracheal aspirates for the evaluation of inflammatory biomarkers to be shipped to a central laboratory for analysis, when feasible, in a subgroup of patients;
- Any adverse events occurring after the Informed Consent Form Signature, in the AE Form of the eCRF. If the abnormality starts prior to the study drug administration and it is listed among the Morbidities or Complications of Prematurity (see Section 10.8) which are clinically expected outcomes in preterm neonates aged between 24^{+0} and 29^{+6} weeks, it should be recorded in the dedicated form.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.2 Phase 2 - Up to 24 hours after test treatment administration

Once the neonate's eligibility to take part in the study is confirmed, the Investigator will have to call/check the IRT to receive the kit number to be used and then the test treatment will be administered within 24 hours from birth. The tear-off sticker from the medication box will be applied in the appropriate section of the surfactant accountability log.

At the administration (Time 0) the following information will be collected:

- SpO_2 and heart rate within 5 min from study drug administration;

Then, after the administration, the following information will be collected:

- SpO_2 and vital signs (systolic and diastolic blood pressure, heart rate) at 30 min, 1 hour, 3, 6, 12, 18 and 24 hours after test treatment administration;
- ventilator settings (PIP, FiO_2, PEEP, MAP), RSS and OSI at 30 min, 1 hour, 3, 6, 12, 18 and 24 hours after test treatment administration. Mechanical ventilation will be monitored and any change in invasive and non-invasive ventilation will be recorded with its date and time;
- Invasive oxygen measurements (A-a oxygen gradient, OI), when feasible, in a subgroup of infants at 3, 6, 12, 18 and 24 hours after treatment;
- Use of oxygen alone (supplemental oxygen without invasive or non-invasive ventilator support) will be monitored and any occurrence will be recorded with its date and time of start and end;
- Blood tests within 24 hours after test treatment administration, defined as full blood count (FBC), urea, creatinine and electrolytes (sodium, potassium, calcium, phosphorus), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, C-reactive protein;
- RDS or other respiratory anomalies 's diagnosis confirmation (Chest X-ray when applicable, at discretion of the Investigator);
- At least one cranial ultrasound within the first 7 days of life;
- Tracheal aspirates for the evaluation of inflammatory biomarkers to be shipped to a central laboratory for analysis, when feasible, in a subgroup of patients;
- new neonatal concomitant medications and adverse events as well as changes in those already reported.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.3 Phase 3 - 2 days after test treatment administration (from 24hrs + 1min to 48hrs after dosing)

The following information will be collected:

- SpO_2
- vital signs (systolic and diastolic blood pressure, heart rate)
- ventilator settings (PIP, FiO_2, PEEP, MAP) and RSS;
- Oxygen Saturation Index (OSI);
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- Invasive oxygen measurements (A-a oxygen gradient, OI), when feasible, in a subgroup of infants;
- Use of oxygen alone (supplemental oxygen without invasive or non-invasive ventilator support) will be monitored and any new occurrence will be recorded with the relevant dates and times;
- At least one cranial ultrasound within the first 7 days of life;
- Tracheal aspirates for the evaluation of inflammatory biomarkers to be shipped to a central laboratory for analysis, when feasible, in a subgroup of patients;
- new neonatal concomitant medications and adverse events as well as changes in those already reported.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.4 Phase 4 - 3 days after test treatment administration (from 48 hrs+ 1 min to 72hrs after dosing)

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- ventilator settings (PIP, FiO_2, PEEP, MAP);
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- Use of oxygen alone will be monitored and any new occurrence will be recorded with the relevant dates and times;
- At least one cranial ultrasound within the first 7 days of life;
- new neonatal concomitant medications and adverse events as well as changes in those already reported.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.5 Phase 5 - 5 days after test treatment administration (from 96 hrs to 120 hrs after dosing)

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- ventilator settings (PIP, FiO_2, PEEP, MAP);
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- use of oxygen alone will be monitored and any new occurrence will be recorded with the relevant dates and times;
- At least one cranial ultrasound within the first 7 days of life;
- new neonatal concomitant medications and adverse events as well as changes in those already reported.

In the event that multiple measurements are available collect the measurements closest to the required time point.

7.1.6 Phase 6 - 7 days after test treatment administration (from 144hrs to 168 hrs after dosing)

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);

- ventilator settings (PIP, FiO_2, PEEP, MAP);
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- use of oxygen alone will be monitored and any new occurrence will be recorded with the relevant dates and times;
- At least one cranial ultrasound within the first 7 days of life;
- new neonatal concomitant medications and adverse events as well as changes in those already reported.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.7 Phase 7 (Follow-up) – 28±2 days postnatal age (PNA)

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- FiO_2;
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- use of oxygen alone will be monitored and any new occurrence will be recorded with the relevant dates and times;
- cranial sonography when applicable at phases 7 or 8 or 9 just before discharge home.
- new neonatal concomitant medications and adverse events as well as changes in those already reported
- 400 µL blood sample for immunogenicity assessments in serum when applicable at Phase 7 or 8 or 9 (to be taken only once), approximately 5 weeks after administration (with a window from 3 to 6 weeks).

In case the baby was discharged home prior to 28 days of life, the baby should be recalled for an on-site clinic visit at the recruiting site. If not feasible, any effort should be made to collect information about the occurrence of any adverse events as well as about any change in concomitant medications. The Investigator should contact the family pediatrician to collect the relevant information.
In case the treated baby was transferred to and discharged home by a continuing care site prior to 28 days of life or still hospitalized there, the baby should be recalled at the recruiting site for the visit. If this is not feasible, the visit can be replaced by a contact/phone call by the Investigator to the physician of the continuing care site or to the family pediatrician to check the occurrence of any other adverse event as well as of any change in concomitant medications.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.8 Phase 8 (Follow-up) – Discharge home

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- FiO_2;
- any change in non-invasive/invasive ventilation with the relevant dates and times;
- use of oxygen alone will be monitored and any new occurrence will be recorded with the relevant dates and times;
- cranial sonography if not already performed at phases 7 or 9;
- new neonatal concomitant medications and adverse events as well as changes in those already reported;
- 400 µL blood sample for immunogenicity assessments in serum if not already taken at day 28 PNA or 36 weeks PMA, according to what described in paragraph 7.2

The discharge home can occur whenever deemed appropriate by the investigator according to the clinical condition of the baby. In case the baby is transferred to a continuing care site, the recruiting site is responsible for the proper continuation of data collection and documentation of the primary and secondary outcomes. The investigator of the recruiting site should keep contacts and provide the continuing care site with written instruction about management of the above as well as for the reporting of serious AE, if any. The recruiting site should receive a discharge letter clarifying as much detailed information as possible the progress in patient's conditions.
Routine clinical safety data included in the discharge home summary letter should be sought to complete data collection and register the end of the trial, if applicable.
If the discharge home coincides with the 36 wks PMA follow-up, only the assessments foreseen at 36 wks PMA will be performed and reported in the eCRF.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.9 Phase 9 (Follow-up) – 36-week PMA (from 36^{+0} to 36^{+6} weeks)

The following information will be collected:

- SpO_2 along with vital signs (systolic and diastolic blood pressure, heart rate);
- FiO_2;
- any change in non-invasive/invasive mechanical ventilation with the relevant dates and times;
- use of oxygen alone (supplemental oxygen without invasive or non-invasive ventilator support) will be monitored and any new occurrence will be recorded with the relevant dates and times;
- cranial sonography if not already performed at phases 7 or 8;
- new neonatal concomitant medications and adverse events as well as changes in those already reported;
- BPD diagnosis (see definition in Appendix II) ;
- 400 µL blood sample for immunogenicity assessments in serum if not already taken at day 28 PNA or at Discharge home, according to what described in paragraph 7.2;

In case the baby was discharged home or transferred to a continuing care site prior to this phase, the baby should be recalled as soon as possible for an on-site clinic visit at the recruiting site. If not feasible, any effort should be made to collect information about the occurrence of BPD, adverse events as well as any change in concomitant medications. The investigator should therefore contact the physician of the continuing care site or the family pediatrician to collect the relevant information.

In the event that multiple measurements are available, the measurements closest to the required time point are to be collected.

7.1.10 Phase 10 – Clinical assessment at 24 months (±3 months) corrected age

A further clinical assessment will be performed at 24 months (±3 months) corrected age by physicians of participating study centers. This clinical assessment will be based on:

- Demographic data
- Vital signs
- Bayley Scales of Infant Development (***see Section 7.2.5***)
- Health status questionnaire (health problems, illnesses, injuries, well-being, diet, respiratory assessment).

7.2 Investigations

7.2.1 Immunogenicity assessment

An immunogenicity assessment in serum will be carried out in about 400 µL blood sample additionally collected prior to test treatment administration and approximately at 5 weeks after administration (with a window from 3 to 6 weeks).In case there is no possibility to obtain the blood sample pre-dose without delaying the baby clinical care, a window of maximum 2 hours is allowed after surfactant administration.

To obtain serum, after collection the blood is allowed to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes. The clot is than removed by centrifugation at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge and serum is the resulting supernatant.
It is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2-8°C while handling and then stored below –65°C until shipped to the central laboratory ([redacted]) for the immunogenicity assay. A laboratory manual will be provided to the clinical sites by [redacted].

In case the treated baby is transferred to another hospital site, the blood sample will be taken by a trained study team member of the original site in which the baby received the study treatment and

transported back to the original site in refrigerated (not frozen) condition within maximum two hours, to be processed according to the above procedures.

The measure of anti-SP-B and anti-SP-C antibodies will be evaluated by a titration versus a positive control serum (GP) diluted in buffer solution. The titer is expressed as the dilution at which the sample exhibited a 0.050 absorbance (background). The entire range of dilutions will be plotted versus optical density. If a sigmoidal profile of the curve will be observed, it will show that the serum contains a significant level of antibodies.
[redacted] is allocated as reference laboratory in charge for this assessment and the samples will be shipped to the following address:

[redacted]

7.2.2 Biomarkers of Inflammation

CXCL8, Interleukin 1β (IL1β), Interleukin 6 (IL6), Tumor Necrosis Factor-alpha (TNF-α), myeloperoxidase (MPO) will be collected prior to study drug administration, at 24±1 hours and on day 2 (48±1 hours) (in a subgroup of babies who require endotracheal intubation for mechanical ventilation, when feasible). In case the baby is extubated earlier than 24 or 48 hours and when feasible, the tracheal aspirate can be collected at any time closest to the above time points.
The suggested procedure to collect the tracheal aspirates is the following:

- After instillation of 0.5 ml of 0.9% saline through the endotracheal tube, a 5 F catheter is inserted slightly beyond the distal tip of the endotracheal tube and all the secretions are aspirated. The tracheal secretions are collected through a Luken's trap. Three to five manual or ventilator breaths are given between instillation of saline and suctioning.
- The whole procedure is then repeated.
- The suction catheter is then flushed with 0.5mL of normal saline to collect the residual sample in the catheter. The final volume should be 1 ml.

The infant's heart rate, respiratory rate and oxygen saturation will be monitored and allowed to stabilize during the suctioning procedure.
The collected tracheal aspirate need to be immediately chilled in ice bath and centrifuged at 2000 x g for 15 min within 1 h. The supernatant will be recovered, divided into aliquots and kept below -65 °C until shipment to the central laboratory for analysis. Further details will be described in the laboratory manual that will be provided to each clinical site.

[redacted] is allocated as reference laboratory in charge for this assessment and the samples will be shipped to the following address:

[redacted]

7.2.3 A-a oxygen gradient and Oxygenation Index

Alveolar-arterial (A–a) oxygen gradient is a measure of the difference between the alveolar concentration of oxygen and the arterial concentration of oxygen obtained from the following equation:

$$Aa\ Gradient = \left(F_iO_2(P_{atm} - P_{H_2O}) - \frac{P_aCO_2}{0.8}\right) - P_aO_2$$

Oxygenation Index (OI) is defined as the ratio of RSS and partial pressure of oxygen in arterial blood (PaO_2).

Both OI and A-a gradient require an arterial blood gas analysis to calculate PaO_2. Given the challenges to placing arterial catheters in pre-term neonates, these parameters will be measured at 3, 6, 12, 18, 24 and 2 days post treatment (only in a subgroup of babies when feasible).

7.2.4 Vital signs and Blood tests

- Heart rate and $SatO_2$ are measured by pulse oximetry
- Systolic and diastolic blood pressures are measured in a non-invasive way
- Blood tests can be performed with an arterial, capillary or venous sample.

The following blood tests are to be performed: FBC, urea, creatinine, electrolytes, glucose, ALT, AST, CRP: 1-3 ml (depending on centers' assays).

7.2.5 Bayley scale

Bayley Scales of Infant Development is a standard series of measurements used primarily to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants and toddlers, aged 0-3. This measure consists of a series of developmental play tasks and takes between 45 - 60 minutes to administer. Raw scores of successfully completed items are converted to scale scores and to composite scores. These scores are used to determine the child's neurodevelopmental performance compared with norms taken from typically developing children of their age (in months). Completed by the parent or caregiver, this questionnaire establishes the range of adaptive behaviors that the child can currently achieve and enables comparison with age norms.

8. EFFICACY ASSESSMENTS

- SpO_2/FiO_2 at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA.

- Fraction of inspired oxygen (FiO_2) at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA;
- Respiratory Severity Score (RSS) will be calculated from ventilator setting as FiO_2 x MAP in the 2 days post treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours and on day 2);
- Oxygen Saturation Index (OSI) will be calculated as FiO_2 x MAP x 100 / SpO2 in the 2 days post treatment (at 30 minutes, at 1, 3, 6, 12, 18, 24 hours and on day 2);
- Arterial oxygen saturation determined by pulse oximetry (SpO_2) at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, at Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA;
- Mean airway pressure (MAP) at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, on Days 2, 3, 5, 7;
- Peak inspiratory pressure (PIP) at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, on Days 2, 3, 5, 7;
- Positive end-expiratory pressure (PEEP) at 30 minutes, at 1, 3, 6, 12, 18, 24 hours, on Days 2, 3, 5, 7
- Alveolar-arterial (A–a) at 3, 6, 12, 18, 24 hours and 2 days post treatment (only in a subgroup of babies when feasible).
- Oxygenation Index (OI) at 3, 6, 12, 18, 24 hours and 2 days post treatment (only in a subgroup of babies when feasible).
- Mortality/BPD rate defined as the incidence of patients who are dead or alive with a diagnosis of BPD at 36-week PMA;
- BPD incidence at 36-week PMA;
- Mortality rate at Day 28 PNA and 36-week PMA;
- RDS-associated mortality rate through 14 days of age, defined as deaths associated with RDS through the first 14 days of life, resulting from severe respiratory failure, from pulmonary hemorrhage if the RDS had not resolved before the development of this complication, or from air leaks that occurred in the presence of severe RDS;
- Percentage of patients requiring at least one surfactant rescue dose;
- Percentage of patients with at least one reading of FiO_2=0.21 (i.e. room air) within 24 hours from first dosing with test treatment;
- Time (hours) to reach FiO_2=0.21 (i.e. room air);
- Duration of invasive mechanical ventilation, defined as the time until the first successful extubation lasting more than 24 hours (days);
- Duration of non-invasive ventilation, defined by nasal continuous positive airway pressure (nCPAP), Bi-level positive airway pressure (BiPAP), nasal intermittent positive pressure ventilation (NIPPV), or high flow nasal cannula (HFNC) (days);
- Duration of oxygen use alone, defined as receiving supplemental oxygen without invasive or non-invasive ventilator support, at 24 hours after first surfactant dosing, at Days 2, 3, 5, 7, at Day 28±2 PNA, at discharge home and at 36 weeks PMA
- Biomarkers of inflammation in tracheal aspirates: CXCL8, Interleukin 1β (IL1β), Interleukin 6 (IL6), Tumor Necrosis Factor-alpha (TNF-α), myeloperoxidase (MPO), prior to study drug administration, at 24 hours and on day 2 (48 hours) (in a subgroup of babies who require endotracheal intubation for mechanical ventilation when feasible).

The following time deviations from theoretical post-dose times will be allowed:

Post-dose time assessments	Allowed deviations
30 minutes	± 5 minutes
1 hour	± 10 minutes
3, 6, 12, 18 and 24 hours	± 30 minutes
28 days PNA	± 2 days

9. SAFETY ASSESSMENTS

The following clinical and laboratory assessments will be performed to explore the safety of the study drug:

- AEs and ADRs
- Vital signs (systolic and diastolic blood pressure, heart rate) and SpO_2 during administration
- Incidence of major neonatal morbidities (for the list of neonatal morbidities and complications of prematurity ***see section 10.8***)
- Hematology and blood chemistry
- Immunogenicity assessment in serum will be carried out in samples collected at baseline prior to test treatment administration and approximately at 5 weeks after administration (with a window from 3 to 6 weeks)
- Bayley Scales of Infant Development at 24 months (±3 months) corrected age
- Health status questionnaire (health problems, illnesses, injuries, well-being, diet, respiratory assessment) at 24 months (±3 months) corrected age.

The following time deviations from theoretical post-dose times will be allowed:

Post-dose time assessments	Allowed deviations
30 minutes	± 5 minutes
1 hour	± 10 minutes
3, 6, 12,18 and 24 hours	± 30 minutes
28 days PNA	± 2 days

10. ADVERSE EVENTS REPORTING

10.1 Definitions

An **Adverse Event** is, "any untoward medical occurrence in a patient or clinical trial subject administered a medicinal (investigational or non-investigational) product and which does not necessarily have a causal relationship with this treatment." An adverse event can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease

temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not considered related to the medicinal (investigational or non-investigational) product.

An **Adverse Drug Reaction** is an, "untoward and unintended response to an investigational medicinal product related to any dose administered."
All adverse events judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression, "reasonable causal relationship," means to convey in general that there are facts (evidence) or arguments meant to suggest a causal relationship.
The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

A **Serious Adverse Event (SAE)/Serious Adverse Drug Reaction** is any untoward medical occurrence or effect that at any dose falls in one or more of the following categories:

- **Results in death**
  Death is not an adverse event but an outcome. It is the cause of death that should be regarded as the adverse event. The only exception to this rule is, "sudden death," where no cause has been established; in this latter instance, "sudden death," should be regarded as the adverse event and, "fatal," as its reason for being serious.
- **Is life-threatening**
  Life-threatening refers to an event in which the subject was at risk of death at the time of the event (e.g., aplastic anaemia, acute renal failure, and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- **Requires hospitalisation or prolongation of existing hospitalization**
  Hospitalization refers to a situation whereby an AE is associated with unplanned overnight admission into hospital, usually for purpose of investigating and/or treating the AE. Hospitalization for the treatment of a medical condition that occurs on an, "elective," or, "scheduled," basis or for a pre-existing condition that did not worsen during the study should not necessarily be regarded as an AE. Complications that occur during the hospitalization are AEs. If a complication prolongs hospitalization, the event is an SAE.
  Emergency room visits that do not result in a formal admission into hospital should be evaluated for one of the other seriousness criteria (e.g., life-threatening; persistent or significant disability or incapacity; medically significant).

- **Results in persistent or significant disability or incapacity.**
  The term significant disability should be viewed as any situation whereby an AE has a clinically important effect on the subject's physical or psychological well-being to the extent that the subject is unable to function normally.
- **Is a congenital anomaly or birth defect (not applicable for this study)**
- **Is a medically significant adverse event**

This criterion allows for any situations in which important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalization may jeopardize the subject's health or may require intervention to prevent one of the above outcomes.

Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether an event is serious because medically significant.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

A **Non-Serious Adverse Event/Non-Serious Adverse Drug Reaction** is an adverse event or adverse drug reaction that does not meet the criteria listed above for a serious adverse event/serious adverse drug reaction.

10.2 Expectedness

An expected adverse reaction is an adverse reaction, the nature or severity of which is consistent with the applicable Reference Safety Information (Investigator's Brochure for CHF 5633 and Summary of Product Characteristics for Curosurf®), otherwise it is considered unexpected.

Reports which add significant information on specificity or severity of a known, already documented serious adverse drug reaction constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered as "unexpected". General examples of such events are: (a) acute renal failure as a labelled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

10.3 Intensity of Adverse Event

The investigator is asked to assess the intensity of all the adverse events and to report them in the eCRF.

The intensity (severity) of a specific event is defined as mild, moderate, or severe; the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Each Adverse Event must be rated on a 3-point scale of increasing intensity:

Mild: The event causes a minor discomfort, or does not lead to establishment of a correcting treatment.

Moderate: The event perturbs the usual functioning of the subject. The event leads to the establishment of a correcting treatment.

Severe: The event prevents any usual functioning of the subject.

10.4 Causality Assessment

The following "binary" decision choice will be used by the Investigator to describe the causality assessment with the study medication:

- Reasonable possibility of a relatedness
- No reasonable possibility of relatedness

The expression "reasonable possibility of relatedness" is meant to convey, in general, that there are facts (evidence) or arguments meant to suggest a causal relationship.
The Investigator will be asked to consider the following before reaching a decision on causality assessment:

- Time relationship between study drug intake and event's onset;
- Medical history;
- Lack of efficacy/worsening of existing condition;
- Study treatment(s);
- Mechanism of action of the study drug;
- Class effects;
- Other treatments-concomitant or previous;
- Erroneous treatment with study medication (or concomitant);
- Protocol related process.

10.5 Action taken with study drug due to the AE

Since patients will receive a single dose of study drug, "action taken with study drug" will be not applicable.

10.6 Other actions taken

- Specific therapy/medication
- (Prolonged) Hospitalization
- Surgical/medical procedure

10.7 Outcome

Each Adverse Event outcome must be described by choosing among:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal
- Unknown

10.8 Recording Adverse Events

All AEs occurring during the course of the study must be documented in the Adverse Event page of the eCRF. Moreover, if the Adverse Event is serious, the Serious Adverse Event Form must also be completed.

It is responsibility of the Investigator to collect all adverse events (both serious and non-serious).
As a general rule, the recording period for Adverse Events is the period starting from the Informed Consent signature until the subject's study participation ends.
However, **the complications of prematurity**, listed below, are clinically expected outcomes in preterm neonates aged between 24^{+0} and 29^{+6} weeks. Therefore, **if started before drug administration,** they **haven't to be reported as AEs but in the relevant section of the eCRF**.
While **the occurrence or worsening of these complications of prematurity after test treatments' administration must be recorded as an AE in the case report form**.

Morbidities or Complications of prematurity

- Air leaks (pneumothorax, pneumomediastinum, pneumopericardium, pneumoperitoneum);
- anemia of prematurity;
- apnea of prematurity;
- necrotizing enterocolitis (NEC);
- patent ductus arteriosus (PDA);
- pneumonia;
- pulmonary interstitial emphysema (PIE);
- pulmonary hemorrhage;
- germinal matrix/intraventricular hemorrhage (GMH/IVH);
- cerebral parenchymal haemorrhage;
- neonatal jaundice;
- periventricular leukomalacia (PVL);
- sepsis and/or meningitis;
- Presence of the following abnormal laboratory values:
    - hypoglycaemia
    - hyperglycaemia
    - hypocalcaemia
    - hypercalcaemia
    - hyponatraemia
    - hypernatraemia
    - hypokalaemia
    - hyperkalaemia.

For study definitions see appendix II and its references.

The investigator should carefully check inclusion and exclusion criteria because some of these morbidities may not allow the neonate to be randomized.

If a clinically significant abnormal laboratory finding or other abnormal assessment detected during the study period meets the definition of an AE, then the AE eCRF page must be completed, as appropriate. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, should be reported on the AE eCRF

page. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded.

For pharmacovigilance purposes, all SAEs should be followed-up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or until the subject is lost to follow-up. Follow-up may therefore continue after the subject has left the study. In this case, the follow-up will continue with no timelines for related SAEs, while for unrelated SAEs the type and extent of follow-up undertaken will be determined for each individual case and will depend upon the nature (e.g. events with poor prognosis or which do not resolve), severity and medical significance of the event.
Follow-up may therefore continue after the patient has been discharged from the NICU.

10.9 Reporting Serious Adverse Events to the Sponsor

The Investigator must report all Serious Adverse Events to the [redacted] Safety Contact within 24 hours of awareness. The information must be sent by providing the completed Serious Adverse Event form. At a later date, the [redacted] Safety Contact will report all information to Chiesi Global Pharmacovigilance, the Clinical Project Manager and the Clinical Research Physician.

Name and Title	Telephone no.	Fax no.	E-mail
[redacted]	[redacted]	[redacted]	[redacted]
[redacted] Global Pharmacovigilance Chiesi Farmaceutici S.p.A., Parma (Italy)	[redacted]	[redacted]	[redacted]

- Reporting of SAEs from the investigator site is from the time of subject's signature of informed consent and until the subject's study participation ends. Moreover, even after the end of the study, if the investigator becomes aware of an SAE and deems it as related to study drug, he/she should report this SAE.
- Up to the closure of the recruiting site, these SAE reports should be reported to the CRO Safety Contact. All related SAEs occurring after the recruiting site is closed should be directly reported to the Sponsor Safety Contact.

10.10 Reporting Serious Adverse Events to Regulatory Authorities/Ethics Committees/IRB

The Sponsor or designated CRO will report adverse events to the regulatory authorities in compliance with the timelines and standards of reporting according to local regulations (Guidance for industry and Investigators-Safety Reporting Requirements for INDs and BA/BE studies,

December 2012). All suspected unexpected serious adverse reactions (SUSARs), which occur with the investigational medicinal product or marketed active comparator, within or outside the concerned clinical trial, will be reported by the Sponsor or designated CRO to regulatory authorities, as required, as well as to the Investigators and Central IRB, if applicable, by MedWatch/CIOMS form. The Investigator (or Sponsor/CRO where required) must inform the IRB per Sponsor instruction upon receipt of the SUSAR notification. An IND and/or NDA Safety Report will be submitted to regulatory authorities unblinded. Participating Investigators and IRB will receive a blinded IND Safety Report, unless otherwise specified.

10.11 General Notes

- In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and provided to the [redacted] Safety Contact together with the Serious Adverse Event form, retaining a copy on site;
- If an autopsy is performed, copy of autopsy report should be actively sought by the Investigator and provided to the [redacted] Safety Contact as soon as available, retaining a copy on site;
- If a baby is transferred to a continuing care site prior to discharge home, the investigator should ensure that the receiving hospital site is aware that the baby is participating to the present study and communication should be maintained in order to record any adverse event. Moreover, the investigator of the recruiting site will have to provide the continuing care site with written instruction about management and reporting of SERIOUS AE. After discharge home from the continuing care site a copy of the discharge summary letter should be requested in order to cross reference any adverse events and to determine if the baby is suffering from BPD.

10.12 Independent Safety Monitoring Board

An Independent Safety Monitoring Board (ISMB) will be established to ensure the safety of study subjects on an on-going basis. The involvement of independent expert advisors will provide an unbiased evaluation of the overall safety in the study, with particular regard to the incidence of major adverse outcomes (i.e. Serious Adverse Events) following study treatment administration.
The ISMB will be composed by independent Clinicians and one independent Biostatistician.
The ISMB will have periodical face-to face or telephone meetings, as appropriate, and a Safety Assessment Report will be issued after each meeting.
The Sponsor (and other study personnel) may be involved in some parts of the ISMB meetings, however, they will never have access to unblinded data and/or unblinded/coded comparisons.
All ISMB members will keep as confidential all information and data deriving from the ISMB activity, without disclosing them to others.
The complete ISMB guidelines including details regarding the members, duties and responsibilities, work and communication processes, frequency of meetings will be established by the ISMB members during the first meeting and reported in a separate charter.

11. DATA MANAGEMENT

An electronic case report form (eCRF) to be filled in by the investigator and/or his/her designee will be used.
Front-end edit checks will run at the time of data collection and back-end edit checks will be run by the Data Manager to check for discrepancies and to ensure consistency and completeness of the data.
Complications of prematurity, procedures and adverse events will be coded using the MedDRA dictionary; medications will be coded using the WHO Drug dictionary and Anatomical Therapeutic Chemical classification (ATC). The same dictionaries will be used to code mother's medical history, complications during pregnancy and mother's prenatal medications.
External data (Immunogenicity and Biomarkers of Inflammation) will be processed centrally and reconciled against data recorded in the eCRF as part of cleaning activities.
Laboratory normal ranges will be collected prior to FPFV at each site and provided to CRO data management that will enter them in the eCRF. Laboratory results will be entered in the eCRF by the investigator.
After cleaning of data, a review meeting will be held to determine the occurrence of any protocol violation and to define the patient populations for the analysis.
Once the database has been declared to be complete and accurate, it will be locked, the randomization codes will be opened and the planned statistical analysis will be performed. Only authorised and well-documented updates to the study data are possible after database lock.
A CD-ROM of the patient data will be sent after database lock at the investigational site for archiving.
The 24-month clinical assessment data will be collected by a paper CRF and recorded in a separate database.

12. STATISTICAL METHODS

12.1 Sample Size

According to the exploratory nature of this this study, no formal power calculation was performed.
A maximum number of sixty-three (63) randomized neonates per treatment group (126 in total) is deemed sufficient to describe the efficacy and safety profile of CHF5633 compared to Poractant Alfa.

12.2 Populations for analysis

The following populations will be considered for the analysis:

- **Intention-to-Treat population (ITT):** all randomized patients who received at least one dose of study medication and with at least one available evaluation of efficacy after the baseline.
- **Safety population:** all randomized patients who took at least one dose of study medication.

The efficacy analyses population will be performed in the ITT population. The Safety population will be used in the analysis of all safety variables.
In case of deviation between as-randomized treatment and treatment actually received, the treatment actually received will be used in the safety analyses (i.e. an as-treated analysis will be performed).

12.3 Statistical analysis

A detailed statistical analysis plan (SAP) will be described in a separate document. The plan might be reviewed and updated as a result of the blind review of the data and will be finalized before breaking the blind.

12.3.1 Descriptive Statistics

General descriptive statistics for numeric variables will include the n (number of observed values), the mean, the standard deviation, 95% CI, the median, the minimum, and the maximum values. For categorical variables, the number and percent of subjects with a specific level of the variable will be presented.

12.3.2 Missing data

Details on dealing with missing data, along with the handling of possible outliers, will be described in the SAP. Critical missing data, if any, will be discussed during the review of the data. Decisions will be fully documented in the Data Review Report.

12.3.3 Description of the population-description of baseline characteristics

Demographics and baseline variables will be summarized by treatment group using descriptive statistics for the ITT population.
The following variables will be summarized by treatment group and overall in order to describe the study population: neonate baseline characteristics and demographics (birth weight, gestational age, sex, race, APGAR score), maternal history including the mother's age, race, medical and pregnancy history, current and pre-birth medications and delivery conditions.

12.3.4 Efficacy variables

Arterial Oxygen Saturation and Ventilator settings

SpO2/FiO2

- SpO_2/FiO_2 will be analyzed using a linear mixed model for repeated measures (MMRM) including treatment, time point, treatment by time point interaction, investigational site and gestational age group as fixed effects and pre-dose SpO_2/FiO_2 as covariate. The adjusted means in each treatment group, the adjusted mean difference between treatments and their 95% confidence intervals (CIs) at each time point and averaged over the first 24 hours will be estimated by the model.

Time profile plot of mean SpO_2/FiO_2 in the first 24 hours post-treatment will be presented by treatment group.

- SpO_2/FiO_2 will be compared between treatments at the remaining post-treatment time points (i.e., Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA) using mixed model including treatment, investigational site and gestational age group as fixed effects and pre-dose SpO_2/FiO_2 as covariate.
- SpO_2/FiO_2 will be analyzed by subgroups based on ventilator support used.

FiO2

- FiO_2 will be analyzed at each time point and averaged over the first 24 hours post dose using the same model used for the SpO_2/FiO_2. Pre-dose FiO_2 will be used as covariate.
  Time profile plot of mean FiO_2 in the first 24 hours after treatment will be presented by treatment group.
- FiO_2 will be compared between treatments at the remaining post-treatment time points (i.e., Days 2, 3, 5, 7, 28±2 PNA, at discharge home and at 36 weeks PMA) using linear mixed model including, treatment, investigational site and gestational age group as fixed effects and pre-dose FiO_2 as covariate.
- Percentage of patients with at least one reading of FiO_2 equal to 0.21 (i.e. at room air) within 24 hours from first intake will be compared by treatment group by means of a Fisher's exact test at 5% significance interval. Odds Ratio (OR) and related exact 95% CI will be also provided.
- The median duration time to reach FiO_2 equal to 0.21 will be compared between treatment groups by the Mann-Whitney U-test.

Respiratory Severity Score

- RSS, calculated from ventilator settings as MAP x FiO_2, will be analyzed at each time point and averaged over the first 24 hours post study drug intake using the same model used for the SpO_2/FiO_2.
  Time profile plot of mean RSS in the first 24 hours after treatment will be presented by treatment group.
- RSS will be compared between treatments at day 2 using linear mixed model including, treatment, investigational site and gestational age group as fixed effects.

Oxygen saturation index

- OSI, will be calculated as MAP x FiO_2 x 100/ SpO2 and analyzed at each time point and averaged over the first 24 hours post study drug intake using the same model used for the SpO_2/FiO_2.
  Time profile plot of mean OSI in the first 24 hours after treatment will be presented by treatment group.
- OSI will be compared between treatments at day 2 using linear mixed model including, treatment, investigational site and gestational age group as fixed effects.

SpO2

- SpO_2 values as well as changes from baseline will be summarized at any post-treatment time point by treatment group by means of descriptive statistics.

MAP, PIP, PEEP

- MAP, PIP, PEEP values as well as changes from baseline will be summarized at any post-treatment time point by treatment group by means of descriptive statistics.

Duration of ventilation/oxygen use

- The median duration time (days) of invasive mechanical ventilation, oxygenation, non-invasive ventilation will be compared between treatment groups by using the Mann-Whitney U-test.

Mortality and BPD

- Mortality/BPD rate at 36-week PMA will be compared by treatment by means of Cochran-Mantel-Haenszel (CMH), adjusting for stratification factors (i.e. GA group and investigational site). Relative Risk (RR) and related 95% confidence interval will be provided.
- The incidence of BPD at 36-week PMA will be compared by treatment as for Mortality/BPD rate.
- The mortality rate at 36-week PMA and at Day 28 will be compared by treatment as for Mortality/BPD rate.
- RDS-associated mortality rate through 14 days of age will be compared by treatment as for Mortality/BPD rate.

Rescue surfactant use

The percentage of patients requiring at least one rescue surfactant dose will be compared by treatment group by means of a Fisher's exact test at 5% significance interval. Odds ratio (OR) and related exact 95% CI will be also provided. Patients with pulmonary haemorrhage will be excluded from this summary.

Biomarkers of inflammation

Mean values of biomarkers of inflammation (i.e., CXCL8, IL1β, IL6, TNF-α, MPO) in the tracheal aspirates as well as change from baseline to 24 and day 2 (48 hours) will be summarized by treatment by means of descriptive statistics.

Alveolar-arterial gradient and Oxygenation Index

A–a gradient and OI values as well as change from baseline in the first 48 hours (i.e., to 3, 6, 12, 18, 24 hours and to day 2) be summarized by treatment by means of descriptive statistics.

12.3.5 Safety variables

Adverse Events
Incidence of AEs, related AEs (ADRs), serious AEs and AEs leading to death will be summarized by treatment group both in term of frequency of neonates with at least one AE and in term of frequency of AEs (number of events). All the aforementioned categories of AEs will be summarized by System organ Class (SOC) and Preferred Term (PT).

Major neonatal morbidities (see section 10.8) will be summarized separately by treatment group in term of frequency of neonates with at least one complication and in term of number of complications.

Vital Signs and SpO2
At each time point (at 0 -for HR only-, 30 minutes, at 1, 3, 6, 12, 18, 24 hours, Days 2, 3, 5, 7 and 28 PNA, discharge home and at 36 weeks PMA), the absolute value and the change from baseline in vital signs (SBP, DBP and HR) will be summarized by treatment group using descriptive statistics.
SpO2 during administration (time 0) will be summarized as well.
Time curve of the mean change from baseline in the first week of life will be presented by treatment group.

Laboratory data
Hematology and chemistry parameters will be listed together with their normal ranges when available.

Immunogenicity data
The levels of antibodies in serum will be listed.

Bayley Scales
Bayley Scales of Infant Development measured at 24 months (±3 months) corrected age will be summarized by treatment by means of descriptive statistics.

Health status questionnaire
Health status questionnaire (health problems, illnesses, injuries, well-being, diet, respiratory assessment) assessed at 24 months (±3 months) corrected age will be summarized by treatment by means of descriptive statistics.

12.3.6 Interim Analysis
No formal interim analysis is planned in this study.

13. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD APPROVAL

The study proposal will be submitted to the Ethics Committee/Institutional Review Board in accordance with the requirements of each country.

The EC/IRB shall give its opinion in writing -clearly identifying the study number, study title and informed consent form approved-, before the clinical trial commences.
A copy of all communications with the EC/IRB will be provided to the Sponsor.
The Investigator should provide written reports to the EC/IRB annually or more frequently if requested on any changes significantly affecting the conduct of the trial and/or increasing risk to the subjects (according to the requirements of each country).

14. REGULATORY REQUIREMENTS

The study will be notified to the Health Authorities (or authorized by) according to the legal requirements in each participating country.
Selection of the subjects will not start before the approval of the Ethics Committee/Institutional Review Board has been obtained and the study notified to Health Authorities (or authorized by).
The study will be conducted in accordance with the Declaration of Helsinki, with the Good Clinical Practices guidelines and following all other requirements of local laws.

15. INFORMED CONSENT

It is the responsibility of the Investigator to obtain written consent from each subject's parent or the parents' legal representative prior to any study related procedures taking place.
If the subject's parents or the parents' legal representative are unable to read, the informed consent will be obtained in the presence of an impartial witness, e.g. a person independent of the study who will read the informed consent form and the written information for the parents.
Consent must be documented by the dated signature. The signature confirms that the consent is based on information that has been understood. Moreover, the Investigator must sign and date the informed consent form.
Each signed informed consent must be kept on file by the Investigator. One copy must be given to the subject.

16. DIRECT ACCESS TO SOURCE DOCUMENTS/DATA

The Investigators must permit trial-related monitoring, audits, Ethics Committee/Institutional Review Board review or regulatory inspection, providing direct access to source data/documents.

17. STUDY MONITORING

Monitoring will be performed by the CRO [redacted], who has been designated by Chiesi Farmaceutici S.p.A..
It is understood that the monitor(s) will contact and visit the Investigator/center before the study, regularly throughout the study and after the study had been completed, and that they will be permitted to inspect the various study records: case reports form, Investigator study file and source data (source data is any data that is recorded elsewhere to the case report forms), provided that subject confidentiality is respected.

The purposes of these visits are:

- to assess the progress of the study;
- to review the compliance with the study protocol;
- to discuss any emergent problem;
- to check the eCRFs for legibility, accuracy and completeness;
- to validate the contents of the CRFs against the source documents;
- to assess the status of drug storage, dispensing and retrieval.

Prior to each monitoring visit, the Investigator or staff will record all data generated since the last visit on the eCRF. The Investigator and/or study staff will be expected to be available for at least a portion of the monitoring visit to answer questions and to provide any missing information.

It is possible that the Investigator site may be audited by Sponsor personnel or regulatory national and/or international regulatory agencies during and after the study has been completed.

18. QUALITY ASSURANCE

The R&D Quality Assurance Department of the Sponsor may perform an audit at any time according to the Sponsor's Standard Operating Procedures, in order to verify whether the study is being conducted in agreement with Good Clinical Practices.

19. INSURANCE AND INDEMNITY

Chiesi Farmaceutici S.p.A. holds and will maintain an adequate insurance policy covering damages arising out of Chiesi's sponsored clinical research studies.
Chiesi Farmaceutici S.p.A. will indemnify the Investigator and hold him/her harmless for claims for damages arising out of the investigation, in excess of those covered by his/her own professional liability insurance, providing that the drug was administered under his/her or deputy's supervision and in strict accordance with accepted medical practice and with the study protocol.
The Investigator must notify Chiesi Farmaceutici S.p.A. immediately upon notice of any claims or lawsuits.

20. CONFIDENTIALITY

All study documents are provided by the Sponsor in confidence to the Investigator and his/her appointed staff. None of this material may be disclosed to any party not directly involved in the study without written permission from Chiesi Farmaceutici S.p.A..
The Investigator must assure the subject's anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subject's study numbers, names, addresses and *(optional)* telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from Chiesi Farmaceutici S.p.A..

21. PREMATURE TERMINATION OF THE STUDY

Both the Sponsor and the Investigator reserve the right to terminate the study at any time. Should this be necessary, the procedures for an early termination or temporary halt will be arranged after consultation by all involved parties.
The Sponsor should submit a written notification to the Regulatory Authority concerned and Ethics Committee/Institutional Review Board providing the justification of premature ending or of the temporary halt.

22. CLINICAL STUDY REPORT

The clinical study report, including the statistical and clinical evaluations, shall be prepared and sent to the coordinating Investigator for agreement and signature.
At the end of the trial a summary of the clinical study report will be provided to all Ethics Committees/Institutional Review Boards, to the Competent Authority of each participating Country and to the Investigators.

23. RECORD RETENTION

After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file.

Regulations require that essential documents must be retained for at least two years after the final marketing approval in an ICH region or until two years have elapsed since the formal interruption of the clinical development of the product under study.

It is the responsibility of the Sponsor to inform the Investigator of when these documents can be destroyed. The Investigator must contact Chiesi Farmaceutici S.p.A. before destroying any trial-related documentation. In addition, all subjects' medical records and other source documentation will be kept for the maximum time permitted by the institution.

24. PUBLICATION OF RESULTS

Chiesi Farmaceutici S.p.A. is entitled to publish and/or present any results of this study at scientific meetings, and to submit the clinical trial data to national and international Regulatory Authorities. Chiesi Farmaceutici S.p.A. furthermore reserves the right to use such data for industrial purposes.
In the absence of a Study Steering Committee, Investigators will inform Chiesi Farmaceutici S.p.A. before using the results of the study for publication or presentation, and agree to provide the Sponsor with a copy of the proposed presentation. Data from individual study sites must not be published separately.
Negative as well as positive results should be published or otherwise made publicly available.

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APPENDIX I

APPROVAL OF THE PROTOCOL BY THE COORDINATING INVESTIGATOR

A MULTICENTER, DOUBLE BLIND, RANDOMIZED, SINGLE DOSE, ACTIVE-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF SYNTHETIC SURFACTANT (CHF 5633) IN COMPARISON TO PORCINE SURFACTANT (PORACTANT ALFA, CUROSURF®) IN THE TREATMENT OF PRETERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME.

Product: CHF 5633 Synthetic surfactant
Pharmaceutical Form: sterile suspension

Approval of Clinical Study Protocol by the Coordinating Investigator:

I have carefully read this protocol and I agree that it contains all the necessary information required to conduct the study and I agree to conduct it as described.
I understand that this trial will not be initiated without Ethics Committee/Institutional Review Board approvals and that the administrative requirements of the governing body of the institution will be fully complied with.
Informed written consent will be obtained from all participating subjects and appropriately documented, prior to their enrolment in the study.
The undersigned agrees that the trial will be carried out in conformity with the Declaration of Helsinki (attention being drawn to Section concerning freely given consent; copy appended), Good Clinical Practices and with all the other local laws and regulations relevant to the use of new and approved therapeutic agents in patients.

Coordinating Investigator's Name: Prof. Rangasamy Ramanathan, MD
Professor of Pediatrics
Division Chief of Neonatology
Medical Director, NICU, LAC+USC Medical Center
Los Angeles, CA, USA

Centre No. : [redacted]

_____ _____
Signature Date

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma (Italy)

APPENDIX II

APPROVAL OF CLINICAL STUDY PROTOCOL BY THE PRINCIPAL INVESTIGATOR

A MULTICENTER, DOUBLE BLIND, RANDOMIZED, SINGLE DOSE, ACTIVE-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF SYNTHETIC SURFACTANT (CHF 5633) IN COMPARISON TO PORCINE SURFACTANT (PORACTANT ALFA, CUROSURF®) IN THE TREATMENT OF PRETERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME.

Product: CHF 5633 Synthetic surfactant
Pharmaceutical Form: sterile suspension

Approval of Clinical Study Protocol by the Principal Investigator:

I have carefully read this protocol and I agree that it contains all the necessary information required to conduct the study and I agree to conduct it as described.
I understand that this trial will not be initiated without Ethics Committee/Institutional Review Board approvals and that the administrative requirements of the governing body of the institution will be fully complied with.
Informed written consent will be obtained from the parents/legal representative of all participating subjects and appropriately documented, prior to the enrolment in the study.
The undersigned agrees that the trial will be carried out in conformity with the Declaration of Helsinki (attention being drawn to Section concerning freely given consent; copy appended), Good Clinical Practices and with all the other local laws and regulations relevant to the use of new and approved therapeutic agents in patients.

Principal Investigator's Name: _____

Centre No. : _____

_____ _____
Signature Date

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma (Italy)

APPENDIX III

MINIMUM LIST OF SOURCE DATA REQUIRED

- Demographic data
- Study number
- Patient identity/number
- Randomization number
- Complications during pregnancy
- Neonatal complications
- Neonatal Concomitant Medications
- Date of informed consent signature
- Date of specific study visits
- Labels of study drugs
- Examination or assessments carried out during the study (please refer to table 2. Flow Chart)
- Laboratory reports (including haematology and biochemistry, chest x-rays and cranial sonography)
- Adverse events / serious adverse events
- 24-month clinical assessment (Bayley scales, health status questionnaire)
- If subject is withdrawn, reason for withdrawal.

APPENDIX IV
LIST OF STUDY DEFINITIONS

- ✓ **Air leaks** will be documented by evidence of pneumothorax, pneumomediastinum, pneumopericardium, pneumoperitoneum on radiograph [(20)].
- ✓ **Anemia of prematurity** is defined by a haemoglobin or haematocrit value that is more than two standard deviation below the mean for age [(21)]
- ✓ **Apnoea of prematurity** is defined as pause in breathing > 20 sec, or <20 sec if accompanied by bradycardia and/or cyanosis [(22)]
- ✓ **Bronchopulmonary dysplasia (BPD)** is defined according to the diagnostic criteria recently proposed by the NICHD / NHLBI / ORD Workshop [(23)]**:**
    - Time point of assessment and general criterion - 36 weeks postmenstrual age (PMA) or discharge home, whichever comes first; treatment with oxygen > 21% for at least 28 days plus the below reported additional criteria:
    - Additional criteria:

      Mild BPD = breathing room air at 36 weeks PMA or at discharge;

      Moderate BPD = need for < 30% oxygen at 36 weeks PMA or at discharge;

      Severe BPD = need for ≥30% oxygen and/or positive pressure at 36 weeks PMA or at discharge.
- ✓ **Hypercalcaemia:** Ca tot > 11 mg/dl
- ✓ **Hyperglycaemia:** > 175 mg/dl (in 2 separate occasions at least 6 hours apart)
- ✓ **Hyperkalaemia: K^+>6,0 mmol/l**
- ✓ **Hypernatraemia:** > 150 mEq/l (operational value > 155 or an increasing trend)
- ✓ **Hypocalcaemia:** Ca tot < 7,0 mg/dl
- ✓ **Hypoglycaemia:** < 40 mg/dl (operational values < 45 in day 1st and 50 further days)
- ✓ **Hypokalaemia: K^+<3 mmol/l**
- ✓ **Hyponatraemia:** < 135 mEq/l (operational value < 130 or a decreasing trend)
- ✓ **Germinal matrix haemorrhage and Intraventricular haemorrhage** will be determined according to Papile's classification of cranial ultrasound findings of echodensity in the

germinal matrix or ventricular extension [24].

- ✓ **Cerebral parenchymal haemorrhage** will be determined according to the cranial ultrasound finding of an echogenic lesion that fans out from the ventricular wall into the brain substance, associated to a GMH/IVH
- ✓ **Necrotizing enterocolitis** is defined as stage 2 or higher as per modified Bell's criteria [25].
- ✓ **Neonatal jaundice is defined as** elevated total serum/plasma bilirubin (TB) levels, which results in yellowish discoloration of the skin, and/or conjunctiva caused by bilirubin deposition
- ✓ **Patent ductus arteriosus** will be documented by echocardiography or if medically treated / surgically ligated / haemodinamically significant.
- ✓ **Periventricular leukomalacia** will be defined by development of periventricular cysts identified by cranial ultrasound.
- ✓ **Pneumonia** will be clinically diagnosed as the presence of infected tracheal aspirate in a neonate with deteriorating oxygenation or chest x-ray appearances of consolidation.
- ✓ **Pulmonary haemorrhage** will be diagnosed when significant amounts of blood from the endtroacheal tube, mouth and nose together with deterioration in oxygenation.
- ✓ **Pulmonary interstitial emphysema** will be diagnosed on chest X-ray appearance.
- ✓ **Retinopathy of prematurity** will be defined as per the International Classification [26].
- ✓ **Sepsis** will be diagnosed by a positive blood culture or suggestive clinical and laboratory presentation resulting in a clinical decision to treat with antibiotics despite the absence of a positive blood culture.
- ✓ **24^{+0} TO ≤ 29^{+6} WEEKS GA:** 24 weeks and 0 days to 29 weeks and 6 days. Gestational age is determined based on certain dates of last menstrual period or early ultrasound scan (i.e. performed at < 20 weeks). If a discrepancy of more than 2 weeks exists choose the early ultrasound scan.