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- (73) Patenthaver: **OAK HILL BIO LIMITED, 3rd Floor , 1 Ashley Road, Altrincham, Cheshire WA14 2DT, Storbritannien**
- (72) Opfinder: **SALAMAT-MILLER, Nazila , Lexington, MA 02421, USA**  
**TAYLOR, Katherine, , Lexington, MA 02421, USA**  
**HE, Bing, , Lexington, MA 02421, USA**  
**BHATTACHARYA, Indranil, , Lexington, MA 02421, USA**  
**HAN, Linda, , Lexington, MA 02421, USA**  
**AMSDEN, Benita, , Lexington, MA 02421, USA**  
**KRANZ, James, , Lexington, MA 02421, USA**
- (74) Fuldmægtig i Danmark: **Cosmovici Intellectual Property, Paul Cosmovici, Rue du Commerce 4, 1204 Geneva, Schweiz**
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# DESCRIPTION

## Description

**[0001]** This application claims priority to and benefit of U.S. Provisional Application No. 63/093,696, filed on October 19, 2020.

## BACKGROUND

**[0002]** There is a great need for compositions optimized for therapeutic use in neonates and/or preterm infants, for example, to treat or reduce the incidence of conditions such as intraventricular hemorrhage, bronchopulmonary dysplasia and chronic lung disease of prematurity.

**[0003]** Intraventricular hemorrhage (IVH) is a life threatening condition in premature infants characterized by bleeding in and around brain ventricles, leading to acute and chronic brain injury, and possible adverse neurodevelopmental outcomes in the long-term.

**[0004]** IVH is a major complication of prematurity. IVH can be associated with serious complications including periventricular hemorrhagic infarction, posthemorrhagic ventricular dilatation, periventricular leukomalacia, and cerebellar hemorrhage, resulting in high mortality and morbidity. Other complications include posthemorrhagic hydrocephalus, cerebral palsy, and mental retardation, and long-term neurodevelopmental disabilities.

**[0005]** IVH can lead to mortality and morbidity in premature infants. Current standard of care is based on early management and stabilization of cerebral hemodynamics and respiratory support. Prognosis is related to the severity of bleeding, damage to the brain parenchyma, the presence of seizures and severity of periventricular hemorrhagic infarction.

**[0006]** Chronic Lung Disease (CLD) of prematurity is another serious and life threatening condition in premature infants. Extremely premature infants in particular are at very high risk for developing CLD. Bronchopulmonary dysplasia (BPD) at 36 weeks post-menstrual age is typically the earliest manifestation of CLD, but infants without BPD may also develop CLD. The term "chronic lung disease of prematurity" encompasses a broad range of symptoms and characteristics that often overlap with manifestations of BPD.

**[0007]** Current standard of care treatment and prevention options for BPD and CLD include respiratory support (such as supplemental oxygen, via non-invasive nasal flow, with continuous positive airway pressure, and mechanical ventilation with endotracheal intubations, pulmonary surfactant administration, bronchodilators to help open the airways, caffeine to help reduce breathing pauses (apnea) and improve breathing muscle function, and steroids to help reduce inflammation, diuretics to help reduce excess fluid in the lungs, vasodilators to help decrease blood pressure in the lungs and antibiotics to fight infections. The long term trajectory of pulmonary outcomes in infants born extremely premature commonly starts with antenatal risk factors, followed by respiratory distress syndrome (RDS) in the first hours or days of life requiring respiratory support, leading up to BPD, typically diagnosed at 36 weeks post-menstrual age, and finally chronic lung disease during infancy, childhood, adolescence and even adulthood. CLD can result in more frequent re-hospitalizations and ER visits for respiratory causes, the need for respiratory medications or home respiratory support, and many children suffer from a form of reactive airway disease (asthma) that continues to limit their quality of life.

**[0008]** Medical treatment of premature neonates, who sometimes have a body mass of about 1 to 2 lbs (450-900 g), presents many challenges not least because the amounts that can be administered are so small. Delivering accurate doses of proteins by infusion to this patient population, and ensuring compatibility of pharmaceutical compositions with clinical administration components (e.g., infusion equipment) is critical.

**[0009]** What is more this class of patients are extremely weak and vulnerable. Toxicological risks posed by exposure to substances such as excipients in the course of pharmaceutical treatments must be carefully evaluated. Neonates may exhibit different sensitivity to chemicals, such as excipients used in formulations, as compared to adults and older children. This creates a huge challenge for those seeking to treat this patient population.

**[0010]** In addition, each therapeutic protein has different properties, for example a balance of hydrophobic and charged moieties on the surface of the folded molecule. Therefore, each therapeutic protein needs a formulation, which is optimized specifically for its properties, e.g., such that the formulation is compatible with the pharmacological activity and stability of the therapeutic protein, and its suitability for administration. This challenge is even greater when the therapeutic entity is a protein complex.

## **SUMMARY**

**[0011]** The present disclosure relates to compositions optimized for administration to neonates and/or preterm infants, and use of the composition in treatment (particularly use in said patient population), for example, in the treatment of intraventricular hemorrhage, bronchopulmonary dysplasia and chronic lung disease of prematurity. The invention is defined in the claims.

**[0012]** Compositions provided herein are suitable for use in neonates and have one or more (such as all) of the following benefits: minimized excipients (to minimize adverse effects and maximize intolerance/toxicity in the patient population); minimized loss of the protein complex when the composition is administered parenterally (for example using tubing, rubber and/or plastic materials and/or filters, for example, for via intravenous (IV) infusion); are stable (for example physically and/or chemically stable which advantageously gives a shelf-life of at least 3-6 months); and have the ability to deliver active therapeutic at the appropriate concentration levels into the neonate patient population.

**[0013]** Thus, in one aspect there is provided a low concentration pharmaceutical composition suitable for administration of a therapeutic protein complex to a neonate comprising: Insulin-like growth factor 1 (IGF-1), and Insulin-like growth factor binding protein 3 complexed (for example, in a range 0.75 to 1.25: 1 or 1: 0.75-1.25 such as in equimolar amounts); and a non-ionic surfactant (for example, a polysorbate surfactant, such as polysorbate 20 or polysorbate 80, in particular polysorbate 20) in the range of 0.0025% to 0.0075%.

**[0014]** In one embodiment, the pharmaceutical composition is isotonic.

**[0015]** In one embodiment, the pharmaceutical composition is liquid.

**[0016]** The concentration of the therapeutic complex is in the range 45-55 mg/L, in particular 50 mg/L.

**[0017]** In one embodiment, the pharmaceutical composition is aqueous.

**[0018]** In one embodiment, the formulation is provided as a unit comprising 50 micrograms/mL rhIGF-1/rhIGFBP-3 solution in 50 mM sodium acetate and 105 mM sodium chloride with 0.005% (v/v) polysorbate 20, at pH 5.5, stored at 2°C to 8°C (36°F to 46°F).

**[0019]** In one embodiment, the pharmaceutical composition according to the present disclosure is provided as final product in an infusion bag, or glass vial, containing approximately 6.5 mL extractable volume.

**[0020]** The pharmaceutical formulation can be administered parenterally without loss of the therapeutic protein complex (such as without loss of the activity of the therapeutic protein complex).

**[0021]** In one embodiment, the composition has a minimal number and/or quantity of excipients, for example 1, 2, 3, 4 or 5 excipients.

**[0022]** The activity or another property of the composition may be measured by an assay or method disclosed herein, such as in the examples.

**[0023]** The polysorbate surfactant, such as polysorbate 20 or polysorbate 80, in particular polysorbate 20 % is in the range of 0.0025% to 0.0075% v/v.

**[0024]** The pH of the composition is in the range pH 5 to pH 7, for example pH 5.3 to pH 5.8, such as pH 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, in particular pH 5.5.

**[0025]** In one embodiment the formulation delivers at least 80% of the dose of the therapeutic protein complex to the neonate in an active form, for example 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

**[0026]** Thus, in a preferred embodiment there is provided a low concentration pharmaceutical composition suitable for administration of a therapeutic protein complex to a neonate (in particular in an active form) comprising 45-55 micrograms/mL, in particular 50 micrograms/mL) of complexed Insulin-like growth factor 1 (IGF-1), and Insulin-like growth factor binding protein 3 (IGFBP-3) (for example in a range 0.75 to 1.25: 1 or 1: 0.75-1.25 such as in equimolar amounts; and a non-ionic surfactant (for example a polysorbate surfactant, such as polysorbate 20 or polysorbate 80, in particular polysorbate 20) in the range of 0.0025% to 0.0075% (e.g. 0.005%) ; a buffer (e.g. 50 mM sodium acetate), salt (e.g. 105 mM sodium chloride) wherein the pH of the formulation is in the range pH 5.3 to 5.8 (e.g. pH 5.5).

**[0027]** In further embodiments, the present invention provides an effective treatment of neonates, and in particular of Intraventricular Hemorrhage (IVH), Bronchopulmonary Dysplasia (BPD), and Chronic Lung Disease (CLD) of prematurity. When administered for example, intravenously immediately after birth to replace IGF-1 lost from the maternal supply, the composition can ameliorate or reduce Intraventricular Hemorrhage (IVH), Bronchopulmonary Dysplasia and/or Chronic Lung Disease of prematurity.

**[0028]** The present invention is based, in part, on the surprising discovery that addition of surfactant (e.g. polysorbate 20) at low concentrations (e.g. 0.0025% - 0.0075% v/v) to the IGF-1/IGFBP-3 pharmaceutical composition results in improved potency and increased stability.

**[0029]** Without wishing to be bound by any particular theory, it is contemplated, for example, that the surfactant (e.g. polysorbate 20) at such low concentrations, minimizes adsorption-related product loss, eliminating the need to prime and flush intravenous infusion sets prior to drug product administration, significantly reducing loss of drug product and improving potency.

**[0030]** The present invention is also based, in part, on the reduced accumulation of oxidized species and improved stability achieved through the use of a contact surface other than stainless steel, such as a single-use bag during compounding the complex with a formulation solution. Improved stability is achieved during storage at about 25°C (e.g. about 23°C to about 27°C), or storage at about 40°C (e.g. about 38°C to about

42°C) for example, during storage for about 3 - 6 months.

**[0031]** In addition to the advantages of reduced aggregation and increased stability and purity, the formulation comprising surfactant allows for accurate dosing and the administration of small dose volumes to extremely premature neonates

**[0032]** As used herein, Chronic Lung Disease (CLD) and Chronic Lung Disease of prematurity are used interchangeably.

**[0033]** The formulation of the present invention is stable and shows reduced aggregation. In contrast, in the absence of polysorbate and when exposed to routine stresses, high molecular weight species accumulate that contribute to decreased stability, increased aggregation and potential increased immunogenicity.

**[0034]** In summary, the present invention provides, among other things, an improved formulation of IGF-1/IGFBP-3 with advantages of increased potency and improved stability during storage, in particular, with minimized toxicity, providing a safe and efficacious product for administration to neonates in treating diseases of prematurity.

**[0035]** In one aspect, stable formulations of rIGF-1/rIGFBP-3 comprising a surfactant can be prepared and delivered at a dose such as about 400 micrograms/kg/24 hours.

**[0036]** In one aspect, stable formulations of rIGF-1/rIGFBP-3 comprising a surfactant can be prepared and delivered at a dose such as about 1000 micrograms/kg/24 hours.

**[0037]** The addition of surfactant also increases the stability of the product by reducing protein loss due to adsorption. In one aspect, the methods provided herein result in high effective exposure of serum IGF-1 by administration of the pharmaceutical rIGF-1/rIGFBP-3 composition at a dose of about 250 micrograms/kg/24 hours or lower in the presence of the surfactant.

**[0038]** In some embodiments, the polysorbate surfactant is polysorbate 20. Polysorbate 20 is also known as polyoxyethylene sorbitan monolaurate.

**[0039]** In some embodiments, the polysorbate surfactant is polysorbate 80.

**[0040]** The polysorbate surfactant is at a concentration of about 0.0025%, about 0.005%, or about 0.0075% v/v. In some embodiments, the polysorbate surfactant is at a concentration of about 0.005% v/v.

**[0041]** In some embodiments, less than about 20% (or less than about: 15%, or 10% or 5% or 4% or 2%) of the IGF-1 in the composition exists as oxidized species after storage for about 6 months at 25 °C. In some embodiments, less than 4% of the IGF-1 exists as oxidized species after storage for about 3 months at 25 °C, such as , less than 2%

**[0042]** In some embodiments, less than 10% (or less than about: 8% or 6% or 5% or 4% or 3%) of the IGF-1 exists as oxidized species after storage for about 3 months at 40 °C. In some embodiments, less than 8% (or 6% or 5% or 4% or 3%) of the IGF-1 exists as oxidized species after storage for about 1 month at 40 °C. In some embodiments, less than 2% of the IGF-1 exists as oxidized species after storage for about 1 month at 40 °C.

**[0043]** In some embodiments, the percentage of oxidized species in the composition is determined by Reversed Phase-Ultra Performance Liquid Chromatography (RP-UPLC). In some embodiments, the IGFBP-3 comprises less than 5% of trisulfide variants.

**[0044]** In some embodiments, the composition further comprises a buffer comprising sodium acetate, acetic acid and/or sodium chloride, such as sodium acetate or acetic acid. In some embodiments, the composition further comprises a buffer comprising sodium chloride.

**[0045]** In some embodiments, the sodium acetate or acetic acid is at a concentration of between about 10 and 100 mM, for example about 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM., such as a concentration of about 50 mM.

**[0046]** In some embodiments, the sodium chloride is at a concentration of about 20 mM and 200 mM. In some embodiments, the sodium chloride is at a concentration of about 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about 150 mM, about 155 mM, about 160 mM, about 165 mM, about 170 mM, about 175 mM, about 180 mM, about 185 mM, about 190 mM, about 195 mM or about 200 mM, such as a concentration of about 105 mM.

**[0047]** The composition is at a pH between about 5.0 and 7.0. In some embodiments, the pH is about 5.0, about 5.2, about 5.4, about 5.6, about 5.8, about 6.0, about 6.2, about 6.4, about 6.6, about 6.8 or about 7.0; or about 5.1, about 5.3, about 5.5, about 5.7; or about 5.9, about 6.1, about 6.3, about 6.5, about 6.7 or about 6.9, such as between about 5.3 and 5.8, in particular about 5.5.

**[0048]** In some embodiments, the pharmaceutical composition is suitable for intravenous injection.

**[0049]** In some embodiments, the pharmaceutical composition has a storage life of 20 months or more (for example at least 24 months) at a temperature of between 2-8 °C.

**[0050]** In some embodiments, the pharmaceutical composition is stable at room temperature and/or ambient light, for example for at least 8 hours, 12 hours, 24 hours, 36 hours, or 48 hours, such as at least 8 hours; or at least 12 hours; or at least 24 hours; or at least 48 hours.

**[0051]** In some embodiments, the pharmaceutical composition comprises rIGF-1 and rIGFBP-3 complexed in a range of 0.75 to 1.25: 1 or 1: 0.75-1.25, for example, in equimolar amounts.

**[0052]** In some embodiments, the IGF-1 is recombinantly produced. In some embodiments, the IGFBP-3 is recombinantly produced.

**[0053]** In some embodiments, the IGF-1 and the IGFBP-3 are complexed prior to administration to the subject.

**[0054]** In some embodiments, the pharmaceutical composition comprising recombinant insulin-like growth factor 1 (rIGF-1), recombinant insulin-like growth factor binding protein 3 (rIGFBP-3), and polysorbate 20 is:

- more stable at room temperature, and/or
- has less oxidation at room temperature

than a pharmaceutical composition comprising recombinant insulin-like growth factor 1 (rIGF-1), recombinant insulin-like growth factor binding protein 3 (rIGFBP-3) without polysorbate 20.

**[0055]** In some embodiments, the percentage of oxidized IGF-1 species does not increase more than 25% upon storage at a temperature of about 25 °C after three months, for examples does not increase more than

10%.

**[0056]** In some embodiments, provided herein is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

**[0057]** There is also provided treatment of or preventing intraventricular hemorrhage (IVH) comprising administering a composition according to the disclosure, for example wherein the subject in need has gestational age of less than 26 weeks, such as a gestational age of between about 23 weeks to 25 weeks + 6 days.

**[0058]** In some aspects, said treatment or prevention of intraventricular hemorrhage (IVH) with a therapeutic amount is sufficient to achieve reduced incidence of intraventricular hemorrhage relative to a control.

**[0059]** In some embodiments, the reduced incidence is achieved between about 36 to 40 weeks post-menstrual age (PMA) (for example 35 to 42 weeks, such as by 35 weeks (or 36 weeks, or 37 weeks, or 38 weeks, or 39 weeks or 40 weeks, or 41 weeks or 42 weeks)) as assessed by ultrasound or MRI.

**[0060]** In some embodiments, the reduced incidence is assessed by ultrasound.

**[0061]** In some embodiments, the reduced incidence for IVH is at least about 30% reduction in incidence of grade II, grade III, or grade IV IVH, such as any one of the same.

**[0062]** Also provided is treatment or prevention of bronchopulmonary dysplasia and/or chronic lung disease of prematurity comprising administering to a subject in need thereof a composition, according to the present disclosure

**[0063]** In some embodiments, the subject in need of treatment is an infant.

**[0064]** In some embodiments, the infant was prematurely born by at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months or 3 months, for example between about 23 to 34 weeks of postmenstrual age (PMA), for example about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks or about 34 weeks of PMA, such as about 23 weeks of PMA.

**[0065]** In some embodiments, the infant is below 36 weeks (or below: 35, 34, 33, 32, 32, 30, 29, 28, 27, 26, 25, 24 or 23 weeks) of PMA.

**[0066]** In some embodiments, the infant is born at between 28-32 weeks of GA, for example infant is born at less than 32 weeks of gestational age (GA), such as less than 31 weeks of GA, alternatively less than 30 weeks of GA, or 29 weeks of GA. In some embodiments, the infant is born at less than 28 weeks of GA.

**[0067]** In some embodiments, the composition is administered at a dosage of about 100 to 1000 micrograms/kg/24 hours, for example about 100 to 800 micrograms/kg/24 hours, such as about 100 to 500 micrograms/kg/24 hours more specifically between 100 micrograms/kg/24 hours and 450 micrograms/kg/24 hours, in particular between 100 micrograms/kg/24 hours and 400 micrograms/kg/24 hours.

**[0068]** In some embodiments, the composition is administered at a dosage of between 150 micrograms/kg/24 hours and 400 micrograms/kg/24 hours, for example between 200 micrograms/kg/24 hours and 400 micrograms/kg/24 hours.

**[0069]** In some embodiments, the composition is administered at a dosage of about 250 to 1000

micrograms/kg/24 hours, for example about 250 to 800 micrograms/kg/24 hours, such as about 800 micrograms/kg/24 hours.

**[0070]** In some embodiments, the composition is administered at a dosage of between 250 micrograms/kg/24 hours and 400 micrograms/kg/24 hours, for example about 250 micrograms/kg/24 hours. In some embodiments, the composition is administered at a dosage of about 400 micrograms/kg/24 hours.

**[0071]** In some embodiments, the composition is administered once, twice, three times, or four times in a 24 hour period. In some embodiments, the composition is administered once in a 24 hour period. In some embodiments, the composition is administered by continuous infusion for the 24 hour period.

**[0072]** In some embodiments, the composition is administered from the time of birth up to post-menstrual age (PMA) of about 23 to 34 weeks, for example from the time of birth up to PMA of about 23 to 32 weeks, such as about 29 weeks plus 6 days.

**[0073]** "PMA" or "postmenstrual age" refers to gestational age plus chronological age.

**[0074]** The corrected age of an infant is the adjusted age of the infant based on his or her delivery due date. Taking the term of the pregnancy to be 40 weeks (i.e., due date), a prematurely born infant gets a corrected age (CA) where the excess time the infant has existed outside the mother's body (i.e. the number of weeks the infant was born premature) is subtracted from the infant's real age.

**[0075]** In some embodiments, the subject has reduced IGF-1 serum levels. In some embodiments, the subject has IGF-1 levels of below 60 micrograms/L, for example below 50 micrograms/L, such as below 40 micrograms/L. In some embodiments, the subject has IGF-1 levels of below 30 micrograms/L. In some embodiments, the subject has IGF-1 levels of between about 30-50 micrograms/L.

**[0076]** In some embodiments, administration of the pharmaceutical composition results in elevated IGF-1 serum levels in comparison to a baseline value, for example IGF-1 serum levels in an untreated infant is below 60 micrograms/L, such as below: 50 micrograms/L, or 40 micrograms/L, or 30 micrograms/L. In some embodiments, the baseline value of IGF-1 serum levels is about 30 to 50 micrograms/L.

**[0077]** In some embodiments, the IGF-1 serum levels are elevated by at least about 25% - 50% in comparison to a baseline value. In some embodiments, the IGF-1 serum levels are elevated by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% in comparison to a baseline value, such as at least about: 10% or 15%, or 20%, or 25%, or 30%, or 35%, or 40%, or 45%, or 50%, or 55%, or 60% in comparison to a baseline value.

**[0078]** In some embodiments, the IGF-1 serum levels remain elevated for at least 24 hours following administration, such as about 48 hours; or about 72 hours. In some embodiments, the IGF-1 serum levels remain elevated for at least about 7 days. In some embodiments, the IGF-1 serum levels remain elevated through postmenstrual age (PMA) of at least about 23 weeks to 34 weeks, such as at least about 23 weeks to 12 months.

**[0079]** In some embodiments, the administration of the pharmaceutical composition results in reduced incidence of chronic lung disease of prematurity, bronchopulmonary dysplasia, right ventricular hypertrophy (RVH), pulmonary hypertension (PH), necrotizing enterocolitis, or intraventricular hemorrhage through postmenstrual age (PMA) 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months or

24 months.

**[0080]** The corrected age (CA) of an infant is the adjusted age of the infant based on his or her due date. Taking the term of the pregnancy to be 40 weeks (i.e., due date), a prematurely born infant gets a corrected age where the excess time it has existed outside the mother's body is subtracted from its real age.

**[0081]** In some embodiments, the administration of the pharmaceutical composition results in elevated IGF-1 serum target levels of between about 28-109 micrograms/mL ( $\mu\text{g/ml}$ ), for example about 30  $\mu\text{g/ml}$ , about 40  $\mu\text{g/ml}$ , about 50  $\mu\text{g/ml}$ , about 60  $\mu\text{g/ml}$ , about 70  $\mu\text{g/ml}$ , about 80  $\mu\text{g/ml}$ , about 90  $\mu\text{g/ml}$ , about 100  $\mu\text{g/ml}$  or about 110  $\mu\text{g/ml}$ .

**[0082]** In some embodiments, the reduced incidence is at least about 20% - 50% relative to an untreated control or a control receiving standard of care. In some embodiments, the reduced incidence is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70% relative to an untreated control or a control receiving standard of care.

**[0083]** In some embodiments, the increased functional status is at least about 20% - 50% relative to an untreated control, for example about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70% relative to a control receiving standard of care.

**[0084]** Increased Functional Status may be Assessed by PREmature Infant Index (PREMI) through postmenstrual age (PMA) 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks or 40 weeks.

**[0085]** In some embodiments, the pharmaceutical composition comprises 50 mM sodium acetate, 105 mM sodium chloride, and 0.005% (v/v) P20 at pH 5.5.

**[0086]** In some embodiments, the sodium acetate or acetic acid is at a concentration of between about 10 and 100 mM, for example about 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM, such as about 50 mM.

**[0087]** In some embodiments, the sodium chloride is at a concentration of about 20 mM and 200 mM, for example about 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about 150 mM, about 155 mM, about 160 mM, about 165 mM, about 170 mM, about 175 mM, about 180 mM, about 185 mM, about 190 mM, about 195 mM or about 200 mM. such as about 105 mM.

**[0088]** In some embodiments, the pharmaceutical composition is at a pH between about 5.0 and 7.0, for example about 5.0, about 5.2, about 5.4, about 5.6, about 5.8, about 6.0, about 6.2, about 6.4, about 6.6, about 6.8 or about 7.0. In some embodiments, the composition is at a pH of about 5.1, about 5.3, about 5.5, about 5.7 or about 5.9, about 6.1, about 6.3, about 6.5, about 6.7 or about 6.9. In some embodiments, the composition is at a pH of between about 5.3 and 5.8. In some embodiments, the composition is at a pH of about 5.5.

**[0089]** In some aspects, provided herein is a pharmaceutical composition comprising a protein complex comprising recombinant insulin-like growth factor 1 (rIGF-1) and recombinant insulin-like growth factor binding protein 3 (rIGFBP-3) in equimolar amounts, polysorbate 20 surfactant at a concentration of about 0.005%, and a buffer comprising sodium acetate, acetic acid, and/or sodium chloride, wherein the pharmaceutical composition has a pH of about 5.3-5.8, wherein the rIGF-1/IGFBP-3 is at a concentration of about 50

micrograms/mL, and wherein less than 2 % of the IGF-1 exists as oxidized species.

**[0090]** In some aspects, a pharmaceutical composition is provided comprising a protein complex comprising recombinant insulin-like growth factor 1 (rIGF-1) and recombinant insulin-like growth factor binding protein 3 (rIGFBP-3) in a range of 0.75 to 1.25: 1 or 1: 0.75-1.25, for example, in equimolar amounts, polysorbate 20 surfactant at a concentration of about 0.005%, and a buffer comprising sodium acetate, acetic acid, and/or sodium chloride, wherein the composition has a pH of about 5.3-5.8, wherein the rIGF-1/IGFBP-3 is at a concentration of about 50 micrograms/mL, and wherein less than 1.5 % of the IGF-1 exists as oxidized species.

#### BRIEF DESCRIPTION OF THE DRAWINGS

##### **[0091]**

**FIG. 1** is a photograph of components of the Swedish Model Infusion Set used in the IGF-1/IGFBP-3 infusion studies.

**FIG. 2** is a graph of protein concentration of IGF-1/IGFBP-3 drug product with and without 0.005% polysorbate 20 before and after buffer correction.

**FIG. 3A** is a graph that shows purity of samples measured by SE-HPLC in the absence of polysorbate 20. **FIG. 3B** depicts SE-HPLC profiles of samples in the presence of polysorbate 20. Percent peak areas are shown for an average of two infusion sets.

**FIG. 4** is a graph of oxidation of IGF-1 in the IGF-1/IGFBP-3 formulation in the presence and absence of P20 over 48 hours, as measured by RP-UPLC.

**FIG. 5** is a graph showing the percentage of oxidized IGF-1 as a function of time, concentration of the drug product and material used.

**FIG. 6** is a graph of percent oxidized IGF-1 as a function of time and head space for single-use bag and glass containers.

**FIG. 7A** is a graph showing stability (percent main peak) of the IGF-1/IGFBP-3 as a function of time at between 2°C -8°C (e.g. 5°C). **FIG. 7B** is a graph showing stability (percent main peak) of the IGF-1/IGFBP-3 as a function of time at 23°C to 27°C (25°C). **FIG. 7C** is a graph showing stability (percent main peak) of the IGF-1/IGFBP-3 as a function of time at between 38°C to 42°C.

**FIG. 8A** is a graph of percent oxidized IGF-1 plotted as function of time at 5°C. **FIG. 8B** is a graph of percent oxidized IGF-1 plotted as function of time at 25°C. **FIG. 8C** is a graph of percent oxidized IGF-1 plotted as function of time at 40°C.

**FIG. 9** depicts graphs of IGF-1 concentration over time for samples treated with either 250 micrograms/kg/24 hours or 400 micrograms/kg/24 hours of IGF-1/IGFBP-3 as compared to subjects receiving standard of care.

**FIG. 10** shows graphs that validate the simulation model based on simulated and observed IGF-1 concentration in untreated controls, subjects receiving standard of care, and subjects receiving treatment with rIGF-1/IGFBP-3 at a low dose or at 250 micrograms/kg/24 hours.

**FIG. 11** is a graph that shows results of a clinical trial simulation wherein the probability of subjects developing no bronchopulmonary dysplasia (BPD) or mild BPD was determined based on serum IGF-1 exposure levels as a result of treatment with either 250 micrograms/kg/24 hours or 400 micrograms/kg/24 hours of IGF-1/IGFBP-3 as compared to subjects receiving standard of care.

**FIG. 12** is a model of simulated 95% prediction intervals of IGF-1 PK concentrations over duration of treatment to predict where the mean and 5th and 95th intervals would fall with respect to target therapeutic range (28-109 ng/ml) in subjects treated with either 250 micrograms/kg/24 hours or 400 micrograms/kg/24 hours of IGF-1/IGFBP-3 as compared to subjects receiving standard of care at day 7 and at end of infusion.

**FIG. 13A** is a graph of serum IGF-1 exposure at day 7 and probability of BPD outcome (mild or no BPD). **FIG. 13B** is a graph of serum IGF-1 exposure at 40 weeks PMA and probability of BPD outcome (mild or no BPD).

## DETAILED DESCRIPTION

**[0092]** The present invention provides, in some aspects, a composition optimized for administration to neonates and/or preterm infants, and methods for treating diseases and complications of prematurity. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

### *Definitions*

**[0093]** "Preterm" or "preterm birth" or "prematurity" or "premature infant" or "premature baby", or grammatical equivalents, refers to birth of an infant prior to 37 weeks of gestation or weighing 10% less than the average for the infant's gestational age. For example, infants born between 22-37 weeks would be considered preterm. "GA" or "Gestational age" is a common term to describe how far along a pregnancy has progressed, measured in weeks from the first day of the woman's last menstrual cycle to the current date. In some embodiments, a premature infant refers to an infant that was prematurely born by at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, or 3 months. In some embodiments, a premature infant refers to an infant born at less than 32 weeks of gestational age (GA). In some embodiments, a premature infant refers to an infant born at less than 28 weeks of gestational age (GA). "PMA" or "postmenstrual age" refers to gestational age plus chronological age. "CA" or "corrected age" is the chronological age reduced by the number of weeks born before 40 weeks of gestation.

**[0094]** As used herein, the term "Gestational age" (completed weeks) means: time elapsed between the first day of the last menstrual period and the day of delivery. If pregnancy was achieved using assisted reproductive technology, gestational age is calculated by adding 2 weeks to the conceptional age.

**[0095]** As used herein, the term "Chronological age" (days, weeks, months, or years) means: time elapsed from birth.

**[0096]** As used herein, the term "Postmenstrual age" (weeks) means: gestational age plus chronological age.

**[0097]** As used herein, the term "Corrected age" (weeks or months) means: chronological age reduced by the number of weeks born before 40 weeks of gestation; the term should be used only for children up to 3 years of age who were born preterm.

**[0098]** During the perinatal period neonatal hospital stay, "postmenstrual age" is preferred to describe the age of preterm infants. After the perinatal period, "corrected age" is the preferred term.

**[0099]** "IGF-I" refers to insulin-like growth factor I from any species, including bovine, ovine, porcine, equine, and human, preferably human, and, if referring to exogenous administration, from any source, whether

natural, synthetic, or recombinant, provided that it will bind IGF binding protein at the appropriate site. IGF-I can be produced recombinantly, for example, as described in PCT publication WO 95/04076.

**[0100]** "IGFBP-3" refers to insulin-like growth factor binding protein 3. IGFBP-3 is a member of the insulin-like growth factor binding protein family. IGFBP-3 may be from any species, including bovine, ovine, porcine and human, in native-sequence or variant form, including but not limited to naturally-occurring allelic variants. IGFBP-3 may be from any source, whether natural, synthetic or recombinant, provided that it will bind IGF-I at the appropriate sites. IGFBP-3 can be produced recombinantly, as described in PCT publication WO 95/04076.

**[0101]** A "therapeutic composition," as used herein, is defined as comprising IGF-I, an analog thereof, or IGF-I in combination with its binding protein, IGFBP-3 (IGF-I/IGFBP-3 complex). The therapeutic composition may also contain other substances such as water, minerals, carriers such as proteins, and other excipients known to one skilled in the art. In some embodiments, the therapeutic composition comprises a surfactant such as polysorbate 20 (P20) or polysorbate 80 (P80).

**[0102]** As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of a therapeutic composition (e.g., IGF-1/IGFBP-3) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, prevents, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition (e.g., Intraventricular Hemorrhage, Bronchopulmonary Dysplasia, Chronic lung disease of prematurity). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. Accordingly, in some embodiments, treatment means preventing onset or progression of a disease.

**[0103]** "Baseline levels of IGF-1" refers to the serum levels of IGF-1 in a subject before receiving any treatment.

**[0104]** "Storage life" or "shelf life" refers to a period of storage of drug product when it retains the characteristics and properties making it suitable for therapeutic use. For example, in some embodiments, during the storage life of the drug product, there are reduced amounts (e.g. below 10%) of undesirable species including oxidized species, high-molecular weight species, degradation products. Storage life is described, for example, at particular temperatures of storage (e.g. 5 °C, 25 °C, 40 °C, etc.).

**[0105]** "Stability" or "stable" refers to the extent to which a drug product retains the characteristics and properties making it suitable for therapeutic use possessed at the time of manufacture during its period of storage and use. Stability includes aspects with regard to its formulation, the stability of IGF-1 and IGFBP3 ingredients, the integrity and stability of the IGF-1/IGFBP3 complex, container and closure, manufacturing and processing conditions, packaging components, storage and shipping conditions, temperature, light, and humidity, and the anticipated duration and conditions of pharmacy shelf-life and patient use. In some embodiments, stability refers to reduced amounts of undesirable species including oxidized species, reduced high molecular weight species that result in aggregation and/or reduced low molecular weight species represented by degradation products. In some embodiments, stability refers to less than 10% of undesirable species. In some embodiments, stability refers to less than 5% of undesirable species. In some embodiments, stability refers to less than 2% of undesirable species.

**[0106]** "Standard Neonatal Care" or "Standard of Care" refers to standard care at a level III neonatal intensive care unit (NICU) described by the American Academy of Pediatrics for infants born below 32 weeks gestational age.

[0107] "Standard of Care for CLD and/or BPD" refers to treatment measures including respiratory support, such as supplemental oxygen, continuous positive airway pressure, and mechanical ventilation with endotracheal intubation. Symptoms are managed with medications such as bronchodilators to help open the airways, steroids to help reduce inflammation, diuretics to help reduce excess fluid in the lungs, vasodilators to help decrease blood pressure in the lungs and antibiotics to fight an infection.

[0108] "Standard of Care for IVH" refers to preventive and/or treatment measures including antenatal steroids and the early management and stabilization of cerebral hemodynamics and respiratory support. IVH treatment refers to supportive management of symptoms with medications to reduce bleeding and damage to the brain, and prevent seizures.

[0109] As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein, or historical reference or data. A "control individual" is an individual afflicted with the same form of disease (e.g., IVH, BPD, CLD, among others) of prematurity as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

### **IGF-1/IGFBP-3**

[0110] IGF-I and IGF-I binding proteins such as IGFBP-3 may be purified from natural sources or produced by recombinant means. For instance, purification of IGF-I from human serum is well known in the art (Rinderknecht et al. (1976) Proc. Natl. Acad. Sci. USA 73:2365-2369). Production of IGF-I by recombinant processes is shown in EP 0128733, published in December of 1984. IGFBP-3 may be purified from natural sources using a process such as that shown by Baxter et al. (1986, Biochem. Biophys. Res. Comm. 139:1256-1261). Alternatively, IGFBP-3 may be synthesized recombinantly as discussed by Sommer et al., pp. 715-728, Modern Concepts Of Insulin-Like Growth Factors (E. M. Spencer, ed., Elsevier, N.Y., 1991). Recombinant IGFBP-3 binds IGF-I in a 1:1 molar ratio (equimolar amounts).

### ***Pharmaceutical composition and therapeutic use***

[0111] The present invention provides a pharmaceutical composition suitable for administration of a therapeutic protein complex to a neonate.

[0112] In some embodiments, a pharmaceutical composition comprising rIGF-1/IGFBP-3 at a concentration of about 50 micrograms/mL is suitable for intravenous administration.

[0113] In some embodiments, a pharmaceutical composition comprising IGF-1 and IGFBP-3, in a range of 0.75 to 1.25: 1 or 1: 0.75-1.25, for example, equimolar amounts of IGF-I and IGF-binding protein 3 may be used. In some embodiments, the IGF-I and IGF binding protein 3 are complexed prior to administration.

[0114] The complex may be formed by mixing approximately equimolar amounts of IGF-I and IGF binding protein 3 dissolved in physiologically compatible carriers such as normal saline, or phosphate buffered saline solution. In some embodiments, a concentrated solution of recombinant human IGF-I and a concentrated solution of recombinant human IGF binding protein 3 are mixed together for a sufficient time to form a complex in a range of 0.75 to 1.25: 1 or 1: 0.75-1.25, for example, in an equimolar complex. In some embodiments, recombinant human IGF-I and recombinant human IGF binding protein 3 are combined to form a complex during purification as described in International Patent Application No. WO 96/40736.

**[0115]** In some embodiments, a pharmaceutical composition is provided comprising the composition described herein and one or more suitable pharmaceutical excipients. In one embodiment, the composition has a minimal number and/or quantity of excipients, for example, 1, 2, 3, 4 or 5 excipients.

**[0116]** In some embodiments, the polysorbate 20 surfactant is at a concentration of about 0.0025%, about 0.005%, or about 0.0075% v/v. In some embodiments, the polysorbate 20 is at a concentration of 0.005%.

**[0117]** In some embodiments, the polysorbate 80 surfactant is at a concentration of about 0.0025%, about 0.005%, or about 0.0075% v/v. In some embodiments, the polysorbate 80 is at a concentration of 0.005%.

**[0118]** In some embodiments, the surfactant % is w/w.

**[0119]** In one embodiment, the surfactant % is w/v, i.e., weight of the composition to volume of the surfactant or weight of the surfactant to volume of the composition.

**[0120]** In one embodiment, the surfactant % is v/v.

**[0121]** In some embodiments, the pharmaceutical composition further comprises a buffer comprising sodium acetate, acetic acid and/or sodium chloride. In some embodiments, the composition further comprises a buffer comprising sodium acetate or acetic acid. In some embodiments, the composition further comprises a buffer comprising sodium chloride.

**[0122]** In some embodiments, the composition is administered intravenously.

**[0123]** Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

**[0124]** The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

**[0125]** In some embodiments, the methods disclosed herein provide for the parenteral administration of a low concentration isotonic pharmaceutical composition suitable for administration of a therapeutic protein complex to a neonate (in particular in an active form) comprising IGF-I complexed with IGF binding protein 3 in the presence of a non-ionic surfactant, e.g., 0.005% polysorbate 20 or polysorbate 80 to infants in need of such treatment.

**[0126]** In one embodiment, the pharmaceutical formulation can be administered parenterally without loss of the therapeutic protein complex (such as without loss of the activity of the therapeutic protein complex).

**[0127]** In one embodiment, activity is measured by an assay disclosed herein, such as in the examples. In one embodiment, the formulation delivers at least 80% of the dose of the therapeutic protein complex to the neonate in an active form, for example, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

**[0128]** Accordingly, in some embodiments, the methods disclosed herein provide for the intravenous administration of a pharmaceutical composition comprising IGF-I complexed with IGF binding protein 3 in the

presence of a surfactant, e.g., 0.005% v/v polysorbate 20 or polysorbate 80 to infants in need of such treatment. In some embodiments, the methods disclosed herein provide for the subcutaneous administration of a pharmaceutical composition comprising IGF-I complexed with IGF binding protein 3 in the presence of a surfactant, e.g., 0.005% v/v polysorbate 20 or polysorbate 80 to infants in need of such treatment.

**[0129]** A pharmaceutical composition according to the present invention may be administered at various doses. For example, a suitable dosage may range from about 100 to 1000 micrograms/kg/24 hours. In some embodiments, a suitable dosage may be or greater than about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 micrograms/kg/24 hours.

**[0130]** In some embodiments, a suitable dosage is about 400 micrograms/kg/24 hours. In some embodiments, a suitable dosage is about 750 micrograms/kg/24 hours. In some embodiments, a suitable dosage is about 1000 micrograms/kg/24 hours.

**[0131]** In some embodiments, a pharmaceutical composition according to the invention is administered from the time of birth up to postmenstrual age (PMA) of about 23 to 34 weeks, up to PMA of about 28 to 32 weeks, up to PMA of about 29 weeks plus 6 days.

### **Chronic Lung Disease**

#### ***Chronic Lung Disease of Prematurity***

**[0132]** Extremely premature infants are at very high risk for developing chronic lung disease of prematurity. Premature babies may need a breathing machine (ventilator) and extra oxygen to breathe. Chronic Lung Disease of prematurity may occur when a breathing machine or oxygen injures a premature baby's lungs. With a lung injury, the tissues inside a baby's lungs get inflamed. The tissue can break down, causing scarring. The scarring can cause trouble breathing, and the baby may need more oxygen. Lung injury may be caused by:

- *Prematurity*: A premature baby's lungs aren't fully formed. This is especially true of the air sacs.
- *Low amounts of surfactant*: This is a substance in the lungs that helps keep the tiny air sacs open.
- *Oxygen use*: High amounts of oxygen can harm the cells in the lungs.
- *Breathing machine (mechanical ventilation)*: Air pressure can harm the lungs. This pressure may come from breathing machines, suctioning of the airways, and use of an endotracheal (ET) tube. An ET tube is a tube placed in your baby's windpipe (trachea) and connected to a breathing machine.

**[0133]** The long term trajectory of pulmonary outcomes in infants born extremely premature commonly starts with antenatal risk factors, followed by respiratory distress syndrome (RDS) in the first hours or days of life requiring respiratory support, often leading up to a diagnosis of BPD in those who survive to term equivalence, and finally chronic lung disease of prematurity as they grow into infancy, early childhood and often even school age or adolescence that results in more frequent re-hospitalizations and ER visits for respiratory causes, the need for respiratory medication or home respiratory support, and many suffer from a form of reactive airway disease that continues to limit their quality of life.

**[0134]** Chronic lung disease of prematurity is a broad term that includes infants diagnosed with bronchopulmonary dysplasia, but may also include infants that never received a diagnosis for bronchopulmonary dysplasia after extremely premature birth and manifest with persistent lung disease.

Chronic lung disease of prematurity may be diagnosed by prolonged time to weaning off of oxygen support, but clinical endpoints of chronic lung disease of prematurity can include, in practice, but are not limited to ongoing oxygen dependence, symptoms of cough and wheezing, respiratory illnesses and hospitalizations, dependence on continuous airway pressure, mechanical ventilation and dependence on respiratory medications, and diagnosis of pulmonary hypertension (PH). In some embodiments, Chronic Lung Disease (CLD) of prematurity may persist into adulthood. In some embodiments, CLD involves a spectrum of diseases and disorders, including but not limited to COPD (emphysema and chronic bronchitis), asthma, cystic fibrosis, restrictive lung disease, and persistent infections.

**[0135]** In some embodiments, the compositions and methods described herein result in reduced incidence of CLD. In some embodiments, the compositions and methods described herein are used to treat CLD.

**[0136]** In some embodiments, reduced incidence or treatment of CLD is assessed by the Time to Final Weaning off Respiratory Technology Support (RTS) from Day 1 through 12 Months Corrected Age (CA) (Time Frame: Baseline through 12 months Corrected Age (CA)). In this context, RTS is defined as any one of the following: (1) supplemental oxygen less than (<) 2 Liter per minute (L/min) without positive pressure (including nasal cannula), (2) positive pressure support (including continuous positive airway pressure [CPAP], nasal cannula oxygen greater than (>) 2 L/min), (3) positive pressure ventilation (high frequency oscillation ventilation and technologies with positive pressure tidal volume breaths, such as mechanical ventilation, nasal intermittent positive pressure ventilation [NIPPV]). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the Time to Final Weaning off RTS as compared to a baseline control.

**[0137]** In some embodiments, reduced incidence or treatment of CLD is assessed by the Incidence of Bronchopulmonary Dysplasia (BPD) or Death through Postmenstrual age (PMA) 36 Weeks (Time Frame: Baseline through 36 weeks postmenstrual age (PMA)). BPD can be assessed, for example, by modified National Institute of Child Health and Human Development (NICHD) severity grading and standardized by oxygen challenge testing. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by Incidence of Bronchopulmonary Dysplasia (BPD) or Death through Postmenstrual age (PMA) 36 Weeks as compared to a baseline control.

**[0138]** In some embodiments, reduced incidence or treatment of CLD is assessed by the Total Number of Days on Respiratory Technology Support (RTS) from birth through 12 Months corrected age (CA) (time frame: birth through 12 months CA). In this context, RTS can be defined as any one of the following: (1) any fraction of inspired oxygen (FiO<sub>2</sub>) greater than (>) 21 percent (%), (2) noninvasive respiratory support delivered via a nasal interface (e.g., continuous positive airway pressure [CPAP], bilevel positive airway pressure [BiPAP], high flow therapy, nasal intermittent positive pressure ventilation [NIPPV], nasal cannula), (3) invasive respiratory support

**[0139]** (mechanical ventilation) via an endotracheal tube or tracheostomy. Total number of days on RTS from birth through 12 months CA will be reported. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by Total Number of Days on Respiratory Technology Support (RTS) from birth through 12 Months corrected age (CA) (time frame: birth through 12 months CA) as compared to a baseline control.

**[0140]** In some embodiments, reduced incidence or treatment of CLD is assessed by the duration of re-hospitalizations (Time Frame: From neonatal intensive care unit (NICU) discharge through 12 months CA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the duration of re-hospitalizations as compared to a baseline control.

**[0141]** In some embodiments, reduced incidence or treatment of CLD is assessed by the number of

emergency room visits (Time Frame: From NICU discharge through 12 months CA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the number of emergency room visits.

**[0142]** In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the number of days of respiratory medication use (Time Frame: From NICU discharge through 12 months CA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the number of days of respiratory medication use as compared to a baseline control. Respiratory medication use can include for example bronchodilators, steroids, leukotriene inhibitors, diuretics.

**[0143]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of signs and/or symptoms of respiratory disease as Assessed by a 28-day Caregiver-administered Diary ending at 12 Months Corrected age (CA) (Time Frame: 11 months CA through 12 months CA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by a 28-day Caregiver-administered Diary ending at 12 Months Corrected age (CA) as compared to a baseline control.

**[0144]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of Chronic Respiratory Morbidity (CRM1) through 12 Months Corrected age (CA) (Time Frame: From NICU discharge through 12 months CA). A subject is defined as having CRM1 if he or she experienced/required at least 1 of the 3 following clinical/treatment events, as reported by parents/caregivers and captured by the pulmonary morbidity assessment on at least two 3-month quarters over a 12-month time period: 1. Emergency room visit or hospitalization associated with a respiratory diagnosis, 2. Home RTS, 3. Daily use of respiratory medications (e.g., bronchodilators, steroids, leukotriene inhibitors, diuretics) as reported by caregivers on the pulmonary morbidity assessment. Incidence of CRM1 through 12 months CA will be reported. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of Chronic Respiratory Morbidity (CRM1) through 12 Months Corrected age (CA) as compared to a baseline control.

**[0145]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of chronic respiratory morbidity including symptoms of respiratory disease (CRM2) through 12 Months Corrected age (CA) (Time Frame: From NICU discharge through 12 months CA). A subject is defined as having CRM2 if he or she experienced/required at least 1 of the 4 following clinical/treatment events, as reported by parents/caregivers and captured by the pulmonary morbidity assessment on at least two 3-month quarters over a 12-month time period: 1. Emergency room visit or hospitalization associated with a respiratory diagnosis, 2. Home RTS, 3. Daily use of respiratory medications (e.g., bronchodilators, steroids, leukotriene inhibitors, diuretics) as reported by caregivers on the pulmonary morbidity assessment, 4. Symptoms of respiratory disease as defined by presence of cough without cold, or wheeze at least once per week. Incidence of CRM2 through 12 months CA will be reported. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of chronic respiratory morbidity including symptoms of respiratory disease (CRM2) through 12 Months Corrected age (CA) as compared to a baseline control.

**[0146]** In some embodiments, reduced incidence or treatment of CLD is assessed by the severity of chronic respiratory morbidity (CRM3) through 12 Months Corrected age (CA), as determined by the Chronic Lung Disease (CLD) of Infancy Severity Score (Time Frame: From NICU discharge through 12 months CA). This is determined by, for example, the CLD of infancy severity score that will include components such as respiratory hospitalizations, RTS use, and use of respiratory medications. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the severity of chronic respiratory morbidity (CRM3) through 12 Months Corrected age (CA), as determined by the Chronic Lung Disease (CLD) of Infancy Severity Score as compared to a baseline control.

**[0147]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) 40 weeks, as assessed by cranial ultrasound (Time Frame: Baseline through 40 Weeks PMA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) 40 weeks, as assessed by cranial ultrasound as compared to a baseline control.

**[0148]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) of between about 36-40 weeks, as assessed by cranial ultrasound (Time Frame: Baseline through 36-40 weeks Weeks PMA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) 36-40 weeks, as assessed by cranial ultrasound as compared to a baseline control.

**[0149]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) 36 weeks, as assessed by cranial ultrasound (Time Frame: Baseline through 36 Weeks PMA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of Intraventricular Hemorrhage (IVH) through postmenstrual age (PMA) 36 weeks, as assessed by cranial ultrasound as compared to a baseline control.

**[0150]** In some embodiments, reduced incidence or treatment of CLD is assessed by the motor function at 12 Months Corrected age (CA), as measured by Alberta Infant Motor Scales (AIMS) (Time Frame: At 12 months CA). The AIMS is a measure of early motor maturation used for assessment of infants at risk for motor delay, focusing on attainment of motor milestones and development of postural control. It consists of 58 items, including assessments in 4 postural positions: prone (21 items), supine (9 items), sitting (12 items) and standing (16 items). Each item is scored as 'observed' or 'not observed'. The scorer identifies the least and most mature item observed. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the motor function at 12 Months Corrected age (CA), as measured by Alberta Infant Motor Scales (AIMS) as compared to a baseline control.

**[0151]** In some embodiments, reduced incidence or treatment of CLD is assessed by the functional status as assessed by PREMature Infant Index (PREMII) at Postmenstrual Age (PMA) 36 Weeks (Time Frame: At 36 weeks PMA). PREMII is a Clinician-Reported Outcome (ClinRO) assessment used to capture overall functional maturation of extremely preterm neonates. Functional Status is defined as what the infant can do with respect to 8 key functional areas (feeding, weight gain, thermoregulation, respiratory support, apnea, bradycardia, desaturation events, and oxygen administration), as a reflection of the infant's overall health and development. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the functional status as assessed by PREMature Infant Index (PREMII) at Postmenstrual Age (PMA) 36 Weeks as compared to a baseline control.

**[0152]** In some embodiments, reduced incidence or treatment of CLD is assessed by the incidence of mortality (Time Frame: From birth through 12 months CA). In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the incidence of mortality as compared to a baseline control.

**[0153]** In some embodiments, reduced incidence or treatment of CLD is assessed by the exposure-response of Insulin-like Growth Factor-1 (IGF-1) (Time Frame: Up to 12 months CA). IGF-1 is evaluated for example using (Pharmacokinetic/Pharmacodynamic [PK/PD]) relationship of IGF-1 between respiratory and neurologic endpoints. In some embodiments, the compositions and methods described herein result in reduced incidence of CLD as assessed by the exposure-response of Insulin-like Growth Factor.

**Intraventricular Hemorrhage**

[0154] Intraventricular hemorrhage (IVH) is a life threatening condition in premature infants characterized by bleeding in and around brain ventricles, the spaces in the brain containing cerebrospinal fluid. Intraventricular hemorrhage is most common in premature babies, especially those born before 28 weeks gestational age.

[0155] Babies with respiratory problems, such as respiratory distress syndrome (RDS), or other complications of prematurity, are more likely to have IVH.

[0156] IVH is associated with serious complications including periventricular hemorrhagic infarction, post hemorrhagic ventricular dilatation, periventricular leukomalacia, and cerebellar hemorrhage, resulting in mortality and morbidity. Other complications include posthemorrhagic hydrocephalus, cerebral palsy, and mental retardation, and long-term neurodevelopmental disorders.

[0157] Current standard of care for prevention and/or treatment of IVH is based on antenatal steroids and the early management and stabilization of cerebral hemodynamics and respiratory support. Prognosis is related to the severity of bleeding, damage to the brain parenchyma, the presence of seizures and severity of periventricular hemorrhagic infarction. IVH treatment comprises supportive management of symptoms with medications to reduce bleeding and damage to the brain, and prevent seizures.

[0158] IVH grades are qualified as follows: grade I - hemorrhage in the region of germinal matrix, very little hemorrhage or no hemorrhage in the ventricle; grade II - hemorrhage filling 10-50% of the ventricle; grade III - hemorrhage filling more than 50% of the ventricle; grade IV (PVHI) - periventricular echodensity. This hemorrhage, which was named grade IV previously, is currently classified as periventricular hemorrhagic infarction (PVI). Grade I and II are defined as mild grades and grade III and PVHI are defined as advanced grades. Table 1 shows cranial ultrasound image grading for IVH.

**Table 1: Cranial ultrasound image grading for IVH**

<b>IVH Grades by Volpe Method</b>	
<b>Severity</b>	<b>Description</b>
Grade I	GMH with no or minimal IVH (<10% of ventricular area on parasagittal view)
Grade II	IVH in 10-50% of ventricular area on parasagittal view
Grade III	IVH >50% of ventricular area on parasagittal view; usually distends lateral ventricle
Grade IV	IVH compounded by hemorrhagic venous infarction in the periventricular white matter

[0159] In some embodiments, the compositions and methods described herein result in reduced incidence of Grade II IVH, Grade III IVH and Grade IV IVH or PVHI. In some embodiments, the compositions and methods described herein result in reduced incidence of Grade II IVH. In some embodiments, the compositions and methods described herein result in reduced incidence of Grade III IVH. In some embodiments, the compositions and methods described herein result in reduced incidence of PVHI. In some embodiments, the compositions and methods described herein are used to treat Grade II IVH, Grade III IVH and Grade IV IVH or PVHI. In some embodiments, the compositions and methods described herein are used to treat Grade II IVH. In some embodiments, the compositions and methods described herein are used to treat Grade III IVH. In some embodiments, the compositions and methods described herein are used to treat Grade IV IVH or PVHI.

**[0160]** In some embodiments, incidence of IVH is assessed by cranial ultrasonography or ultrasound. Screening is performed at various intervals to detect and assess cerebral hemorrhage, including, for example, in some embodiments, on day 0, 1, 2, 3, 7, 14, and 21 days. In some embodiments, ultrasound is further carried out at 32 weeks PMA, 33 weeks PMA, 34 weeks PMA, 35 weeks PMA, 36 weeks PMA, 37 weeks PMA, 38 weeks PMA, 39 weeks PMA or 40 weeks PMA. In some embodiments, ultrasound is further carried out at 36 weeks PMA. In some embodiments, ultrasound is further carried out at 40 weeks PMA. In some embodiments, a single reader (masked to treatment) is used to evaluate all ultrasound images for the highest grade of GMH-IVH, according to Papile and Bowerman methods. (Papile LA, et al. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J. Pediatr. (1978). 92: 529-34; Bowerman RA, et al. Natural history of neonatal periventricular/intraventricular hemorrhage and its complications: sonographic observations. AJR Am J. Roentgenol. (1984) 143: 1041-52.)

**[0161]** In some embodiments, no IVH and grade I IVH are grouped together. In some embodiments, two independent readers are used for a post-hoc analysis which is also masked to treatment to assess periventricular hemorrhagic infarction (PVHI), and discrepancies were resolved by consensus agreement. IVH was graded based on severity according to Volpe. In some embodiments, PHI is graded by Dudink method by assessing the localization of caudate vein, temporal vein, anterior terminal vein and complete terminal vein infarction. (Dudink J. et al., Venous subtypes of preterm periventricular haemorrhagic infarction. Arch Dis Child Fetal Neonatal Ed. (2008) 93:F201-6.)

**[0162]** In some embodiments, a limited PHI is assessed when only caudate vein or temporal vein is affected or a small anterior terminal vein infarction is observed in cranial ultrasounds. In some embodiments, extensive PHI is assessed with complete terminal vein infarction or combination of caudate vein, temporal vein and anterior vein infarctions. Cranial ultrasound images are also used to detect post-hemorrhagic ventricular dilatation (PHVD), and white matter injury (WMI). (Davies, M.W. et al., Reference ranges for the linear dimensions of the intracranial ventricles in preterm neonates. Arch. Dis. Child Fetal Neonatal Ed., 2000 82:F218-23; Govaert P. et al. An Atlas of Neonatal Brain Sonography. 2nd Ed. London: Mac Keith Press, 2010).). In some embodiments, WMI is scored using a four-grade scale as shown in Table 2.

**Table 2. Grades of Post-Hemorrhagic Ventricular Dilatation (PHVD) and White Matter Injury**

<b><i>PHVD Grades by Davies Method</i></b>	
<b>Severity</b>	<b>Description</b>
0	Normal, Anterior Horn Width < 3mm
1	Mild, Anterior Horn Width 3 to < 5mm
2	Moderate, Anterior Horn Width 5-10 mm
3	Severe, Anterior Horn Width >10 mm
<b><i>White Matter Injury by Govaert and deVries Method</i></b>	
<b>Scoring</b>	<b>Description</b>
0	Persistent periventricular hyperechogenicity or punctate lesions, no cysts, no obvious white matter loss (white matter loss was identified as ventricular dilatation without hemorrhage)
1	Persistent periventricular hyperechogenicity or extensive punctate lesions evolving into diffuse white matter loss without cysts
2	Persistent periventricular hyperechogenicity evolving into small localized frontoparietal cystic lesions (limited cystic PVL)
3	Persistent periventricular hyperechogenicity evolving into extensive cystic lesions (extensive cystic PVL)

<b>Brain Injury Severity Score</b>	
Scoring	Description
0	No brain abnormalities
1	GMH, periventricular hyperechogenicity without white or gray matter loss, and mild-moderate cerebral injury
2	IVH II, mild PHVD, stroke of a perforating artery
3	IVH III, persistent moderate PHVD without shunt or Rickham device, persistent periventricular hyperechogenicities with diffuse white and/or gray matter loss at term cranial ultrasound
4	Limited PHI, limited PVL, PHVD with shunt or Rickham device, anterior cerebral artery stroke, posterior cerebral artery stroke, severe cerebellar injury
5	Unilateral extensive PHI, extensive cystic PVL, mild cerebral artery stroke, severe global brain atrophy
6	Bilateral extensive PHI

**[0163]** In some embodiments, IVH is assessed by magnetic resonance imaging (MRI). MRI evaluates white matter injury and dimensions and localization of hemorrhage associated with neurodevelopmental outcomes.

**[0164]** In some embodiments, a consensus IVH grade is allotted to each subject based on the highest grade of IVH observed by single, or in the case of multiple readers, joint masked readers.

**[0165]** In some embodiments, reduced incidence of IVH relates to assessing a subject as manifesting a lower grade of IVH as compared to the grade of IVH in the absence of treatment. In some embodiments, for example, reduced incidence refers to Grade I IVH instead of Grade II, Grade III or Grade IV IVH prior to treatment. In some embodiments, reduced incidence refers to Grade II IVH instead of Grade III or Grade IV IVH prior to treatment. In some embodiments, reduced incidence refers to Grade III IVH instead of Grade IV IVH prior to treatment.

**[0166]** In some embodiments, the severity of IVH according to treatment group was determined from the maximum-grade hemorrhage observed for each infant in the population. In some embodiments, reduced incidence of IVH refers to a reduction of incidence of IVH in the population by 20-50%, for example by: 20%, or 30%, or 40%, or 50%.

**[0167]** In some embodiments, reduced incidence of IVH refers to a reduction of incidence of Grade IV IVH (or PHVI) in the population by 20-50%, for example by: 20%, 30%, 40%, or 50%.

**[0168]** In some embodiments, reduced incidence of IVH refers to a reduction of incidence of Grade III IVH in the population by 20-50%, for example by: 20%, 30%, 40%, or 50%.

**[0169]** In some embodiments, reduced incidence of IVH refers to a reduction of incidence of Grade II IVH in the population by 20-50%, for example by: 20%, or 30%, or 40%, or 50%.

**[0170]** In some embodiments, reduced incidence of IVH refers to a reduction of incidence of Grade I IVH in the population by 20-50%, for example by: 20%, or 30%, or 40%, or 50%.

**[0171]** In some embodiments, the treatment of IVH refers to administration of a therapeutic composition (e.g., IGF-1/IGFBP-3) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, prevents, reduces severity of and/or reduces incidence of one or more symptoms or features of Intraventricular

Hemorrhage. In some embodiments, the subject receiving treatment does not exhibit signs of IVH and/or exhibits only early or mild signs of IVH. In some other embodiments, the subject receiving treatment exhibits one or more established signs of IVH. Accordingly, in some embodiments, treatment refers to preventing onset or progression of IVH from mild to severe forms.

### ***Bronchopulmonary Dysplasia***

**[0172]** Bronchopulmonary dysplasia (BPD) is a serious lung condition that affects newborns. BPD mostly affects premature infants and is a breathing disorder where an infant's lungs become irritated and do not develop normally. BPD mostly affects premature newborns who need oxygen therapy, which is oxygen given through nasal prongs, a mask, or a breathing tube. It occurs most often in low-weight infants born more than 10 weeks early or weigh less than 2 pounds at birth.

**[0173]** Since it was first described in 1967, the definition for BPD has been evolving. The original definition included infants with oxygen requirement for 28 days with associated radiographic changes. This definition of BPD was then refined as infants requiring oxygen support at 36 weeks PMA in infants born with birth weight < 1,500 g. In 2001, the NIH defined BPD as "For those born at gestational age < 32 weeks, BPD referred to requirement of oxygen support (>21%) for at least 28 days and a subsequent assessment at 36 weeks PMA or discharge, whichever comes first. In those born with GA >32 weeks, BPD referred to the requirement of supplemental oxygen (<21%) for at least 28 days and a subsequent assessment at 56 days post-natal age or discharge, whichever comes first." Infants with no oxygen requirement were classified as having mild BPD. Moderate BPD was diagnosed in those requiring <30% oxygen and severe BPD in those with a need for positive pressure ventilation/continuous positive pressure and/or oxygen requirement >30%. The "physiologic definition" of BPD proposed in 2003 classified as infants as having BPD that "required positive pressure and oxygen  $\geq 0.3$  at 36 weeks PMA" In contrast, those that required  $FiO_2 < 0.3$ , required an oxygen reduction test for up to 2 weeks based on oxygen saturation. However, the physiologic definition remained less popular than the functional criterion that if premature newborns still require oxygen therapy by the time they reach 36 weeks of post menstrual age, they are diagnosed with BPD.

**[0174]** Babies who develop BPD are often born with respiratory distress syndrome (RDS). Bronchopulmonary dysplasia is often referred to interchangeably with chronic lung disease of premature babies, but BPD is in fact a single time-point assessment at 36 weeks, while CLD broadly refers to chronic and prolonged or persistent lung disease, sometimes in infants previously diagnosed with BPD, or in infants that have not yet received a diagnosis for BPD. For practical purposes, BPD provides a relevant, easily measured clinical endpoint to measure outcomes on CLD as it is more well-defined. Accordingly, in some embodiments, the methods described herein result in reduced incidence of BPD. In some embodiments, the methods described herein are used to treat BPD. In some embodiments, the methods described herein result in no, mild or less severe BPD.

**[0175]** Some newborns may need long-term oxygen or breathing support from nasal continuous positive airway pressure (NCPAP) machines, ventilators, and medicines like bronchodilators. They may continue to have breathing problems due to chronic lung disease of prematurity throughout childhood and even into adulthood.

**[0176]** In some embodiments, the compositions and methods described herein result in reduced incidence of bronchopulmonary dysplasia. In some embodiments, the compositions and methods described herein are used to treat bronchopulmonary dysplasia.

**[0177]** In some embodiments, there is also provided an article of manufacture comprising packaging material and a pharmaceutical agent contained within the packaging material. The packaging material comprises a

label which indicates that the pharmaceutical may be administered, for a sufficient term at an effective dose, for treating and/or preventing complications associated with preterm birth. The pharmaceutical agent comprises the IGF-I/IGFBP-3 composition together with a pharmaceutically acceptable carrier.

**[0178]** In one embodiment, the formulation is provided as a unit comprising 50 micrograms/mL rhIGF-1/rhIGFBP-3 solution in 50 mM sodium acetate and 105 mM sodium chloride with 0.005% (v/v) polysorbate 20, at pH 5.5, stored at 2°C to 8°C (36°F to 46°F).

**[0179]** The invention will be further characterized by the following examples which are intended to be exemplary of the invention.

**[0180]** Comprising in the context of the present specification is intended to mean "including".

**[0181]** Where technically appropriate, embodiments of the invention may be combined, in particular where methods describe composition elements this combination of features may be employed to define the composition per se, and vice versa.

**[0182]** The background contains technical information and may be used as basis for amendments.

**[0183]** Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

**[0184]** Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

## **EXAMPLES**

### **Example 1. *Assessing the in-use stability of Mecasermin Rinfabate Drug Product in the presence and absence of 0.005% (v/v) polysorbate 20 (P20)***

**[0185]** This example illustrates the in-use stability and/or compatibility of the rhIGF-1/rhIGFBP-3 drug product in the presence and absence of 0.005% polysorbate 20 (P20) during a worse case administration simulation in a Phase 2 Swedish Model infusion set over a period of 48 hours at room temperature.

**[0186]** Briefly, an assessment was conducted with two drug product formulations: the Phase 2 (Process A) drug product formulation (50 µg/mL mecaseimerin rinfabate in 50 mM sodium acetate with 105 mM sodium chloride, at pH 5.5) in the absence of polysorbate 20, and the Phase 3 (Process B) drug product formulation that consisted of the Phase 2 formulation containing 0.005% (v/v) polysorbate 20.

**[0187]** During Phase 2 clinical studies, infusion sets had to be primed and flushed with the drug product for at least one hour to prevent adsorption-related product loss to the infusion sets, and ensure that the premature neonates received the administered dose of 250 µg/kg/24 hours of drug product. It was observed that when "dry" infusion sets were used without any priming or flushing with the drug product, or any other solution, prior to administration, adsorption-related product loss occurred to the infusion set ("worse case administration").

**[0188]** As the concentration of the drug product is extremely low (50 µg/mL) and the Phase 2 formulation buffer does not contain any surfactant, which prevents or reduces protein adsorption to different polymeric surfaces, a study was carried out in order to investigate the effect of inclusion of 0.005% P20 in the drug product for adsorption-related product loss and overall quality attributes of the drug product such as purity, pH,



Sampling Time (hr)	Drug Product Formulation with no P20					Drug Product Formulation with P20				
	Visual observation	pH	Conc. Solo VPE	RP-UPL C	SE-HP LC	Visual observation	pH	Conc. Solo VPE	RP - UP LC	SE-HPL C
48	+	+	+	+	+	+	+	+	+	+

[0194] Visual observation of the drug product as well as the formulation buffer controls for both formulations (with and without P20) was performed at each time point. Drug product and formulation samples demonstrated no trend with regard to the presence of particulates or fibers. No change in the pH of either drug product formulation (with and without P20) was observed at any time during the study.

[0195] The protein concentration of the drug product was measured over the 48-hour study, before and after buffer correction (FIG. 2). During protein concentration measurements, an increase in the concentration at the later time points was observed that was attributed to potential unknown species that interfered with the protein concentration measurements. Therefore, formulation controls with and without P20 were also generated to correct the protein concentration for the unknown species.

[0196] The data showed that less adsorption-related protein loss was observed when P20 was present in the formulation. For example, over the initial infusion period of 4 hours, and after protein content correction for the unknown light absorbing species, a 3 fold less protein loss was observed for the drug product in the formulation that contained P20 as compared with the drug product in the formulation that did not contain P20. The increase in protein concentration at the 48 hour time point even after buffer correction is attributed to background due to leachates.

[0197] Overall, the results demonstrated that the addition of P20 prevented the adsorption related product loss in the model infusion set over 48 hours at room temperature. The improvement was seen when the drug product was first exposed to the "dry" infusion set for up to 4 hours. There is no need to prime and flush the infusion sets prior to the drug product administration after the inclusion of 0.005% polysorbate 20 in the drug product formulation.

[0198] In-line filter compatibility was assessed for two exemplary in-line filters. An average dose of about 5.5 mL for about 1 kg neonate was tested.

Table 4. Protein Content Assessment Before and After In-line Filtration

Filter	Drug Product	Protein Concentration (micrograms/mL)	
		Before Filtration	After Filtration
MX1480 Filter Assembly	Drug product	48.1	32.1
	Drug product with 0.005% (v/v) polysorbate 20	48.6	45.5
Neonatal/Pediatric 0.22 micrometers, 96 hour IV filter	Drug product	46.6 ± 1.1	31.5 ± 0.6
	Drug product with 0.005% (v/v) polysorbate 20	50.8 ± 0.8	53.8 ± 0.4

[0199] In the absence of polysorbate 20, a significant loss of drug product was observed in both instances of exemplary in-line filters.

[0200] Loss of protein content observed in the absence of P20 is undesirable since increasing dose volumes is not feasible in neonates to deliver higher effective doses due to daily limited fluid intake in neonates.

**Example 2. Measuring purity of the Mecasermin Rinfabate drug product in formulations in the presence and absence of 0.005% (v/v) polysorbate 20 (P20) by SE-HPLC and RP-UPLC**

[0201] This example illustrates the purity of drug product in formulations in the presence and absence of 0.005% (v/v) polysorbate 20 (P20).

[0202] In order to compare the purity of the drug product in the presence and absence of 0.005% (v/v) polysorbate 20 (P20), the Size Exclusion High Performance Liquid Chromatography (SE-HPLC) assay was performed. The assay was characterized with the optimization of the column loading for the best profile and signal detection.

Table 5. Stability Summary of Drug Product in the Infusion Set without Polysorbate 20 at the Ambient Temperature<sup>1</sup>

Assay	Proposed Target Criteria	Baseline <sup>2</sup>	Syringe Exposed Baseline	1 hour	4 hours	8 hours	24 hours	48 hours	
<b>Appearance and Description</b>									
Appearance	Clear to slightly opalescent, colorless to slightly colored, essentially free of particles	T E S T	Conforms	Clear, colorless, small and large fibers'	Conforms	Clear, colorless, 1 small white flake	Clear, colorless with 1 short fiber	Conforms	Clear, colorless, with a few small fibers
		B U F F E R	NA	Clear, colorless, small and large fibers	Clear, colorless, small and 1 large fiber	NT	NT	NT	Clear, colorless with 1 small fiber
<b>Purity and Impurities</b>									
Size exclusion HPLC	≥92% main peak area		93.9%	94.4%	94.5%	948%	946%	94.9%	95.3%
	≤8% high molecular weight peak area <sup>4</sup>		4.1%	3.8%	3.8%	3.7%	3.7%	3.7%	3.1%
RP-UPLC	≥95% main peak area		97.3%	97.3%	97.0%	96.1%	960%	95.3%	95.6%
	Report results for oxidized rhIGF1 peak area		2.1%	2.1%	2.3%	3.2%	3.2%	3.4%	35%
<b>Potency</b>									
Cell Proliferation Assay	50% to 150% Relative Potency					76%	NT	57%	
<b>Content</b>									
Protein concentration	45 to 55 µg/ml		43 <sup>5</sup>	32	38	38	NT	39	48

<b>General</b>								
pH	5.5±0.3	5.5	5.5	5.5	5.5	5.5	5.5	5.5
NT: Not Tested								
NA: Not Applicable								
1. Average data from two infusion sets is shown except appearance and selected time points for concentration.								
2. Baseline sample is the drug product in the vial (n=1).								
3. The data is reported for the worse case when the particle was observed in at least 1 of the 2 vials. Although the receiving vials were washed and depyrogenated, a controlled study of purified water in the receiving vials (3 ml) demonstrated that small and large fibers were observed with water. The receiving vials were changed to clean 5 ml. receiving vials for time points 4 to 48 hours.								
4. High molecular weight species are the sums of peaks 4, 5 and 6 in the SE-HPLC profile. The reported value is the average of data from two independent in-use studies.								
5. The concentration value is reported after buffer correction: the value without buffer correction is 47 µg/ml.								

Table 6. Stability Summary of Drug Product in the Infusion Set with Polysorbate 20 at the Ambient Temperature<sup>1</sup>

<b>Assay</b>	<b>Proposed Target Criteria</b>		<b>Baseline<sup>2</sup></b>	<b>Syringe Exposed Baseline 1</b>	<b>1 hour</b>	<b>4 hours</b>	<b>8 hours</b>	<b>24 hours</b>	<b>48 hours</b>
<b>Appearance and Description</b>									
Appearance	Clear to slightly opalescent, colorless to slightly colored, essentially free of particles	Test sets	Conforms	Clear, colorless, small and large fibers'	Clear, colorless, small and large fibers'	Clear, colorless, 1-2 small fibers	Clear, colorless, 1 long fiber	Conforms	Clear, colorless, small and large fibers
		Buffer sets	NA	Clear, colorless, 1 small and 1 large fiber <sup>4</sup>	Clear, colorless, 1 large fiber <sup>3</sup>	Clear, colorless, 1 small fiber, 1 small flake	Clear, colorless, large, medium and small fibers <sup>4</sup>	Clear, colorless, 1 long fiber, 1 short fiber	Conforms
<b>Purity and Impurities</b>									
Size Exclusion HPLC	≥92% main peak area		95%	94.6%	93.8%	94.4%	94.6%	94.7%	95.3%
	≤8% high molecular weight		3.7%	3.7%	3.8%	3.6%	3.5%	3.5%	3.1%

<b>Purity and Impurities</b>									
	peak area <sup>5</sup>								
RP-UPLC	≥95% main peak area	98%	97.7%	97.5%	97.1%	96.3%	95.8%	95.6%	
	Report results for oxidised rhIGF1 peak area	1.7%	1.7%	1.9%	2.3%	2.9%	3.4%	35%	
<b>Potency</b>									
Cell Proliferation Assay	50 to 150% Relative Potency		90%	Not Tested				83%	
<b>Appearance and Description</b>									
Protein concentration	45 to 55 µg/ml		49	45	45	47	45	41	49
<b>General</b>									
pH	5.5±0.3		5.5	5.5	5.5	5.5	5.5	5.5	5.5
NA: Not Applicable									
NT: Not Tested									
1. Average data from two infusion sets is shown except appearance and selected time points for concentration.									
2. Baseline sample is the drug product in the vial (n=1).									
3. The data is reported for the worse case when the particle was observed in at least 1 of the 2 vials. Although the receiving vials were washed and depyrogenated, a controlled study of purified water in the receiving vials (3 ml) demonstrated that small and large fibers were observed with water. The receiving vials were changed to clean 5 ml. receiving vials for time points 4 to 48 hours.									
4. A separate infusion step was set up to observe the appearance of the formulation buffer with P20 4 days after the original study.									
5. High molecular weight species are the sums of peaks 4, 5 and 6 in the SE-HPLC profile. The reported value is the average of data from two independent in-use studies.									

[0203] The data showed that less adsorption-related protein loss was observed when P20 SE-HPLC data demonstrated a similar purity (percent complex peak area) for the drug product in formulations held in the infusions set for up to 48 hours (Tables 5 and 6). Three high molecular weight (HMW) species were observed, which were summed to report a qualitative value for HMW species in formulations in the absence of P20 (FIG. 3A) and in the presence of P20 (FIG. 3B). HMW are undesirable because they contribute to decreased stability of the product, increased aggregation and increased immunogenicity.

**Example 3. Measuring degradation of the Mecasermin Rinfabate drug product in formulations in the presence and absence of 0.005% (v/v) polysorbate 20 (P20) by RP-UPLC**

[0204] This example illustrates exemplary degradation of mecasermin rinfabate by oxidation.

[0205] To investigate the extent of degradation of mecasermin rinfabate, Reversed Phase-Ultra-High Performance Chromatography (RP-UPLC) was performed to monitor the oxidation of IGF1 in the IGF-1/IGF1-

BP3 protein complex.

[0206] The RP-UPLC data demonstrated a small increase in the percent oxidized IGF1 after 48 hours hold time in the infusion sets when compared to baseline in both formulations (FIG. 4).

[0207] This assay also monitors the percent peak areas for IGF-1 and IGFBP-3 proteins separately; the increase in the IGF-1 oxidation coincided with the decrease in the percent IGF-1 peak area while the percent IGFBP-3 did not change significantly. The percent decrease in IGFBP-3 was approximately 0.6% for non-P20 containing formulation as compared with 0.1% when there was P20 present in the formulation. Tables 5 and 6 report the percent oxidized IGF-1 and the sum of the IGF-1 and IGFBP-3 peak areas; the latter is reported as the percent main peak area.

**Example 4. Measuring potency of the Mecasermin Rinfabate drug product in formulations in the presence and absence of 0.005% (v/v) polysorbate 20 (P20)**

[0208] This example illustrates exemplary effects of the presence of 0.005% (v/v) polysorbate 20 (P20) in a mecasermin rinfabate formulation on potency of the drug product.

[0209] To investigate the effects of the presence or absence of 0.005% (v/v) polysorbate 20 (P20) in a formulation on the potency of mecasermin rinfabate drug product, selected samples, at baseline and 48-hour hold time, in formulations containing 0.005% (v/v) P20 or in the absence of 0.005% P20 were tested for potency.

[0210] In the worst case scenario where the infusion sets were not primed and flushed with the drug product, there was a 25% potency reduction when the formulation buffer did not contain P20 as compared with only 8% potency reduction when polysorbate 20 was present (Table 7). Although both values may be considered in the range of a typical cell based assay variation (70% to 130%), an approximately 3 fold lower potency is observed when no polysorbate 20 was present in the drug product formulation.

Table 7. Percent Relative Potency of Drug Product after 48 hours of Hold Time in Formulation with and without Polysorbate 20

Drug Product Sample	Percent Relative Potency <sup>1</sup>	
	No Polysorbate 20	With Polysorbate 20
Baseline	76%	90%
Drug product samples after 48 hrs hold time	57%	83%

<sup>1</sup>Relative potency is expressed as percent of the Drug Substance Demo run

[0211] Addition of polysorbate 20 improved the overall quality of the product as a higher potency was observed in the presence of polysorbate 20 in the drug product.

[0212] Collectively, the results of these studies demonstrated that the quality of the Phase 3 drug product is maintained for up to 48 hours in the infusion set when stored at room temperature with exposure to ambient light and temperature.

**Example 5. Mecasermin Rinfabate drug product in formulations in the presence and absence of 0.005% (v/v) polysorbate 20 (P20) or polysorbate 80 (P80)**

[0213] In this example, the safety and toxicity of polysorbate 20 and polysorbate 80 exposure in formulations of mecasecmin rinfabate drug product were compared.

[0214] The nonclinical data for polysorbates 20 and 80 were used to derive IV permitted exposure values of 0.02 mg/kg body weight/day for premature neonates and 0.08 mg/kg body weight/day for full-term infants and children up to 1 year of age (Table 8).

Table 8. Summary of Exposures to Polysorbate 20 from the rhIGF-1/rhIGFBP-3 Drug Product and Other Sources

Patient weight	Daily P20 exposure from rhIGF-1/rhIGFBP-3	Daily P20 exposure from TPN	Daily P20 exposure from rhIGF-1/rhIGFBP-3 and TPN	Daily P80 exposure from TPN	Daily total P20 + P80 exposure from TPN	Daily P20 exposure with no signs of E-Ferol Toxicity	Daily P80 exposure with no signs of E-Ferol Toxicity
0.4 kg	0.11 mg	0.24 mg	0.36 mg	15 mg	15.24 mg	3.2 mg	28.8 mg
1.7 kg	0.47 mg	0.52 mg	0.99 mg	32.5 mg	33.02 mg	13.6 mg	122.4 mg
TPN = Total parenteral nutrition							

[0215] Based on these comparisons, there are no toxicological concerns related to the minor additional amount of polysorbate 20 that patients would receive from the rhIGF-1/rhIGFBP-3 drug product, relative to the amount of polysorbate 20 and polysorbate 80 that patients already would be receiving from total parenteral nutrition products.

#### **Manufacture of Mecasermin Rinfabate in a single-use bag**

[0216] This example demonstrates the role of contact material in percent oxidation and stability of IGF-1/IGFBP-3.

[0217] The drug product manufactured using stainless steel containers or single use bag were analyzed for oxidation. Briefly, the excipients (sodium chloride, sodium acetate, acetic acid and polysorbate 20) were mixed. The excipients were subsequently filtered and mixed with a thawed drug substance which was compounded to drug product, IGF-1/IGFBP-3 complex. The buffer compounded bulk drug product was taken in either a 1000L stainless steel container or 500L single use bag (Mobius MIX0500L bag Gold including magnetic stirrer) and filtered through a filter (Opticap XL10 0.22  $\mu$ m, Durapore PVDF membrane) into a receiving stainless steel container or bag (including magnetic stirrer). The two batches of the drug product were analyzed for percent of oxidized IGF-1 by RP-UPLC at release and during storage.

[0218] FIG. 5 shows the percentage of oxidized IGF-1 as a function of time and concentration of the drug product in stainless steel in a small-scale model. It was observed that drug product that did not have any contact with the stainless steel was less prone to oxidation of IGF-1, across all concentrations.

[0219] Similar experiment was repeated by compounding the drug product in a glass container and compared to a compounded drug product in a single-use bag. FIG. 6 summarizes the percent oxidized IGF-1 as a function of time and head space for single-use bag and glass containers. It was observed that drugs compounded in single-use bags yielded the lowest oxidation, followed by the formulations compounded in glass having 20% fill. Next, the stability of the IGF-1 was tested by plotting the percentage of main peak (un-oxidized IGF-1) as a function of time, at different temperatures. It was observed that single-use bag generated drug product preserved the stability of IGF-1 across all temperatures tested, compared to stainless

steel vessel-generated drug product. The stability (plotted as percent main peak) are shown in FIG. 7A-FIG. 7C. Additionally, the percent oxidized IGF-1 was plotted as a function of time. It was observed that single use bag generated drug product had lower percent oxidized IGF-1 across all temperatures tested, compared to stainless steel vessel-generated drug product. The percent oxidized IGF-1 plotted as function of time are shown in FIG. 8A-FIG. 8C.

**Example 6. Treatment of CLD in extremely premature infants**

[0220] An investigational drug comprising insulin like growth factor -1/insulin-like growth factor binding protein-3 (rhIGF-1/rhIGFBP-3) complex was studied for therapeutic effect in CLD. The drug product formulation used was 50 µg/mL mecasermin rinfabate in 50 mM sodium acetate with 105 mM sodium chloride, at pH 5.5, containing 0.005% (v/v) polysorbate 20.

[0221] It is designed as a multicenter, randomized, open-label, controlled, 3-arm study to evaluate the clinical efficacy and safety of the therapeutic composition in preventing human chronic lung disease of prematurity. This study is undertaken on subjects through 12 months corrected age (CA) compared to standard neonatal care in extremely premature infants. The study is reviewed and approved by the institutional review board (IRB)/independent ethics committee (IEC) of the responsible institution.

[0222] Purpose: The purpose of this study is to determine if an investigational drug comprising rhIGF-1/rhIGFBP-3 (henceforth, the therapeutic composition) can reduce respiratory complications in extremely premature babies through 12 months corrected age (CA), as compared to extremely premature babies receiving standard neonatal care alone.

[0223] Study Subjects: The subjects are between gestational age (GA) of 23 weeks + 0 days and 27 weeks +6 days. Subjects include both sexes. At least fifty subjects are included in the study.

[0224] Exclusion criteria: The exclusion criteria include detectable gross malformation, known or suspected chromosomal abnormality, genetic disorder or syndrome, according to the investigator's opinion. The exclusion criteria also include persistent blood glucose level less than (<) 2.5 millimoles per liter (mmols/L) at the baseline visit to exclude severe congenital abnormalities of glucose metabolism; clinically significant neurological disease according to the investigator's opinion; monozygotic multiples; and any other condition that may pose risk to the subject or interfere with the subject's ability to be compliant with the protocol or interfere with the interpretation of results. If the subject is participating or plans to participate in a clinical study of another investigational study drug, device, or procedure (participation in observational studies is permitted on a case-by-case basis) are excluded. If the subject or subject's parent or legally authorized representative(s) is unable to comply with the protocol or is unlikely to be available for long-term follow-up as determined by the investigator, the subject is also excluded.

[0225] Details of the Study Design: The primary purpose of the study is prevention of Bronchopulmonary Dysplasia and Chronic Lung Disease of prematurity. It is an open label study, and the intervention model will be Parallel Assignment. The conditions monitored will be BPD and CLD.

[0226] 250 micrograms/kg/24 hours of the therapeutic composition is administered to one group of participants (Group A) by intravenous administration (IV) from birth up to postmenstrual age (PMA) 29 weeks +6 days. To another group of participants (Groups B), 400 micrograms/kg/24 hours of the therapeutic composition is administered by intravenous administration (IV) from birth up to postmenstrual age (PMA) 29 weeks +6 days. To the third group (Group C or control group), standard neonatal care alone is provided.

[0227] The primary outcomes measured is incidence of chronic lung disease (CLD) of prematurity through 12

Months Corrected Age (CA) [Time Frame: Baseline through 12 Months Corrected Age (CA)] CLD is a common adverse outcome of premature birth resulting in recurrent respiratory symptoms requiring treatment with pulmonary medications such as bronchodilators, need for supplementary home oxygen, frequent emergency room visits or hospital readmissions, especially during the first year of life. CLD will be measured by respiratory health care utilization and respiratory symptoms.

**[0228]** The secondary outcomes include incidence of Bronchopulmonary Dysplasia (BPD) at Postmenstrual Age (PMA) 36 Weeks [Time Frame: PMA Week 36]. BPD is a chronic lung disorder characterized by pulmonary immaturity, undifferentiated alveoli with the presence of hyaline membrane and atelectasis, dilated capillaries immersed in the mesenchyme, and a distorted deposition of the extracellular matrix. BPD results in residual effects on pulmonary function and is linked to neurodevelopmental problems during later childhood.

**[0229]** The secondary outcomes also include

- Incidence of Severe Intraventricular Hemorrhage (IVH) Grade III or IV Through Postmenstrual Age (PMA) 40 Weeks [Time Frame: Baseline Through PMA 40 Weeks]
- Incidence of Bronchopulmonary Dysplasia (BPD) at Postmenstrual Age (PMA) 40 Weeks [Time Frame: PMA Week 40]
- Incidence of Chronic Lung Disease (CLD) or Death Through 6 Months Corrected Age (CA) [Time Frame: Baseline through 6 Months Corrected Age (CA)] CLD is a common adverse outcome of premature birth resulting in recurrent respiratory symptoms requiring treatment with pulmonary medications such as bronchodilators, need for supplementary home oxygen, frequent emergency room visits or hospital readmissions, especially during the first year of life. CLD will be measured by respiratory health care utilization and respiratory symptoms.
- Functional Status as Assessed by PREmature Infant Index (PREMII) at Postmenstrual Age (PMA) 40 Weeks [Time Frame: PMA Week 36] PREMII is a Clinician-Reported Outcome (ClinRO) assessment used to capture overall functional maturation of extremely preterm neonates. Functional Status is defined as what the infant can do with respect to 8 key functional areas (feeding, weight gain, thermoregulation, respiratory support, apnea, bradycardia, desaturation events, and oxygen administration), as a reflection of the infant's overall health and development.

**Example 7. Intraventricular Hemorrhage (IVH) treatment in extremely premature low gestational age infants**

**[0230]** An investigational drug comprising insulin like growth factor -1/insulin-like growth factor binding protein-3 (rhIGF-1/rhIGFBP-3) complex (50 µg/mL mecasermin rinfabate in 50 mM sodium acetate with 105 mM sodium chloride, at pH 5.5, comprising 0.005% (v/v) polysorbate 20) is studied for therapeutic effect in intraventricular hemorrhage (IVH). It is designed as a multicenter, randomized, open-label, controlled, 3-arm study to evaluate the clinical efficacy and safety of the therapeutic composition in preventing intraventricular hemorrhage. This study is undertaken on subjects through 36 weeks PMA compared to standard neonatal care (SNC) in extremely premature infants with gestational age <26 weeks. The study is reviewed and approved by the institutional review board (IRB)/independent ethics committee (IEC) of the responsible institution.

**[0231]** Purpose: The purpose of this study is to determine if an investigational drug comprising rhIGF-1/rhIGFBP-3 (henceforth, the therapeutic composition) can reduce incidence of intraventricular hemorrhage in extremely premature babies through 36 weeks PMA, as compared to extremely premature babies receiving standard neonatal care alone.

**[0232] Study Subjects:** The subjects are infants with gestational age (GA) of less than 26 weeks or < 25 weeks + 6 days. Subjects include both sexes. At least fifty subjects are included in the study.

**[0233] Study Design:** 250 micrograms/kg/24 hours of the therapeutic composition is administered to one group of participants (Group A) by intravenous administration (IV) from birth up to postmenstrual age (PMA) 29 weeks +6 days. To another group of participants (Groups B), 400 micrograms/kg/24 hours of the therapeutic composition is administered by intravenous administration (IV) from birth up to postmenstrual age (PMA) 29 weeks +6 days. To the third group (Group C or control group), standard neonatal care alone is provided.

**[0234]** In lower gestational age infants, where the incidence of IVH is higher, the incidence of IVH II/III/PHVI is assessed.

**[0235]** The primary outcomes measured are incidence and severity of intraventricular hemorrhage at Postmenstrual Age (PMA) 36 Weeks [Time Frame: PMA Week 36]. IVH is a common adverse outcome of premature birth and will be monitored by cranial ultrasound.

**[0236]** The secondary outcomes include severity and incidence of Bronchopulmonary Dysplasia (BPD) at Postmenstrual Age (PMA) 36 Weeks [Time Frame: PMA Week 36]. BPD is a chronic lung disorder histologically characterized by pulmonary immaturity, undifferentiated alveoli with the presence of hyaline membrane and atelectasis, dilated capillaries immersed in the mesenchyme, and a distorted deposition of the extracellular matrix. BPD results in residual effects on pulmonary function and is associated with neurodevelopmental disabilities during later childhood.

**[0237]** Efficacy analysis would be carried out on infants <26 week GA at 36 week PMA. Relative reduction in IVH grades II/III/IV (PHVI) is assessed. In some embodiments, subjects are followed out to about 40 weeks, 12 months or 24 months CA.

#### **Example 8. BPD prevention in extremely premature infants**

**[0238]** A randomized study for effect of IGF-1/IGFBP-3 in complications of extreme prematurity, including BPD prevention, was undertaken with an intervention model of parallel assignment. The study was conducted in multiple centers in Italy, the Netherlands, Poland, Sweden, the United Kingdom and the United States between 18 Jun 2010 and 30 March 2016.

**[0239]** The drug Mecasermin Rinfabate, that is IGF-1/IGFBP-3, was administered as continuous intravenous infusion in subjects from Study Day 0 (day of birth) up to and including PMA 29 weeks + 6 days, when the subject's endogenous production of IGF-1 is considered sufficient to maintain physiologic serum IGF-1 levels. After discontinuation of study drug infusion, each subject will be followed to PMA 40 weeks  $\pm$  4 days. The study was intended to determine the rhIGF-1/rhIGFBP-3 Dose, Administered as a Continuous Infusion (CI), required to establish and maintain longitudinal serum IGF-1 levels within physiological levels in premature infants, to prevent retinopathy of prematurity. This was a Phase 2, Randomized Controlled, Assessor-blind, dose confirming, pharmacokinetic, safety and efficacy of rhIGF-1/rhIGFBP-3. 61 Participants received insulin-like growth factor (rhIGF-I)/insulin-like growth factor binding protein-3 (rhIGFBP-3) 250 micrograms per kilogram (mcg/kg) for 24 hours through continuous intravenous (IV) infusion from Day 0 up to 29 weeks 6 days of post-menstrual age (PMA). As a control group, 60 participants received standard of care alone. Table 3 illustrates the participant flow of the overall study.

#### **Table 9 Participant Flow: Overall Study**

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	rhIGF-1/rhIGFBP-3	Standard of Care (Control)
STARTED	61	60
COMPLETED	46	46
NOT COMPLETED	15	14
Withdrawal by Subject	2	1
Adverse Event	11	9
Protocol Deviation	2	2
Administrative Decision	0	1
Other Unspecified	0	1

Table 10 illustrates the population in the study.

	rhIGF-1/rhIGFBP-3		Standard of Care (Control)		Total		
Overall Participants Analyzed	61		60		121		
[Units: Participants]							
Age	25.60 (1.207)		25.62 (1.397)		25.61 (1.300)		
[Units: Weeks]							
Mean (Standard Deviation)							
Sex: Female, Male							
[Units: Participants]							
Count of Participants							
	Female	22	36.1%	21	35.0%	43	35.5%
	Male	39	63.9%	39	65.0%	78	64.5%

[0240] The secondary outcomes included among other parameters:

- Time to Discharge From Neonatal Intensive Care (TDNIC) [ Time Frame: Day 0 to 40 Weeks Post Menstrual Age (EOS)]
- Number of Participants With Bronchopulmonary Dysplasia (BPD) [Time Frame: At 36 Weeks Post Menstrual Age]
- Severity of BPD as mild, moderate and severe were based on the National Institute of Child Health and Human Development (NICHD) guidelines for preterm infants born at gestational age (GA) less than (<) 32 weeks.
- Mild: oxygen requirement during the first 28 days but in room air at PMA 36 weeks or discharge to home, whichever comes first.
- Moderate BPD: oxygen requirement during the first 28 days and oxygen <30 percent (%) at PMA 36 weeks or discharge to home, whichever comes first.
- Severe BPD: oxygen requirement during the first 28 days and oxygen greater than equal ( $\geq$ )30% through head hood or nasal cannula, or continuous positive airway pressure, or mechanical ventilation, or high flow nasal cannula  $\geq$  2 L/min at PMA 36 weeks or discharge to home, whichever comes first.
- Rate of Change in Body Weight [Time Frame: Day 0 to 40 Weeks Post Menstrual Age (EOS) ] The rate of change is the rate of specific body weight change per day in kilogram (kg).
- Rate of Change in Length [ Time Frame: Day 0 to 40 Weeks Post Menstrual Age (EOS) ]
- The rate of change is the length change per day in centimeter (cm). Number of Participants With Treatment Emergent Adverse Event (TEAE) and Treatment Emergent Serious Adverse Event (TESAE) [ Time Frame: Day 0 to 40 Weeks Post Menstrual Age (EOS) ]
- An adverse event (AE) was any untoward medical occurrence in a participant who received study drug

without regard to possibility of causal relationship. A serious adverse event (SAE) was an AE resulting in any of the following outcomes or deemed significant for any other reason: death; initial or prolonged inpatient hospitalization; life-threatening experience (immediate risk of dying); persistent or significant disability/incapacity; congenital anomaly.

- Treatment-emergent adverse event was defined as the onset of any AE or if the severity of a pre-existing AE worsened any time on or after the date of first dose of investigational product.
- Percentage of Serum IGF-1 Concentrations Falling Within Target Range After Infusion of rhIGF-1/rhIGFBP-3 [Time Frame: Day 0 to 40 Weeks Post Menstrual Age (EOS)]
- Serum samples were collected from treated and control participants for quantitation of IGF-1 using validated immunoassays. Target range of serum IGF-1 was 28-109 mcg/L. The percentage of serum IGF-1 levels across treated participants that fall within the range was reported.
- Serum Concentrations of IGFBP-3 After Intravenous (IV) Infusion of rhIGF-1/rhIGFBP-3 [Time Frame: Day 0 and Week 40 Post Menstrual Age]
- Serum Concentrations of Acid Labile Sub-unit (ALS) After Intravenous (IV) Infusion of rhIGF-1/rhIGFBP-3 [Time Frame: Day 7 and Week 40 Post Menstrual Age]

**Table 11 illustrates the measured values of BPD as secondary outcome.**

	rhIGF-1/rhIGFBP-3	Standard of Care (Control)
Participants Analyzed	47	49
[Units: Participants]		
Number of Participants With Bronchopulmonary Dysplasia (BPD)		
[Units: Participants]		
No BPD	4	4
Mild	23	16
Moderate	9	5
Severe	10	22
Unable to determine	1	2

[0241] No statistical analysis provided for Number of Participants With Bronchopulmonary Dysplasia (BPD).

**Example 9. Exposure Response Analysis of IGF-1 Following Administration of IGF-1 and IGFBP-3 in Premature Infants**

[0242] This example illustrates the exposure response of IGF-1 in serum in extremely premature infants treated with IGF-1/IGFBP-3.

[0243] Briefly, a human recombinant form of the naturally occurring protein complex of IGF-1 and insulin-like growth factor binding protein-3 (rhIGF-1/rhIGFBP-3) is administered to premature infants.

[0244] About 96 premature infants were administered rhIGF-1/rhIGFBP-3 daily in an intravenous infusion, starting within 24 hours of birth, and ending at 30 weeks post-menstrual age (PMA). Control subjects received standard neonatal care (SOC). The development of BPD was assessed by oxygen challenge testing at PMA of 36 weeks.

[0245] The median gestational age at birth was 25.9 weeks (and ranged from 23.1-27.9 weeks). Median birth

weight was 0.790 (ranged from 0.425 - 1.22). Logistic regression models were constructed to assess the relationship between IGF-1 exposure metrics and the probability of developing BPD (i.e., No/mild BPD vs. Moderate/Severe BPD). Sources of variability in the BPD response such as gestational age, birth weight, sex, oxygen saturation on day 1, APGAR score at 1 min., and study arm were assessed and considered.

**[0246]** Clinical trial simulations were then performed to identify the dosing regimen that would result in serum IGF-1 levels that correlated with BPD prevention or amelioration, i.e. provided high probability of No/mild BPD symptoms in the premature infants receiving treatment relative to standard of care. A total of 500 trials of 75 subjects treated with 250 and 400 micrograms/kg/day rhIGF-1/rhIGFBP-3 or no dose (SOC) in a 1:1:1 ratio were simulated based on a population PK model.

**[0247]** The results from these simulations showed that higher observed average IGF-1 levels at day 7 (CavD7) were associated with a greater probability of No/Mild BPD, showing a statistically significant exposure-response relationship. The probability of No/Mild or Moderate/Severe BPD was determined based on simulated CavD7 in each trial according to the above exposure-response model.

**[0248]** In addition, effects of other variables were observed. For example, statistically significant effects of weight at birth and sex were observed, whereby larger females are expected to have a higher probability of No/Mild BPD. Neither oxygen saturation at birth nor APGAR score at 1 min were predictors of No/Mild BPD.

**[0249]** The results from the simulations showed that treatment with rhIGF-1/rhIGFBP-3 treatments resulted in a higher probability of developing no BPD or mild BPD relative to standard of care for treating CLD (FIG. 9, Table 12). A comparison between subjects treated with 250 and 400 micrograms/kg/day rhIGF-1/rhIGFBP-3 or no dose (SOC) is seen at FIG. 11.

**[0250]** Based on concentration-time data of IGF-1 in premature infants, the pharmacokinetic characteristics of infused (or exogenous) IGF-1 was evaluated. Overall, a one-compartment disposition model with linear elimination combined to a baseline model taking into account the change in weight over time adequately characterized the concentration-time profiles of infused and endogenous IGF-1, respectively. In addition, the model included between-subject variability (BSV) on volume of distribution and clearance, as well as BSV on endogenous models (IGF-1 concentration at birth ( $C_{endo,0}$ ), and in more mature infants on IGF-1 half-live for the initial decline ( $C_{endo,mat}$ )). Since age and weight are important components for the endogenous production of IGF-1, as well as clearance of IGF-1 following IV infusion of rhIGF-1/rhIGFBP-3, a maturation model was developed to characterize the body weight increase as a function of PMA in premature infants. The model was validated as shown by graphs of IGF-1 concentration over time for subjects administered 250 micrograms/kg/day rIGF-1/rIGFBP-3 or a lower dose, relative to control subjects that received standard of care or untreated controls (FIG. 10).

Table 12. Clinical Trial Simulations in 75 and 150 Premature Infants to Compare Probability of Success (i.e., no/mild BPD) in the Active group Compared to Standard of Care

Arm	CavD7 (µg/L)	Prob (%) 95%CI	Median OR (95% CI)	
			75	150
Number of subjects (1:1:1)				
400 micrograms/kg/day	50.3	73.2 [52.96,86.93]	0.345 [0.831,1.52]	0.351 [0.135,0.901]
250 micrograms/kg/day	36.8	61.9 [47.85, 74.14]	0.531 [0.138,2.21]	0.548 [0.212,1.34]
SOC	19.2	45.1 [34.47, 56.21]		

**[0251]** At the 400 micrograms/kg/24 hours regimen of rIGF-1/rIGFBP-3, a population PK model was

developed using standard of care data and IGF-1 data. The model was used to simulate 95% prediction intervals of IGF-1 PK concentrations over duration of treatment to predict where the mean and 5th and 95th intervals would fall with respect to target therapeutic range (FIG. 12).

**[0252]** The results showed that mean IGF-1 levels were higher at a dose of 400 micrograms/kg/24 hours than 250 micrograms/kg/24 hours. By day 7, approximately 95% of the subjects administered 400 micrograms/kg/24 hours are expected to be in the therapeutic range. At EOI, 90% of subjects are expected to be below 109 ng/ml and 95% of subjects above 28 ng/ml. Majority of the subjects administered a dose of 400 micrograms/kg/24 hours are expected to achieve target IGF-1 levels (28 to 109 ng/ml) by day 7 and are expected to have a low risk for overshooting target IGF-1 levels by end of infusion. End of infusion is, for example, about 28, 29, 30, 31, or 32 weeks post-menstrual age (PMA). In some embodiments, end of infusion is about 28 weeks post menstrual age (PMA). In some embodiments, end of infusion is 29 weeks post menstrual age (PMA). In some embodiments, end of infusion is about 30 weeks post menstrual age (PMA). In some embodiments, end of infusion is about 31 weeks post menstrual age (PMA). In some embodiments, end of infusion is about 32 weeks post menstrual age (PMA).

**[0253]** In order to evaluate whether early or higher IGF-1 serum exposure resulted in better BPD outcomes, IGF-1 serum exposure was measured at day 7 (FIG. 13A) and week 40 PMA (FIG. 13B).

**[0254]** Exploratory logistic regression analyses were conducted to explore the relationship between serum IGF-1 exposure at different time points and BPD outcome. Earlier time points showed significant relationship between higher IGF-1 exposure and favorable BPD outcome (mild or no BPD). To reduce bias, IGF-1 average concentrations over 7 days was selected for model development. Weight at birth (higher WT-better outcome) and gender (females-better outcome) were identified as covariates influencing BPD outcome.

**[0255]** The simulation results further showed that rhIGF-1/rhIGFBP-3 administered at a 400 micrograms/kg/24 hours regimen was associated with a greater probability of no BPD or mild BPD relative to the 250 micrograms/kg/24 hours regimen. Based on simulation studies, the higher dose of 400 micrograms/kg/24 hours was found to be highly correlated with BPD prevention and amelioration of CLD.

**[0256]** While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the examples serve only to illustrate the compounds of the invention and are not intended to limit the same.

**[0257]** The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents.

## **REFERENCES CITED IN THE DESCRIPTION**

Cited references

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## Patentkrav

1. Flydende lægemiddelsammensætning, der er egnet til indgivelse til en nyfødt, hvilken flydende lægemiddelsammensætning omfatter et proteinkompleks omfattende rekombinant insulinlignende væksthormon 1 (rIGF-1), rekombinant insulinlignende væksthormonbindende protein 3 (rIGFBP-3) og en polysorbatsurfactant i en koncentration på mellem ca. 0,0025 % og 0,0075 %, hvor:
  - a. rIGF-1'et og rIGFBP-3'et er kompleksbundne,
  - b. rIGF-1'et/IGFBP-3'et er i en koncentration på ca. 45-55 mikrogram/ml, og
  - c. sammensætningen har en pH på mellem ca. 5,0 og 7,0.
2. Sammensætning ifølge krav 1, hvor rIGF-1'et/IGFBP-3'et er i en koncentration på ca. 50 mikrogram/ml.
3. Sammensætning ifølge krav 1 eller 2, hvor polysorbatsurfactanten er valgt blandt polysorbat 20 (P20) og polysorbat 80 (P80).
4. Sammensætning ifølge krav 3, hvor polysorbatsurfactanten er P20.
5. Sammensætning ifølge et hvilket som helst af krav 1 til 3, hvor polysorbatsurfactanten er til stede i sammensætningen i en koncentration på ca. 0,005 %.
6. Sammensætning ifølge et hvilket som helst af krav 1 til 5, yderligere omfattende en buffer omfattende natriumacetat, eddikesyre og/eller natriumklorid.
7. Sammensætning ifølge krav 6, hvor natriumacetatet eller eddikesyren er i en koncentration på mellem ca. 10 og 100 mM.
8. Sammensætning ifølge krav 6, hvor natriumacetatet eller eddikesyren er i en koncentration på ca. 50 mM.

9. Sammensætning ifølge et hvilket som helst af krav 6 til 8, hvor natriumkloriden er i en koncentration på ca. 20 mM og 200 mM.

10. Sammensætning ifølge krav 9, hvor natriumkloriden er i en koncentration på ca. 105 mM.

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11. Sammensætning ifølge et hvilket som helst af krav 1 til 10, hvor pH'en er ca. 5,3-5,8.

12. Sammensætning ifølge et hvilket som helst af krav 1 til 11, hvor sammensætningen er egnet til intravenøs indgivelse.

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13. Sammensætning ifølge et hvilket som helst af krav 1 til 12, hvor sammensætningen har en holdbarhed på mindst 24 måneder ved en temperatur på mellem 2-8 °C.

14. Sammensætning ifølge et hvilket som helst af de foregående krav, hvor sammensætningen er stabil ved rumtemperatur og/eller omgivende lys i mindst 8 timer, 12 timer, 24 timer, 36 timer eller 48 timer.

15

15. Sammensætning ifølge krav 1 eller 2, hvor mindre end 20 % af IGF-1'et eksisterer som oxideret form efter opbevaring i ca. 6 måneder ved 25 °C.

20

16. Sammensætning ifølge krav 1 eller 2, hvor mindre end 10 % af IGF-1'et eksisterer som oxideret form efter opbevaring i ca. 3 måneder ved 40 °C.

17. Sammensætning ifølge et hvilket som helst af de foregående krav, hvor %'en af oxideret form bestemmes af Reversed Phase-Ultra Performance Liquid Chromatography (RP-UPLC).

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18. Sammensætning ifølge et hvilket som helst af de foregående krav, hvor IGFBP-3'et omfatter mindre end 5 % trisulfidvarianter.

19. Sammensætning ifølge krav 1 omfattende et proteinkompleks omfattende rekombinant insulinlignende vækstfaktor 1 (rIGF-1) og rekombinant insulinlignende vækstfaktorbindende protein 3 (rIGFBP-3) i ækvimolære mængder, polysorbit-20-surfactant i en koncentration på ca. 0,005 % (v/v) og en buffer omfattende natriumacetat, eddikesyre og/eller natriumklorid, hvor sammensætningen har en pH på ca. 5,3-5,8, hvor rIGF-1'et/IGFBP-3'et er i en koncentration på ca. 50 mikrogram/ml, og hvor mindre end 1,5 % af IGF-1'et eksisterer som oxideret form ved frigivelse af produktet.
20. Lægemiddelsammensætning ifølge et hvilket som helst af krav 1 til 19 som et endeligt produkt i hætteglas, indeholdende ca. 6,5 ml ekstraherbar mængde.
21. Sammensætning ifølge et hvilket som helst af krav 1 til 20 til anvendelse ved medicinsk behandling af præmature nyfødte.
22. Sammensætning ifølge et hvilket som helst af krav 1 til 20 til anvendelse ved behandling eller forebyggelse af intraventrikulær blødning (IVH), bronchopulmonær dysplasi (BPD) eller kronisk lungesygdom hos præmature (CLD).
23. Sammensætning til anvendelse ifølge krav 21 eller 22, hvor sammensætningen indgives i en dosis på ca. 400 mikrogram/kg/24 timer.

# DRAWINGS

Drawing

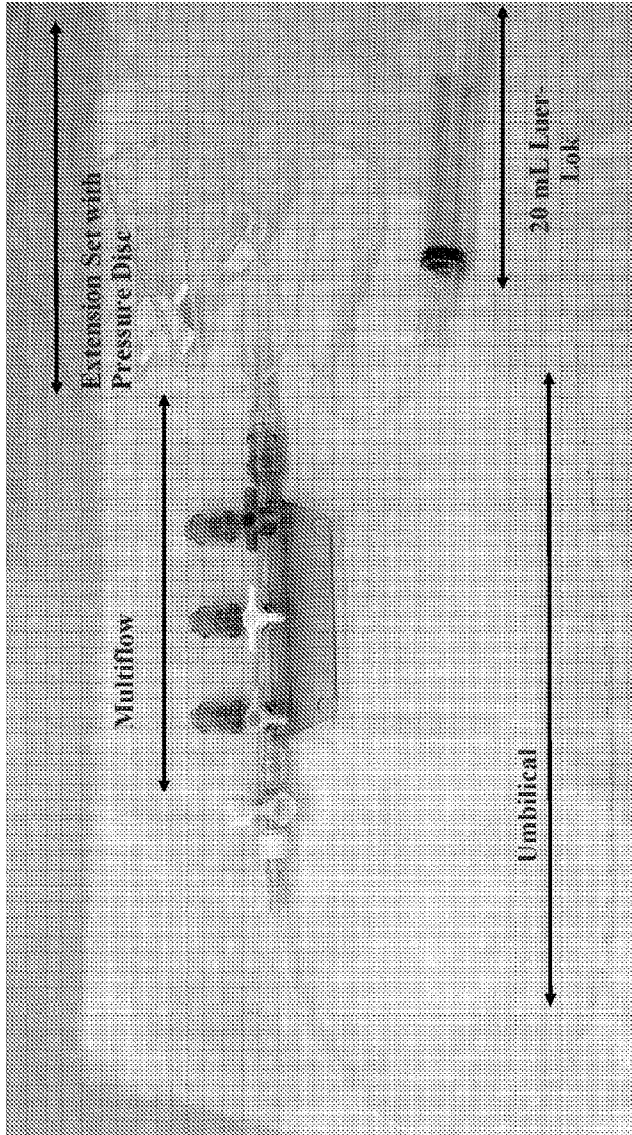


FIG. 1

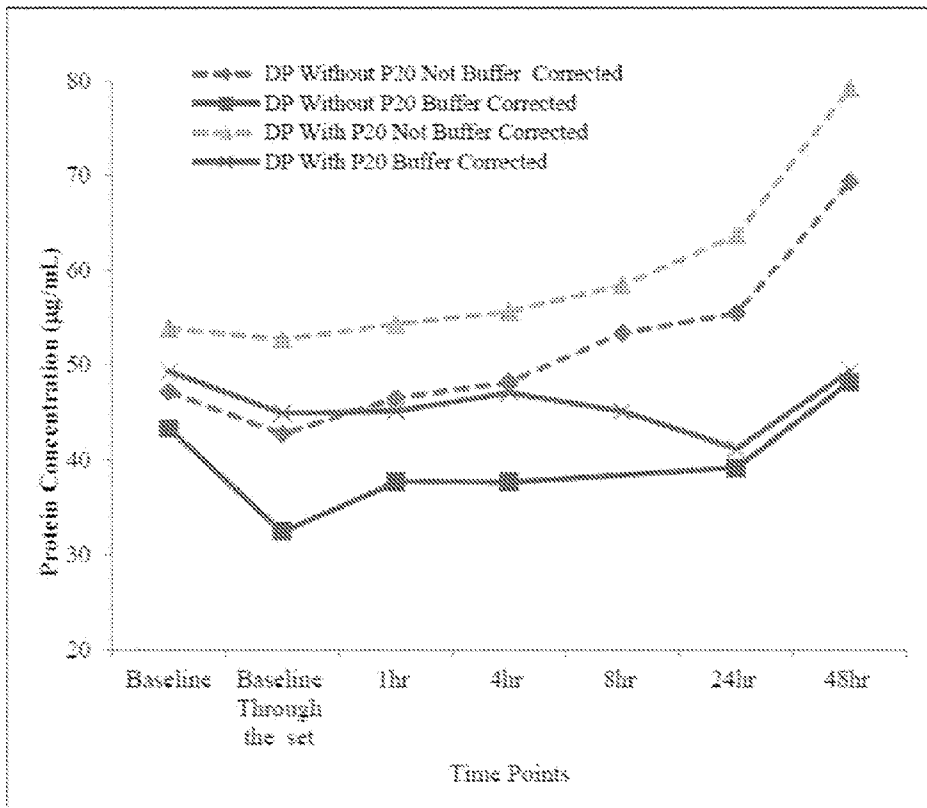


FIG. 2

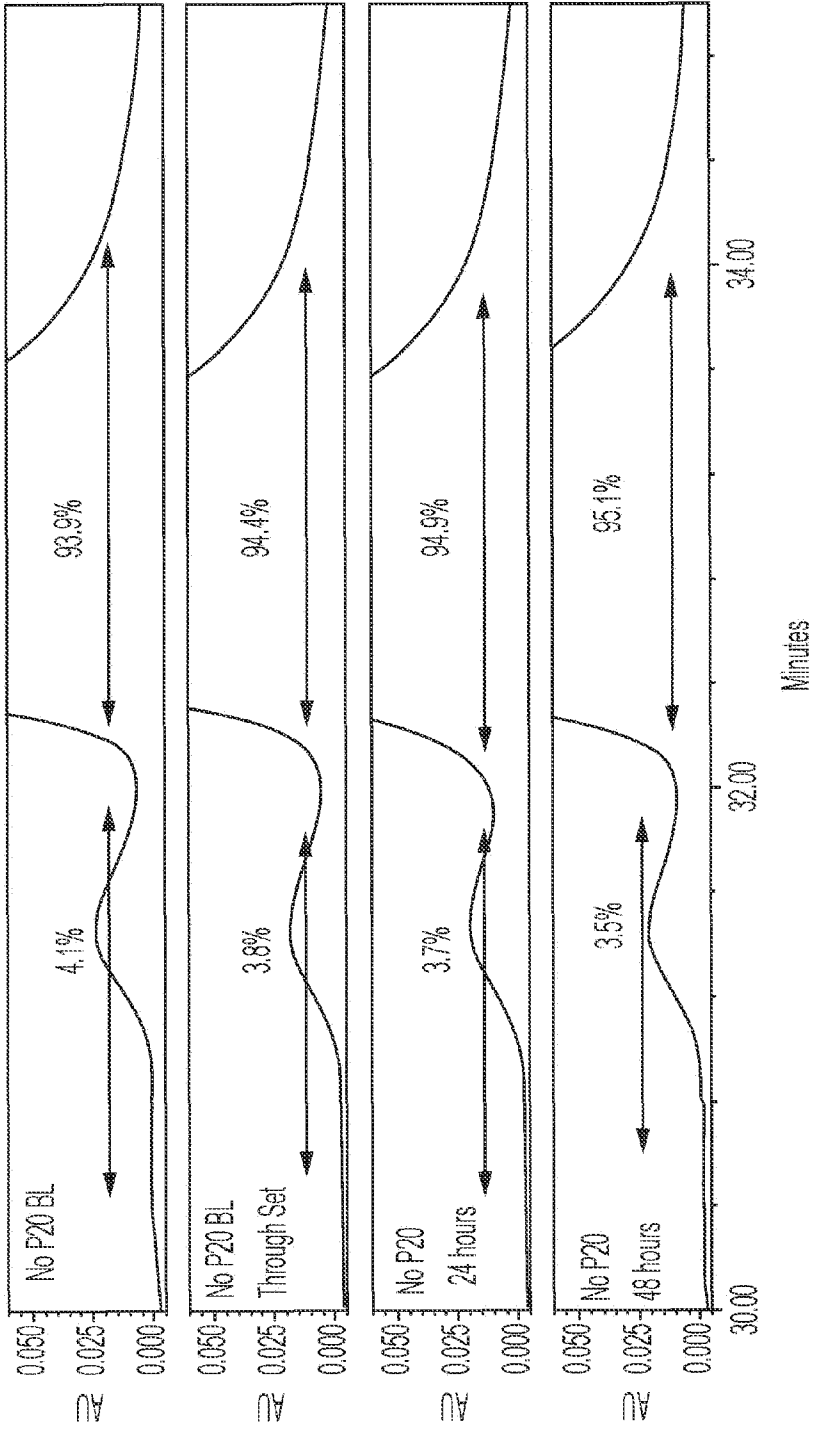


FIG. 3A

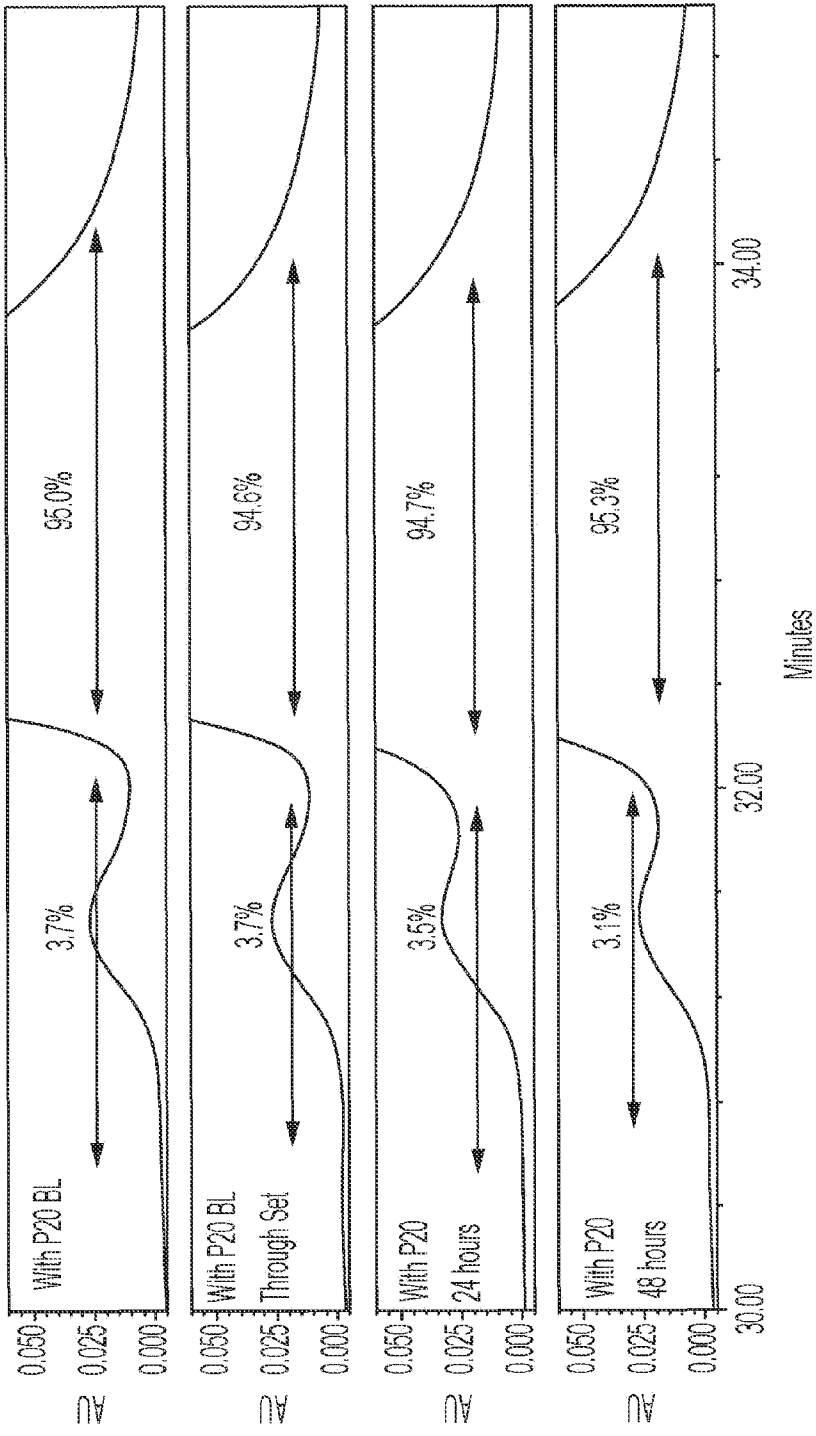


FIG. 3B

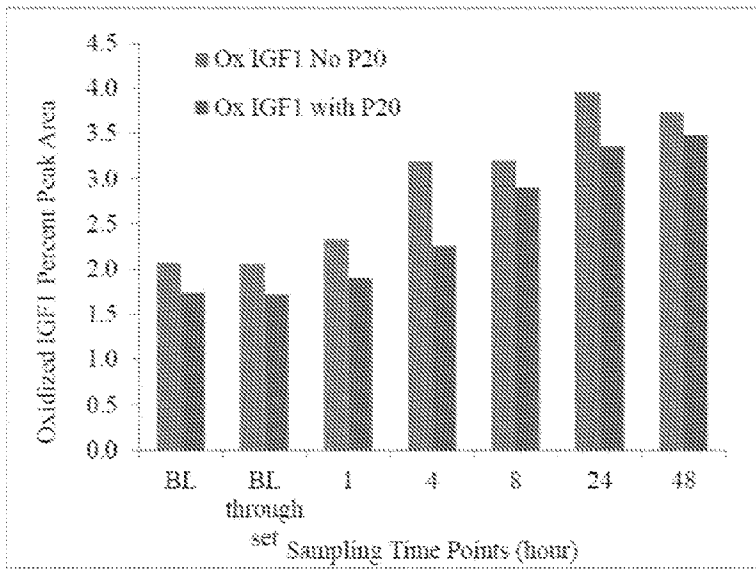


FIG. 4

Comparison of IGF-1 Oxidation in 10 ml, SS Vessel with Increasing Concentrations of Mecasermin Rinfabate and Decreasing Head Space at Room Temperature

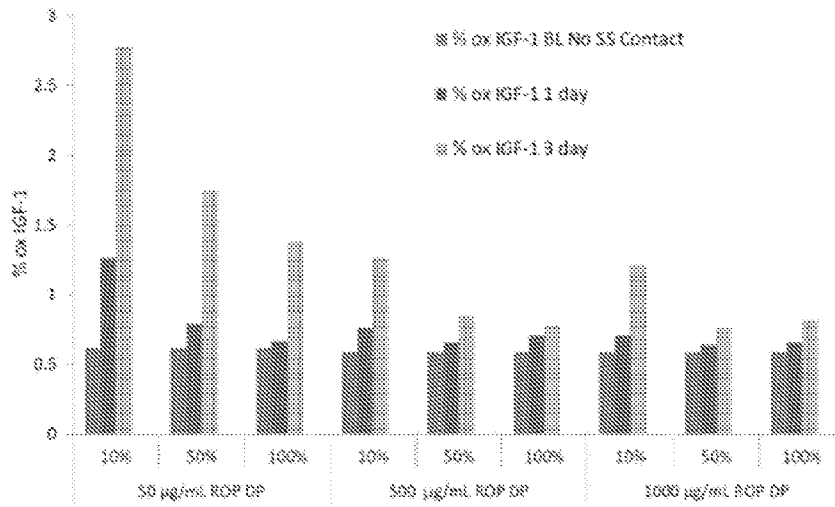


FIG. 5

**Percent IGF-1 Oxidation – All Feasibility Studies at Room Temperature**

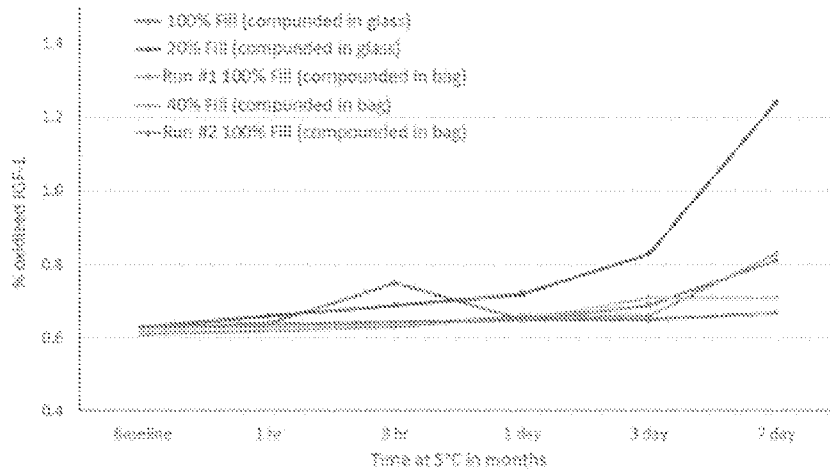


FIG. 6

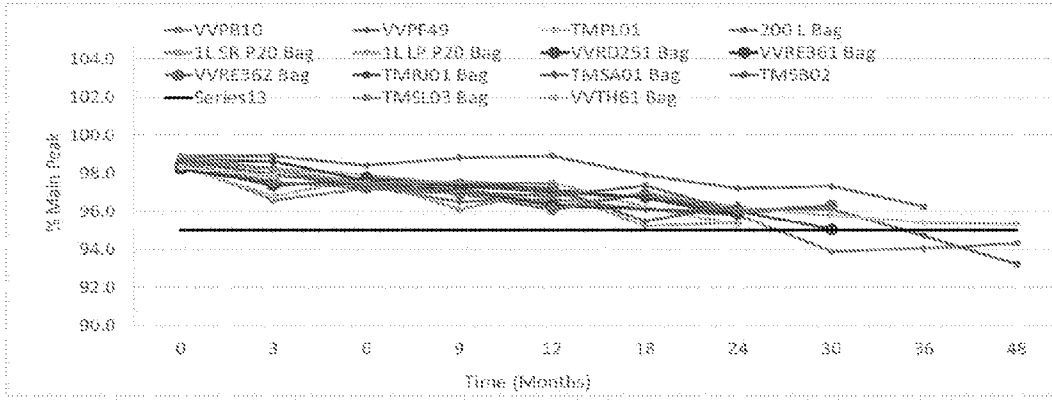


FIG. 7A

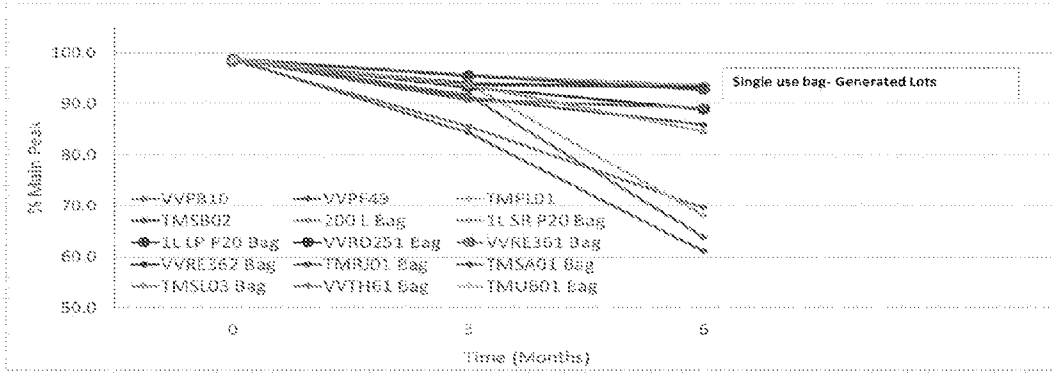


FIG. 7B

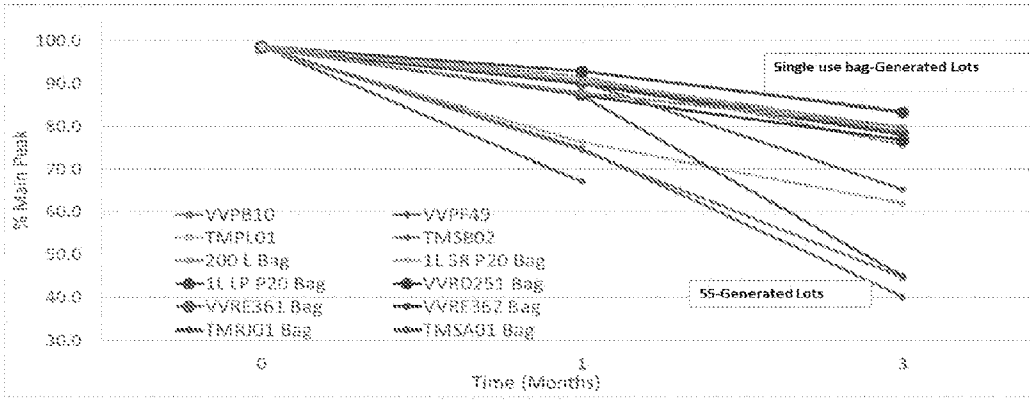


FIG. 7C

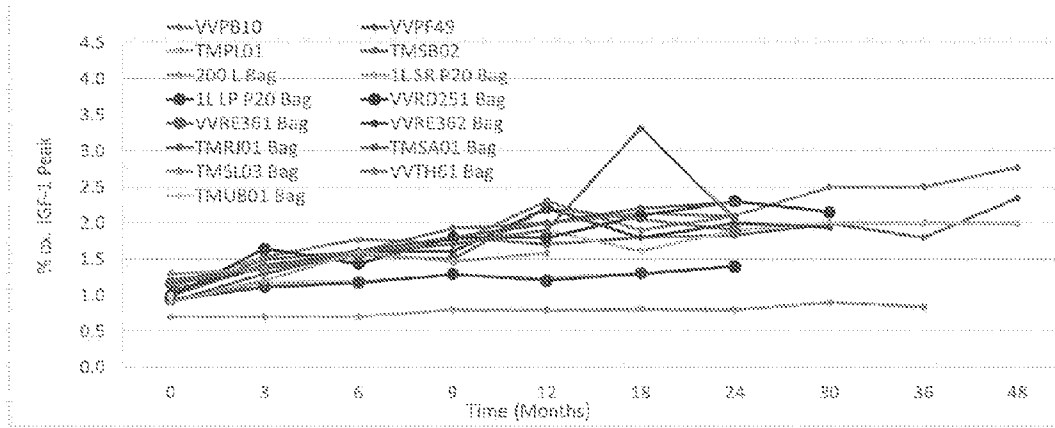


FIG. 8A

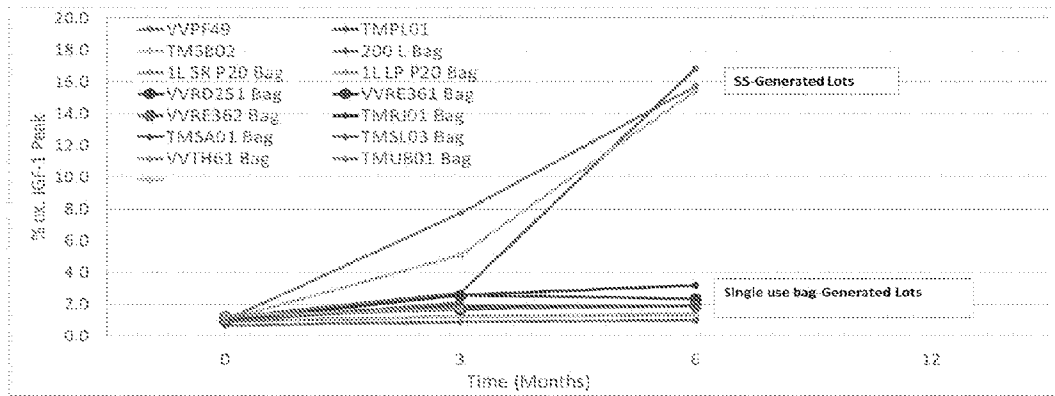


FIG. 8B

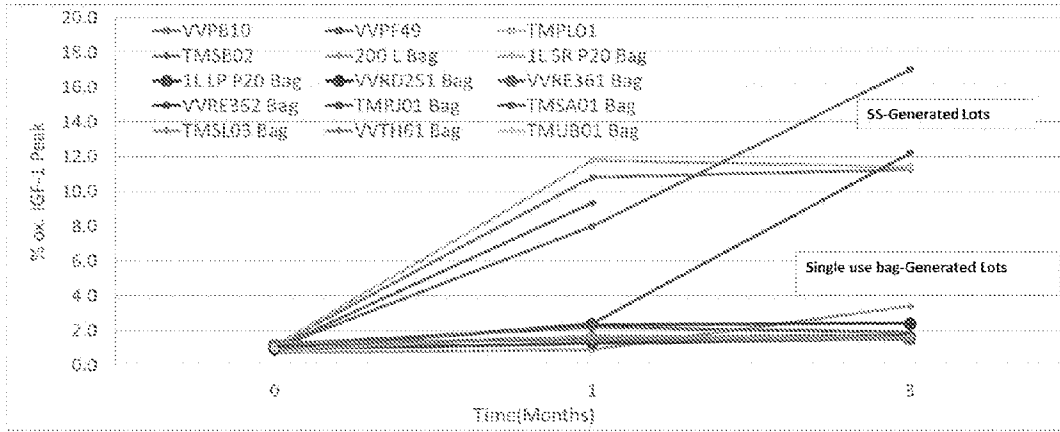


FIG. 8C

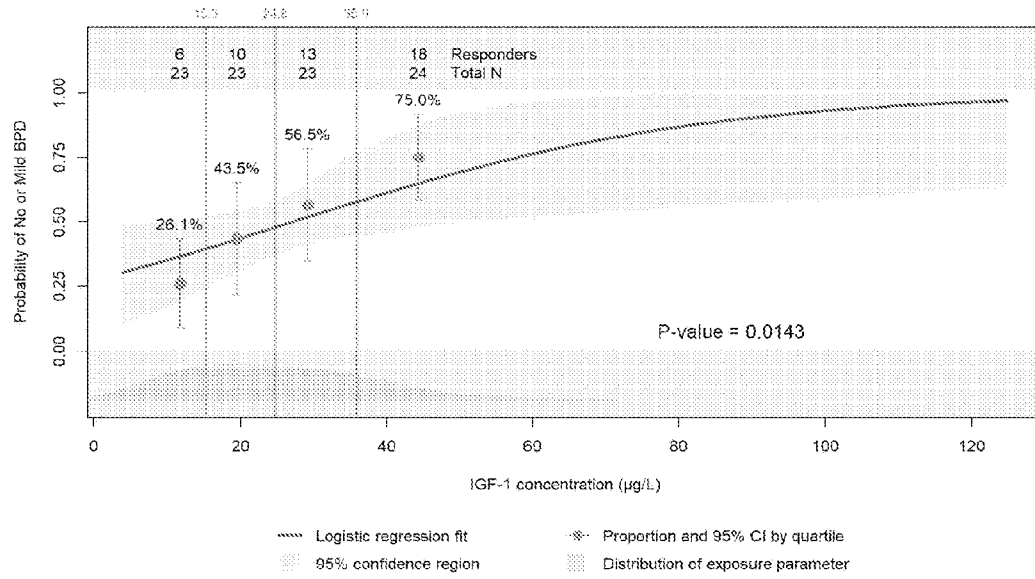


FIG. 9

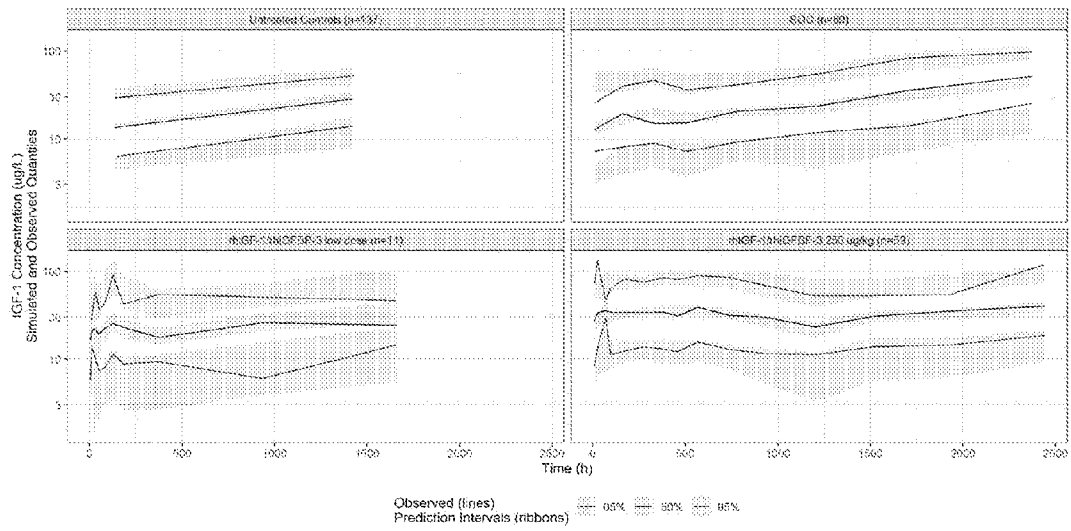


FIG. 10

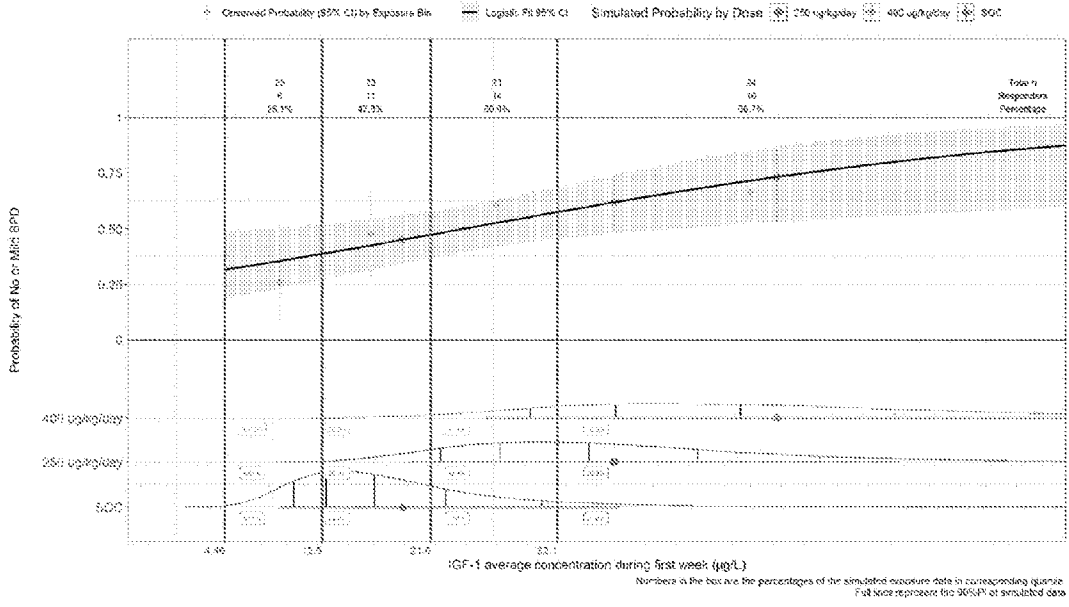


FIG. 11

95% IGF-1 Prediction Interval

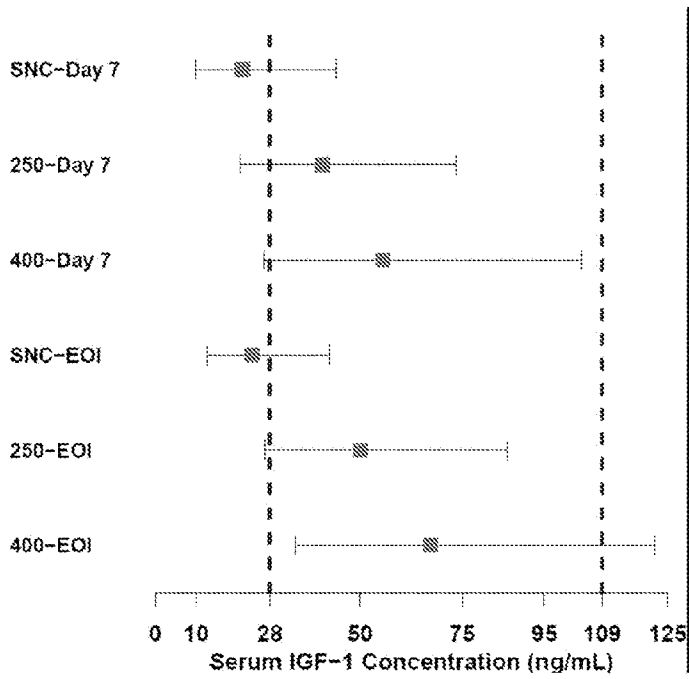


FIG. 12

**Day 7 Serum IGF-1 Exposure Relationship To BPD outcome**

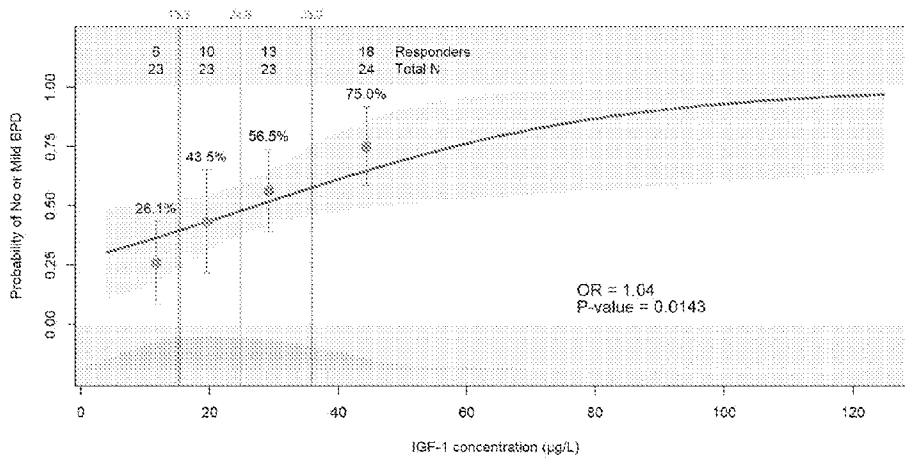


FIG. 13A

**Week 40 PMA Serum IGF-1 Exposure Relationship To BPD outcome**

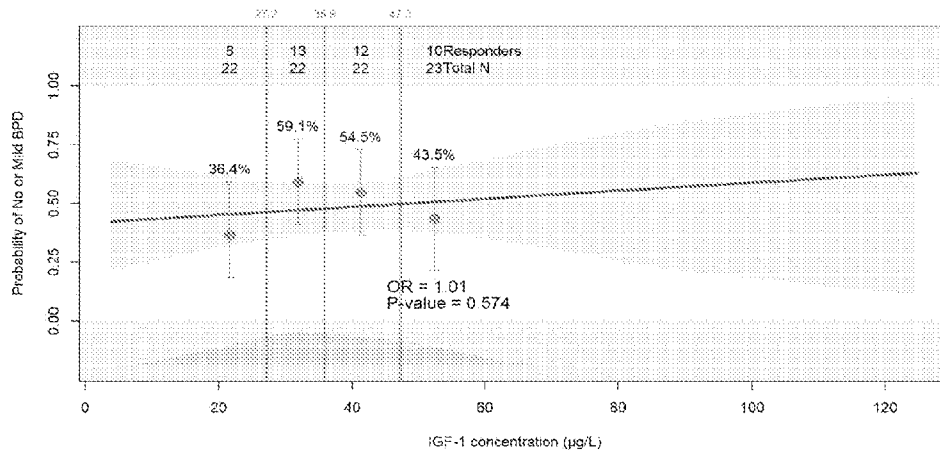


FIG. 13B