



(51) International Patent Classification:

A61K 31/02 (2006.01)

A61P 11/00 (2006.01)

A61K 31/00 (2006.01)

(21) International Application Number:

PCT/US2020/030418

(22) International Filing Date:

29 April 2020 (29.04.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/840,183

29 April 2019 (29.04.2019)

US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: LIQUID VENTILATION FOR BRONCHOPULMONARY DYSPLASIA

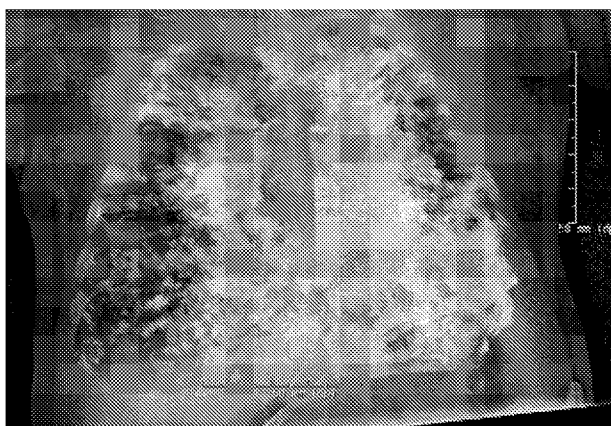


FIG. 1

(57) Abstract: The disclosure is related to the use of liquid fluorocarbon ventilation for the treatment of bronchopulmonary dysplasia (BPD) in neonates, in particular pre-term neonates having severe BPD.



Published:

— *with international search report (Art. 21(3))*

DESCRIPTION

LIQUID VENTILATION FOR BRONCHOPULMONARY DYSPLASIA

PRIORITY CLAIM

This application claims benefit of priority to U.S. Provisional Application Serial No. 62/840,183, filed April 29, 2019, the entire contents of which is hereby incorporated by reference.

FIELD OF THE DISCLOSURE

The present disclosure relates to the fields of biology, medicine and pediatrics. More particularly, the disclosure relates to fluorocarbon liquid compositions and liquid ventilation thereof for treating bronchopulmonary dysplasia (BPD).

BACKGROUND

Of the 135,000 neonates with respiratory distress syndrome in the U.S. each year, 15,000 progress to a comorbid diagnosis of bronchopulmonary dysplasia (BPD). The incidence of BPD has not changed for 30 years. According to the American Lung Association, BPD results from damage to the lungs by IMV and long-term oxygen. However, the exact diagnostic criteria are broad and continue to be debated by neonatologists. BPD is a chronic condition and the long-term morbidity includes respiratory symptoms, poor lung function, delayed cognitive development and lower academic achievement.

Current treatment of BPD is twofold – managing ventilator requirements for IMV (type, settings, duration) and secondly judicious use to corticosteroids. However, corticosteroids have risks to cognitive development and so cannot be used for more than 10 days in treatment, therefore being very limited in use. BPD infants are often long-term inpatients and among the most-costly, with the average patient spending 118 days and incurring on average \$666,000.

Decreasing the severity of BPD is therefore valuable in many domains – from preventing the acute suffering to reducing the long-term illness to reducing burden on valuable ventilator beds. Research in recent times has led to some improvements in care such as caffeine administration and Less Invasive Surfactant Administration (LISA) for RDS, while there are some ongoing studies into the efficacy of inhaled steroids. However, as evidenced by their long hospital stays, even with new improved treatments, BPD is difficult to treat effectively.

Perflurooctylbromide (PFOB) has long been considered as a treatment for pulmonary conditions due to its properties of high gas solubility delivering oxygen to alveoli and anti-inflammatory effects that protect sensitive lung tissue. Promising early research was trumped by adverse events in adult trials (not neonates), causing the FDA to withhold its use and
5 triggering the demise of the manufacturing in 1997. Its use in BPD, particular neonatal BPD, has not been explored.

SUMMARY

In accordance with the present disclosure, there is provided a method of treating an infant subject with broncopulmonary dysplasia (BPD) patient comprising the steps of:

(a) administering to the lung of the patient an effective amount of a fluorocarbon liquid having an equilibrium coefficient of spreading which is a positive number, said amount of administered fluorocarbon liquid not exceeding 35% of the functional residual capacity of the lung of the patient upon exhalation taking into account any positive or negative expiratory pressure applied to said patient's lung; and

(b) introducing a breathing gas into the lung wherein said introduced breathing gas physically admixes with and oxygenates said fluorocarbon liquid within the lung.

The fluorocarbon liquid may comprise a mixture of two or more fluorocarbons, such as a mixture of fluorocarbons comprises at least one fluorocarbon having an equilibrium coefficient of spreading which is a negative number. The fluorocarbon liquid may further comprise a surfactant.

The fluorocarbon liquid may comprise a non-halogenated fluorocarbon or a halogenated fluorocarbon. The fluorocarbon liquid may comprise a fluorocarbon-hydrocarbon compound, such as perfluoro-octyl-bromide. The fluorocarbon liquid may have a surface tension value less than 20 dynes/cm. The fluorocarbon liquid may comprise a cyclic fluorocarbon. The cyclic fluorocarbon may selected from the group consisting of perfluorodecalin, F-adamantane, F-methyladamantane, F-1, 3-dimethyladamantane, F-dimethylbicyclo[3,3,1]nonane, F-dimethylbicyclo[3,3,1]nonane and combinations thereof. The fluorocarbon liquid may comprises a perfluorinated amine selected from the group consisting of F-triethylamine, F-tri-butylamine, F-4-methyloctahydroquinoline, F-n-methyl-decahydroisoquinoline, F-n-methyldecahydroquinoline, F-n-cyclohexylpyrrolidine, F-2-butyltetrahydrofuran and combinations thereof.

The method may further comprise the step of introducing said breathing gas by spontaneous ventilation while the fluorocarbon liquid is in the lung or may further comprise the step of introducing said breathing gas using mechanical ventilation while the fluorocarbon liquid is in the lung. The fluorocarbon liquid may be administered as an aerosol.

The subject may be an infant of 12 months corrected age or less, or is a pre-term infant, or is both. The subject may suffer from mild BPD, from moderate BPD or from severe BPD. The may be dose is between about 1.0 mg/kg/day and about 5.0 mg/kg.day fluorocarbon liquid, such as about 2.5 mg/kg/day. The subject may be dosed twice per day, such as or up to five

consecutive days or up to ten days. The cumulative five-day dosage may be no more than about 12.5 mg/kg, no more than about 10 mg/kg or about 7.5 mg/kg, or no more than about 5 mg/kg. The cumulative ten-day dosage may be is no more than about 25 mg/kg, no more than about 20 mg/kg or about 15 mg/kg, or no more than about 10 mg/kg. The subject maybe a pre-term
5 infant of six months corrected age or less, diagnosed with severe BPD, is dosed twice per day to a total of 2.5 mg/kg/day and is treated for five, six, seven, eight, nine or ten consecutive days.

In some embodiments of the method of treating of the disclosure, the administration of the therapeutically effective amount of the composition is provided as a single dose or provided
10 within a single medical procedure.

In some embodiments of the method of treating of the disclosure, the administration of the therapeutically effective amount of the composition is provided as multiple doses or provided over multiple medical procedures.

As used herein in the specification, “a” or “an” may mean one or more. As used herein
15 in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another”
20 may mean at least a second or more.

Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, for the method being employed to determine the value, or that exists among the study subjects. Such an inherent variation may be a variation of $\pm 10\%$ of the stated value.

Other objects, features and advantages of the present disclosure will become apparent
25 from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art
30 from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Subject 1 after receiving 177.8 mL of PFOB.

FIG. 2. Subject 4 after receiving 46.0 mL of PFOB.

DETAILED DESCRIPTION

The disclosure provides for the use of pulmonary ventilation using liquid fluorocarbon preparations for the treatment of BPD. Based on their preliminary findings, the inventors propose that fluorocarbons like PFOB can be safely used in infants diagnosed with BPD.

5 Although BPD is a chronic condition, they propose that PFOB can be safely administered to the BPD population because BPD patients are a more stable population (less rapidly changing lung function, requiring less frequent ventilator adjustments) than prior neonatal populations studied. Indeed, the previous acutely ill patients had rapidly changing pulmonary conditions and were more labile, thus potentially at a higher risk for side effects attributed to PFOB-PLV, 10 which are not expected in BPD populations. The reasons why BPD patients require long-term hospitalization relate to interrupted lung development and subsequent lung inflammation or sepsis due to ventilator induced lung injury (VILI). The literature suggests that PFOB may work as a substitute surfactant and may also have anti-inflammatory effects. Thus, PFOB may be safer in the BPD population because it may decrease harmful oxygen exposure, which 15 requires additional interventions, resulting in further complications to the patient.

Studies show infants receiving PFOB-PLV for up to 7 days have improvements in gas exchange and pulmonary compliance without adverse events related to the drug or technique. The inventors believe that extending the course of PFOB-PLV will be safe in stable BPD patients up to 10 days and result in continued improvements in gas exchange. They also 20 suggest that extending PFOB-PLV up to 10 days will rest the lungs of BPD patients, similar to inducing paralysis in the same population. These findings suggest that the response of sick term infants to PFOB-PLV is less gradual than that observed in preterm infants with RDS. Differences in this response may be due to a relatively rapid reduction in surface tension and volume recruitment in the preterm infant. Therefore, PFOB should be safely administered 25 for up to 10 days.

Subjects are intubated with an endotracheal (ET) tube and ventilated with the conventional ventilator of choice as determined by clinicians in charge of care prior to dosing and for the duration of the treatment period. If subjects are on a high frequency mode of ventilation, the infant can be changed to CMV for at least two days before entering treatment 30 because evaporation rates of PFOB are too great with high frequency ventilation. In a model protocol, PFOB can be administered in a series of treatment doses equal to 2.5 mL/kg daily not to exceed a total intra-pulmonary volume of up to 12.5 mL/kg over 5 days or up to 25 mL/kg

over 10 days. Some infants may be treated with lower or higher total pulmonary volumes of PFOB depending on their lung pathology and tolerance of the liquid instillation.

These and other aspects of the disclosure are reproduced below.

5 I. Bronchopulmonary Dysplasia

Respiratory distress syndrome (RDS) is a lung condition in neonates, resulting from lack of surfactant, a substance needed to inflate the lungs and the alveoli. RDS affects both adult and pediatric populations. In adults and children, RDS is due to lung injury initiated by pneumonia or sepsis. In infants, RDS can result from insufficient or impaired development of
10 the lungs due to prematurity. It is estimated that some degree of chronic lung disease may occur in up to 60% of infants weighing less than 700 grams (g) at birth and 50% of all infants born less than 1000g at birth.

BPD affects approximately 12,000 premature infants in the United States each year (lungusa.org), with the incidence of the disease inversely proportional to gestational age. Thus,
15 the more premature the patient, the higher the probability the patient will develop BPD. Clinical features of BPD include wheezing, appearance of cysts or infiltrates in the lungs when viewed radiographically, and development of pulmonary infection. As is common with RDS, BPD patients also have decreased blood oxygenation (hypoxia). Even if optimally treated, BPD can cause prolonged breathing problems, respiratory failure, and death.

Many advances in caring for newborns with severe respiratory disease have occurred
20 in the last 30 years. However, the outcomes for infants with BPD remain suboptimal. Infants with BPD continue to suffer significantly higher mortality and morbidity rates compared to the initial therapies in the first weeks of life. Treating BPD is complicated by the fact that often the lungs show heterogeneity and require very different medical approaches to resolve injuries.
25 A single patient may need many different therapies due to some areas of the lungs being stiff, while others are over distended. In the most severe cases of BPD, paralysis for one to two weeks often helps break the pathologic cycle of disease. However, severe cases may still require continuous mandatory ventilation (CMV) therapy for 6 months or more, which can introduce additional problems to the patient's health.

Although CMV is necessary to maintain life, it interrupts normal airway and alveolar
30 development. At present, there is no specific ventilator strategy that has any appreciable effect on changing the course of BPD. Many infants with BPD will also experience some neurodevelopmental delay and/or long-term lung impairment.

CMV is the primary treatment for patients diagnosed with BPD or other severe lung injuries. CMV provides sustained support for oxygenation and gas exchange of the patient during hospitalization using maintained positive pressure. However, CMV has the potential to cause further lung injury. Ventilated individuals are at risk of developing air leaks (pneumothoraces), lung collapse, presence of liquid in the lungs (edema), or tissue scarring caused by the presence of immune cells (fibrosis) [2, 3]. This combination of complications is referred to as VILI. The series of physiological events that VILI induces, often results in further lung complications, including: continued interventions to maintain life, extended hospitalization, and/or possibly death.

BPD patients often receive other supportive respiratory therapies during the course of their hospitalization. Pulmonary vasodilators such as inhaled nitric oxide (iNO) are often used to treat the pulmonary hypertension that develops in some infants with BPD. Although iNO may decrease short term oxygen requirements [4], it may not impact long-term pulmonary health [5]. Glucocorticoids may also be administered to ventilated patients to treat chronic inflammation. The two most commonly administered steroids used to prevent or treat BPD are dexamethasone and hydrocortisone. Clinically, it usually takes approximately 10 days of steroid administration to observe a beneficial effect on BPD. As is the case with CMV, steroid administration is not without complications. Dexamethasone is reported to cause neurodevelopmental issues if administered in high doses [6]. Hydrocortisone is documented as causing gastrointestinal perforation, although this finding may have been confounded by concurrent administration with nonsteroidal anti-inflammatory drugs and was only seen in younger infants. Most of the potential detrimental effects of steroid usage in neonatal populations relates to the need to administer doses systemically [6, 7]. Developing a respiratory therapy to treat lung complications, but does not adversely affect other organs, would be beneficial for treating BPD and other lung injuries in the neonatal population.

II. Fluorocarbons

Compounds useful in this invention, such as those listed below (hereinafter called "fluorocarbons") are generally able to promote gas exchange, and most of these fluorocarbons readily dissolve oxygen and carbon dioxide. There are a number of fluorocarbons that are contemplated for medical use. These fluorocarbons include bis (F-alkyl) ethanes such as $C_4F_9CH=CH_4CF_9$ (sometimes designated "F-44E"), $i-C_3F_9CH=CHC_6F_{13}$ ("F-i36E"), and $C_6F_{13}CH=CHC_6F_{13}$ ("F-66E"); cyclic fluorocarbons, such as $C_{10}F_{18}$ ("F-decalin", "perfluorodecalin" or "FDC"), F-adamantane ("FA"), F-methyladamantane ("FMA"), F-1,3-

dimethyladamantane ("FDMA"), F-di-or F-trimethylbicyclo[3,3,1]nonane ("nonane"); perfluorinated amines, such as F-triethylamine ("FTEPA") and F-tri-butylamine ("FTBA"), F-4-methyloctahydroquinolizine ("FMOQ"), F-n-methyl-decahydroisoquinoline ("FMIQ"), F-n-methyldecahydroquinoline ("FHQ"), F-n-cyclohexylpyrrolidine ("FCHP") and F-2-butyltetrahydrofuran ("FC-75" or "RM101").

Other fluorocarbons include brominated perfluorocarbons, such as 1-bromoheptadecafluoro-octane ($C_8F_{17}Br$, sometimes designated perfluorooctylbromide or "PFOB"), 1-bromopentadecafluoroheptane ($C_{sub.7}F_{sub.15}Br$), and 1-bromotridecafluorohexane ($C_6F_{13}Br$, sometimes known as perfluorohexylbromide or "PFHB"). Other brominated fluorocarbons are disclosed in U.S. Patent 3,975,512 to Long. Also contemplated are fluorocarbons having nonfluorine substituents, such as perfluorooctyl chloride, perfluorooctyl hydride, and similar compounds having different numbers of carbon atoms. In addition, the fluorocarbon may be neat or may be combined with other materials, such as surfactants (including fluorinated surfactants) and dispersed materials.

Additional fluorocarbons contemplated in accordance with this invention include perfluoroalkylated ethers or polyethers, such as $(CF_3)_2CFO(CF_2CF_2)_2OCF(CF_3)_2$, $(CF_3)_2CFO-(CF_2CF_2)_3OCF(CF_3)$, $(CF_3)CFO(CF_2CF_2)F$, $(CF_3)_2CFO(CF_2CF_2)_2F$, $(C_6F_{13})_2O$. Further, fluorocarbon-hydrocarbon compounds, such as, for example compounds having the general formula $C_nF_{2n+1}-C_{n'}F_{2n'+1}$, $C_nF_{2n+1}OC_{n'}F_{2n'+1}$, or $C_nF_{2n+1}CF=CHC_{n'}F_{2n'+1}$, where n and n' are the same or different and are from about 1 to about 10 (so long as the compound is a liquid at room temperature). Such compounds, for example, include $C_8F_{17}C_2H_5$ and $C_6F_{13}CH=CHC_6H_{13}$. It will be appreciated that esters, thioethers, and other variously modified mixed fluorocarbon-hydrocarbon compounds are also encompassed within the broad definition of "fluorocarbon" materials suitable for use in the present invention. Mixtures of fluorocarbons are also contemplated. Additional "fluorocarbons" not listed here, but having those properties described in this disclosure that would lend themselves to pulmonary therapies are additionally contemplated.

Some fluorocarbons have relatively high vapor pressures which render them less suitable for use as a surfactant replacement and for partial liquid breathing. These include 1-bromotridecafluorohexane ($C_6F_{13}Br$) and F-2-butyltetrahydrofuran ("FC-75" or "RM101"). Lower vapor pressures are additionally important from an economic standpoint since significant percentages of fluorocarbon having high vapor pressure would be lost due to vaporization during the therapies described herein. In a preferred embodiment, fluorocarbons having lower surface tension values are chosen as surfactant supplements.

The fluorocarbon of choice should have functional characteristics that would permit its use temporarily as a lung surfactant, for oxygen delivery, in removal of material from the interior of the lung, or for inflation of collapsed portions of the lung. Fluorocarbons are biocompatible and most are amenable to sterilization techniques. For example, they can be
5 heat-sterilized (such as by autoclaving) or sterilized by radiation. In addition, sterilization by ultrafiltration is also contemplated.

One group of preferred fluorocarbons have the ability to reduce the surface tension in the lung. As noted above, surfactants function to decrease the tension between the surface molecules of the alveolar fluid. The lung surfactant is solubilized in a water-continuous fluid
10 lining the alveolus. Typically, the surface tension in the absence of lung surfactant is ca. 60 dynes/cm decreasing to 5-30 dynes/cm in the presence of lung surfactant. Fluorocarbons have low surface tension values (typically in the range of 20 dynes/cm) and have the added benefit of dissolving extremely large quantities of gases such as oxygen and carbon dioxide. Perfluorocarbons are particularly suited for this use, and brominated fluorocarbons are
15 particularly preferred.

A particular fluorocarbon of interest is perfluoro-octyl-bromide ($C_8F_{17}Br$), previously marketed as perflubron. This molecule is a biochemically inert linear perfluorocarbon (PFC) with structural formula, $CF_3(CF_2)_6CF_2Br$. Perfluoro-octyl-bromide (PFOB) is a clear, colorless, liquid that is water-insoluble and chemically stable between 15° and 30°C. PFOB is
20 among a group of PFCs that fall in a molecular weight category that make it ideal for biologic use. PFOB, and also the cyclical perfluorodecalin, are biocompatible and can be manufactured with high purity levels.

PFCs have a long half-life, thus they can remain in the body at physiological temperatures for an extended period of time after minimal exposure (8). There is no
25 documented evidence suggesting adverse effects from the PFOB or metabolic sub-products. Additionally, PFCs have been used commercially since the 1940s and industrial workers have not demonstrated side effects due to exposure. PFCs used for liquid ventilation, such as PFOB, are usually rapidly excreted via the lungs. The PFOB molecule used in this protocol is chemically the same to the one approved for lung lavage in Europe, Canada and South America
30 and is virtually the same molecule approved by the FDA for previous human subjects. Supplemental information regarding the manufacturing of the study PFOB is included in the CMC section of this IND application.

PFOB is an ideal liquid breathing medium due to its ability to dissolve the respiratory gases oxygen (O_2) and carbon dioxide (CO_2). PFOB, and other PFCs, also have a very low

surface tension suggesting that it may work as replacement surfactant [8]. Additionally, some PFCs have radiographic properties that can be used to visualize pneumothoraces or subsequent lung injury [9-11]. Additionally, PFOB liquid itself is twice the weight of water and therefore when the lungs are filled, the weight of the PFOB column may provide additional positive end-expiratory pressure (PEEP) helping to expand the lungs.

III. Partial Liquid Ventilation

Liquid ventilation has been attempted over the last 100 years using various liquids and ventilation techniques. In the 1960s, a study using mice showed that mammals could use PFCs as a liquid breathing medium [12]. In the early 1970s, Moskowitz and colleagues, developed a total liquid ventilation (TLV) system that was composed of a ventilator filled with liquid PFCs [13]. Although many animal studies highlighted the efficacy of TLV, it remained an experimental respiratory therapy due to difficulties in delivering PFC for ventilation. An alternate strategy, termed partial liquid ventilation (PLV), was developed.

Unlike TLV, PLV did not require special equipment to administer the PFC to the patient. A standard conventional gas ventilator, available in neonatal intensive care units (N/IICU), is used for PFC delivery. In PLV, PFOB is administered to the lungs of the patient via an endotracheal tube (ET). The infant is then ventilated with standard pressure on a volume ventilator. These improvements in delivery made PLV using PFOB a more feasible treatment option for the typical N/IICU. This method was first used clinically in severely premature infants as a rescue therapy in the 1980s [1].

In the 1990s, four separate investigational new drug (IND) applications were approved by the FDA to investigate the safety and efficacy of PFCs, mainly PFOB, as liquid breathing medium in neonates. While animal studies showed clear efficacy and safety of PFOB, clinical studies of PLV in adults had mixed outcomes. A summary of the primary findings from non-clinical and clinical studies, including adult and pediatric populations, is provided below.

Over the course of the last 50 years, many animal studies showed liquid ventilation to be an effective treatment for severe lung injury. These studies indicated PLV to be a superior mode of respiratory support when compared to CMV. Various studies also demonstrated short-term beneficial physiologic responses in lung function due to improved alveolar recruitment and significant preservation of normal histological structure of the lung [13-21]. Non-clinical studies in newborn animal models of RDS showed that PFOB enhances uniformity of the lung inflation consistent with PFOB working as an artificial surfactant [22-26]. Animal studies also

showed that PFOB minimizes functional lung impairment due to the high airway pressures and sustained FiO_2 requirements that are characteristics of CMV [1, 26-34].

Recent studies continue to show PFOB improves oxygenation [14, 35-38] in animal models of lung injury consistent with earlier findings. Additionally, recent studies report that PFOB increases lung compliance [14, 23, 37, 39, 40]. Findings from earlier studies indicated that PFOB may have potential anti-inflammatory properties. Animal trials showed administration of PFOB decreased expression of known inflammatory markers [24, 41, 42] and decreased inflammatory cell infiltration into the lungs [43, 44] compared to animals treated with CMV. Additionally, two recent studies reported PFOB does not interfere with cerebral blood flow [45, 46], suggesting PLV with PFOB will have limited impact on cardiac output and circulation.

Animal trials consistently support the safety of PFOB, as few negative effects have been noted. Studies show PFOB is not absorbed systemically and causes no long-term harm. Some studies reported that final concentrations of PFC in animal models after cessation of treatment were minimal [47-49]. However, studies show initiation of PFOB-PLV and removal of PFOB does not produce significant adverse effects. Pneumothorax, a documented adverse event in adult clinical studies, is not reported with any significance in animal models with severe lung injury.

U.S. Patent 8,048,043, Australian Patent 1664801, U.S. Patent 6,013,619, Australian Patent 4013099, Australian Patent 704024, EP 0 904 122, U.S. Patent 5,859,068 and U.S. Patent 5,655,521 all described relevant delivery technologies and formulations and are incorporated herein by reference.

IV. Examples

The following examples are included to demonstrate certain non-limiting aspects of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the disclosure. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 – Pilot Safety and Feasibility Study Protocol

Study Rationale. Perfluorocarbon liquids (PFCs) have been studied for several decades for pulmonary diseases, with perfluoro-octyl-bromide ($C_8F_{17}Br$) (PFOB) demonstrating promise in both animal and clinical studies. While clinical studies from the 1990s indicated the safety of PFOB in neonatal studies, a multi-site clinical study in adults indicated significant morbidity and mortality leading to discontinuation of drug manufacture and halting of further studies.

While the adult study results were problematic, none of the pediatric studies demonstrated significant safety signals, and indeed, indicated varying degrees of efficacy (refer to FDA IND 42,317; IND 44,628; IND 44,076; and IND 49,053). The pediatric studies were primarily conducted in acutely ill neonates with severe lung disease, a generally high-risk population of study subjects.

The study team at Childrens Hospital of Philadelphia (hereafter “CHOP”) considers infants with bronchopulmonary dysplasia (BPD) to be ideally suited for a pilot safety and feasibility study of PFOB. Infants with BPD are chronically ill, and more stable clinically than neonates with RDS, thus a potentially less-risky population to initiate the study. Additionally, preliminary data in several neonates with BPD or BPD-like characteristics shows the potential for feasibility, safety and efficacy.

Study Objectives. Primary Objective. The primary study objective is to assess the safety and feasibility of PFOB as a liquid breathing medium for up to 10 days in subjects with severe BPD as evaluated by: (1) no sustained oxygen desaturations ($SpO_2 \leq 80\%$) for greater than ten minutes without response to increased oxygen therapy, (2) no persistent hypotension (as defined by $\geq 20\%$ decrease in blood pressure) without response to volume expansion and/or inotropic therapy, (3) no major mucus plugging events (defined as events that are unresolved after two bronchoscopes), and (4) no pneumothoraces/pleural effusion with PFOB (5) No sustained CO_2 retention greater than 95 mmHg for over four hours. Feasibility will be assessed by the ability to perform a successful PLV according to protocol parameters.

Secondary Objectives. The secondary objectives are exploratory, to assess the potential efficacy of PFOB for up to 10 days on the duration of mechanical ventilation, concomitant medication, and oxygen requirement. In addition, the anti-inflammatory response of PFOB will be measured to determine its effect on inflammatory cytokines. The effect of PFOB on pulmonary artery pressure will also be evaluated by obtaining an ECHO prior and post PFOB

instillation. Residual perflubron in the lungs will be assessed radiographically as an exploratory endpoint up to 30 days post treatment.

Test Articles. Perfluoro-octyl-bromide ($C_8F_{17}Br$), marketed as perflubron, is a biochemically inert linear perfluorocarbon (PFC) with structural formula, $CF_3(CF_2)_6CF_2Br$.

5 Perfluoro-octyl-bromide (PFOB) is a clear, colorless, odorless liquid that is water-insoluble and chemically stable between 15° and 30°C. PFOB is among a group of PFCs that fall in a molecular weight category that make it ideal for biologic use. PFOB, and also the cyclical perfluorodecalin, are biocompatible and can be manufactured with high purity levels. The liquid has anti-inflammatory properties, surfactant-like properties, and high oxygen-carrying
10 capacity.

Study Design. This is a single center, randomized, non-blinded, safety and feasibility trial to evaluate the use of perfluorooctylbromide (PFOB) partial liquid ventilation in infants with bronchopulmonary dysplasia (BPD). Eligible subjects will be randomized (3:1) to one of two treatment arms (1) PFOB Group or (2) Control Usual Care Group for five days in Part 1,
15 and if safety data allows, for up to 10 days in Part 2. All subjects will be monitored daily. Subjects in the PLV treatment arm will be evaluated for safety after initial PFOB treatment dose of 2.5 mL/kg and up to a total intra-pulmonary volume of 12.5 mL/kg for up to 5 days (Part 1) and 25 mL/kg for up to 10 days (Part 2). Control subjects will be maintained on mechanical ventilation and evaluated in the same manner as the respective Part 1 or Part 2 PLV
20 treatment arm. All participants will be monitored for safety until 30 days post treatment and will be monitored via medical record review until 6 months post treatment or discharge, whichever comes first.

Inclusion Criteria. Inclusion criteria are as below:

- 1) Neonates with severe BPD as defined by 36 weeks post conception age and require
25 positive pressure ventilation
- 2) Infants born at less than 32 weeks post conception age
- 3) Subjects may be up to 6 months corrected age
- 4) On conventional mechanical ventilation for chronic lung disease for at least two days prior to enrollment

- 5) On conventional mechanical ventilation at the time of enrollment and anticipated to continue for 14 days
- 6) Off systemic steroids for lung disease for 72 hours (3 days) prior to T=0
- 7) Hemoglobin value ≥ 8 g/dL: if less than 8 g/dL, transfusion is permitted. Clinical blood transfusions should be administered prior to enrollment.
- 8) Parental/guardian permission (informed consent)

Exclusion Criteria. Patients eligible for study must not meet any of the following criteria:

1. Mechanical ventilation for acute disease, such as for infection or for post-operative complications
2. Severe Pulmonary Hypertension (PAH) (pulmonary pressure greater than 2/3 systemic) as defined by either: ECHO, cardiac catheterizations, or a CT-Angiogram consistent with PAH within the last 3 weeks.
3. Pneumothorax (active air leak) requiring chest tube within 72 hours of T=0
4. Active pulmonary hemorrhage within 72 hours of T=0
5. History of Grade III/IV interventricular hemorrhage without resolution or stability within 3 weeks of verifying eligibility
6. Severe congenital heart disease compromising pulmonary circulation
7. Other major congenital malformation (including but not limited to CDH) or known genetic syndromes at the discretion of the investigator
8. Use of an investigational drug within 7 days prior to confirmation of eligibility.
9. The clinical attending physician believes it is not in the subject's and/or parents'/guardians' best interest to participate in the trial.
10. Airway leak in endotracheal tube $> 20\%$

Participants that do not meet all eligibility criteria may not be randomized to either treatment or control arms until all criteria are met, documented, and confirmed by a study investigator.

Number of subjects. Part 1 will include 9 subjects in the PFOB group and 3 control subjects. Part 2 will include 6 subjects in the PFOB group and 2 control subjects. In total there will be 15 study patients and 5 control patients at CHOP.

Study Duration. Each subject will be on study drug or control arm for up to either 5 days (Part 1) or up to 10 days (Part 2), with a 6-month chart review of status of each subject. The total duration of the study is anticipated to take up to 2 years to recruit, enroll and follow-up subjects.

Study Phases. Screening: The study team will review the medical records of any potential participant in the CHOP neonatal intensive care unit (N/IICU) with a diagnosis of BPD and appear eligible based on medical record review. Parents or legal guardians of BPD infants will be approached about the clinical investigation and given the opportunity to ask questions and consent. Parental/guardian permission (informed consent) will be obtained prior to any study related screening procedures. Participants will be screened, eligibility will be confirmed, and eligible participants will be randomized to one of two arms: (1) PFOB-PLV or (2) control. Participants who are not initially eligible for the trial may be rescreened and monitored for eligibility for the duration of treatment in the CHOP N/IICU.

Study Treatment: Eligible PLV participants will be administered a series of treatment doses/aliquots of PFOB, each equal to 2.5 mL/kg, instilled via the ETT to a total intrapulmonary volume of up to 12.5 mL/kg over a five-day period (Part 1) and 25 mL/kg over a ten-day period (Part 2). Control participants will be kept on mechanical ventilation per usual care, and will be monitored on the same schedule as the PFOB-PLV participants. The proposed subject Schedules of Evaluations for Part 1 and 2 are in Appendix 1 and Appendix 2.

Evaluations and Follow-Up: Between one and seven days prior to dosing, the following baseline safety assessments will be collected: vital signs, ventilator settings, blood gases, laboratory tests, explicit urine output monitoring, chest X-Ray, and concomitant medications. These assessments will be monitored on a regular basis during drug administration, as well as during mechanical ventilation for the control group. Capnography, volumetric capnography, and airway flow and pressure will be assessed in both groups using an NM3 respiratory monitor. An ECHO will be performed at 2 specific time points: (1) within 5 days prior to study treatment; (2) 5-8 days after initial dosing of the lung with PFOB (Part 1) and 10-13 days after initial dosing of the lung with PFOB (Part 2). Tracheal aspirates will be collected at 4 specific time points: (1) within 5 days prior to study treatment; (2) 3-5 days after initial dosing of the

lung with PFOB (Part 1) and 8-10 days after the initial dosing of the lung with PFOB (Part 2);
(3) 10-15 days after initial dosing of the lung with PFOB (Part 1) and 15-20 days after the
initial dosing of the lung with PFOB (Part 2) & 30 days after initial dosing of the lung with
PFOB (Part 2). These assessments will also be collected for the control group. A chest
5 ultrasound will be performed up to three times in the PFOB (Part 2) to see if PFOB can be
visualized sonographically. Exploratory therapeutic endpoints will include effects on
mechanical ventilation, concomitant medications, and oxygen requirements in both groups as
well as residual PFOB 30 days post treatment.

Limited chart review will continue monthly until the participant is discharged, expired
10 or reached one year of age. A follow-up phone survey will occur at 6-8 months to help assess
pulmonary function and weight gain.

Efficacy Evaluations. Efficacy is not the primary endpoint of this study. Exploratory
therapeutic endpoints will be evaluated and include effects of PFOB-PLV on mechanical
ventilation, concomitant medications, oxygen requirements, and pulmonary function.

15 **Pharmacokinetic Evaluations.** This protocol will be evaluating safety, pulmonary
mechanics and respiratory function. We will not be conducting pharmacokinetic analyses.

Safety Evaluations. This is primarily a safety study to determine whether the PFOB-
PLV is safe for use in subjects with BPD. The safety analysis will be a comparison of the
following endpoints both daily and over the five days (Part 1) and up to 10 days (Part 2):
20 number of significant desaturation events, number of mucus plugging events, average change
(drop) in mean blood pressure, percent change from baseline of mechanical ventilator settings
(specifically peak inspiratory pressure (PIP), Mean Airway Pressure (MAP), and inspired
oxygen, , number of pneumothorax/pleural effusion with PFOB events, and evidence of
increased CO₂ retention, renal insufficiency, hyperkalemia, or metabolic acidosis. Blood gases,
25 laboratory values, urine output, ventilator settings, vital signs and oxygen requirements will be
monitored frequently as is standard in N/IICU patients. The rationale for continuous bedside
monitoring is to provide the highest degree of safety monitoring. Pulmonary function will be
evaluated using a non-invasive respiratory monitor.

Statistical and Analytic Plan. This is a pilot study to examine the safety of PFOB-
30 PLV and to gather data for future research aimed at addressing its safety and efficacy.
Therefore, the sample size of 15 PFOB-PLV subjects and 5 control usual care subjects was not
chosen to achieve a predefined level of significance. Results will be reported descriptively for
each study arm. The data for this study will be summarized with descriptive statistics,

frequency tables, and graphs.

Data and Safety Monitoring Plan. Independent medical monitors will include non-study team neonatologists and pulmonary specialists at CHOP. The DSMB will consist of several members with expertise in the field. Prior to study initiation, a complete data and safety monitoring plan will be designed with the assistance of the CHOP IND/IDE Support Program and the Office of Research Compliance and Regulatory Affairs.

Example 2 – Phase II Clinical Protocol

Previous studies of Perfluorooctylbromide (PFOB) partial liquid ventilation (PLV) in critically ill infants with acute respiratory distress found improvements in lung compliance, oxygenation, and survival. However, no studies have tested its safety or efficacy in a chronic disease such as Bronchopulmonary Dysplasia (BPD).

Objectives of the study are to evaluate the safety and feasibility of PFOB-PLV in preterm infants with BPD at age 30-60 days and to determine the maximum tolerated dose (MTD) of PFOB, between the range of 2.5 mL/kg to 15 mL/kg, or visible meniscus, in preterm infants with BPD at age 30-60 days.

The study is a prospective, single-center, dose-escalating, randomized controlled trial conducted in the neonatal intensive care units at CHOP. Participants are infants born less than 32 weeks gestational age, who are 30-60 chronological days and receiving conventional mechanical ventilation for lung disease will be eligible. Infants with a diagnosis of PIE or CLE at the time of enrollment, severe cardiac conditions compromising pulmonary function, or receiving mechanical ventilation for post-operative complications or infection will be excluded. The total number of subjects enrolled will be 16. Subjects will be randomized in blocks of 4 with a 3:1 allocation to receive either PFOB-PLV (n=12) or standard care (n=3) across 5 treatment days.

Subjects in the PLV treatment arm will be evaluated for safety after daily PFOB treatment dose, starting with 2.5 mL/kg, for up to 5 days. Control subjects will be maintained on mechanical ventilation and evaluated in the same manner as the PLV treatment arm. All participants will be monitored for safety until 30 days post-treatment.

The primary study outcome will be a composite of 5 safety endpoints: (1) no sustained oxygen desaturations ($\text{SpO}_2 \leq 80\%$) for greater than ten minutes without response to increased oxygen therapy; (2) no persistent hypotension (as defined by $\geq 20\%$ decrease in blood pressure) without response to volument expansion and/or inotropic therapy; (3) no

pneumothoraces/pleural effusion with PFOB; (4) no sustained CO₂ retention greater than 80 mmHg for over two hours; and (5) no evidence of renal insufficiency, hyperkalemia, or metabolic acidosis.

Study Title. Perfluorooctylbromide (PFOB) Partial Liquid Ventilation (PLV) for Up
5 to 5 days in Preterm Infants with Bronchopulmonary Dysplasia (BPD) at 30-60 Days.

Study Rationale. Respiratory distress syndrome (RDS) affects an estimated 40,000 newborn infants every year. After exposure to inspired oxygen and mechanical ventilation, the lung frequently responds with inflammation, remodeling and developmental arrest in combination with conducting airway disruption. A negative cycle is created in which
10 continued lung injury necessitates continued mechanical support.

While it is clear that newborn outcomes have improved over the last 15 year, it is important to note that the frequency of bronchopulmonary dysplasia (BPD) has not improved. A novel approach to improving short-term ventilation mechanics and physiologic response to mechanical ventilatory support is needed to improve long-term outcomes and break the negative
15 cycle.

Perfluorocarbon liquids (PFCs) have been studied for several decades for pulmonary diseases, with perfluoro-octyl-bromide (C₈F₁₇Br) (PFOB) demonstrating promise in both animal and clinical studies. While clinical studies from the 1990's indicated the safety of PFOB in infants, randomized controlled trials in adults indicated significant mortality leading to
20 discontinuation of drug manufacture and halting further investigations.

Whereas the adult study results were problematic, none of the pediatric studies demonstrated significant safety signals and, indeed, indicated varying degrees of efficacy (refer to FDA IND 42,317; IND 44,628; IND 44,076; IND 49,053). The pediatric studies were primarily conducted in acutely ill infants with severe lung injury, a generally high-risk
25 population of study subjects.

The study team at CHOP has considerable experience and expertise with pediatric respiratory diseases and considers infants with BPD to be ideally suited for safety and feasibility studies of PFOB. Interim safety data from our pilot study demonstrates that treatment with a low dose of PFOB is safe in infants with established BPD. We hypothesize
30 that PFOB can be safely administered earlier in the course of respiratory disease, during the first 30-60 days of life.

Study Objectives. Primary objectives. To assess the safety and tolerability of PFOB-PLV administered for up to 5 days in preterm infants with BPD during the first 30-60 days of

life. Safety will be evaluated by a composite of five safety endpoints and to determine the maximum tolerated dose (MTD) of PFOB between the range of 2.5 mL/kg and 15 mL/kg, or visible meniscus. Secondary objective. To assess the potential efficacy of PFOB on the duration of mechanical ventilation, concomitant medication and oxygen requirement and to evaluate the anti-inflammatory response of PFOB and determine its effect on inflammatory cytokines up to 30-days post-treatment and to evaluate the effect of PFOB on pulmonary artery pressure

Test Article. Perfluoro-octyl-bromide ($C_8F_{17}Br$), marketed as perflubron, is a biochemically inert linear perfluorocarbon (PFC) with structural formula, $CF_3(CF_2)_6CF_2Br$. Perfluoro-octyl-bromide (PFOB) is a clear, colorless, odorless liquid that is water-insoluble and chemically stable between 15° and 30°C. PFOB is among a group of PFCs that fall in a molecular weight category that make it ideal for biologic use. PFOB, and also the cyclical perfluorodecalin, are biocompatible and can be manufactured with high purity levels. The liquid has anti-inflammatory properties, surfactant-like properties, and high oxygen-carrying capacity.

Study Design. This is a single center, randomized, non-blinded Phase 2 study with an interpatient dose escalation study design. Eligible subjects will be randomized (3:1) to one of two treatment arms: (1) PFOB group or (2) Control group. The control group will be maintained on conventional mechanical ventilation and treated according to standard of care. The dose escalation study design will be used to establish the maximum tolerated dose (MTD) of PFOB between the range of 2.5 mL/kg and 15 mL/kg, or a visible meniscus, in preterm infants with BPD during the first 30-60 days of life. Two patients per treatment cohort will be assigned to receive daily treatment doses of PFOB for up to 5 days, starting at a dose of 2.5 mL/kg.

Patients will receive daily treatment doses of PFOB unless there is a serious adverse event (SAE) and/or clinical observation satisfying withdrawal or holding criterion*. All dose-escalation decisions will be based on safety data and doses will not be escalated unless the subjects receiving the highest current dose have been observed for five days (1 cycle).

The dose escalation design will be conducted as follows. Two subjects will be enrolled to a cohort. The occurrence of a SAE in one of the two patients will increase enrollment of up to 2 additional patients to the same cohort. When more than 1 SAE occurs in 2 subjects in a dosing cohort, dose escalation will be stopped and this dose level will be identified as the non-tolerated dose. Doses between the non-tolerated dose and the preceding lower dose, where less than 1 SAE occurred, may be explored to more precisely define the MTD.

All participants will be monitored for safety until 28 days post-treatment and limited chart review will continue monthly until the subject is discharged from the N/IICU, expired, or reached 1 year of age.

Inclusion Criteria. Inclusion criteria are set out below:

- 5 1. Preterm infants born ≤ 32 weeks gestation with respiratory failure
2. Intubated for at least 5 days
3. On conventional mechanical ventilation for at least 2 days prior to enrollment
4. Off systemic steroids for lung disease for 72 hours (3 days) prior to T=0
5. Parental/guardian permission (informed consent)
- 10 6. 30-60 days old at enrollment

Exclusion Criteria. Exclusion criteria are set out below:

1. Mechanical ventilation for acute disease, such as for infection or for post-operative complications
2. No active sepsis*
- 15 3. Severe cardiac condition* compromising pulmonary function
4. Active pulmonary hemorrhage within 72 hours of T=0
5. Infants with a diagnosis of PIE or CLE at time of enrollment
6. Pneumothorax (active air leak) requiring chest tube within 72 hours of T=0, weak from when the chest tube is removed?
- 20 7. Airway leak in endotracheal tube $> 20\%$
8. The clinical attending and/or study team physicians believe it is not in the subject's best interest to participate in the trial

Participants that do not meet all eligibility criteria initially may continue to be screened during their hospitalization to see if they meet eligibility criteria at a later date.

- 25 **Total Number of Subjects.** The total number of subjects will include 12 subjects in the PFOB group and 4 control subjects.

Study Duration. Each subject will be on study drug or control arm for up to 5 days, with a 10-month chart review of status of each subject. The total duration of the study is anticipated to take up to 2 years to recruit, enroll and follow-up subjects.

- 30 **Study Phases. Screening.** The study team will review the medical records of preterm infants in the CHOP Neonatal/Infant Intensive Care Unit (N/IICU) on mechanical ventilation

for lung disease to determine eligibility. Parents or legal guardians of eligible preterm infants will be approached about the clinical investigation and given the opportunity to ask questions and consent. Parental/guardian permission (informed consent) will be obtained prior to any study-related procedures. Participants who are not initially eligible for the trial may be
5 rescreened and monitored for eligibility for the duration of their stay in the CHOP N/IICU.

Study Treatment Eligible participants will be administered treatment doses of PFOB over a five-day period. The dose escalation schedule is as follows:

- Cohort 1: Two subjects will be given up to 2.5 mL/kg/day of PFOB
 - Cohort 2: Two subjects will be given up to 5.0 mL/kg/day of PFOB
 - 10 • Cohort 3: Two subjects will be given up to 7.5 mL/kg/day of PFOB
 - Cohort 4: Two subjects will be given up to 10 mL/kg/day of PFOB
 - Cohort 5: Two subjects will be given up to 15 mL/kg/day of PFOB
 - Cohort 6: Two subjects will be given a loading dose of up to a visible meniscus, and subsequent dosing every 4 hours as needed to maintain a visible meniscus.
- 15 The occurrence of a serious adverse event and/or adverse event satisfying withdrawal or holding criterion in one of the two subjects will increase enrollment of 2 additional patients to the same cohort. A subject that has an SAE will be withdrawn from the study. When more than 1 SAE or 4 AEs satisfying withdrawal or holding criteria occurs in 2 subjects in a dosing cohort, dose escalation will be stopped and this dose level will be identified as the non-tolerated dose.
- 20 Doses between the non-tolerated dose and the preceding lower dose, where less than 1 SAE occurred, may be explored to more precisely define the MTD.

Study Evaluations. The following baseline assessments will be collected in the week prior to dosing: vital signs, ventilator settings, blood gases, laboratory tests, explicit urine output monitoring, chest X-Ray, and concomitant medications. These assessments will be
25 monitored on a regular basis during drug administration. Capnography, volumetric capnography, and airway flow and pressure will be assessed using an NM3 respiratory monitor. Tracheal aspirates will be collected at 3 specific time points: (1) within 5 days prior to study treatment; (2) 5-8 days after initial PFOB treatment dose (3) and 28 days after initial PFOB treatment dose.

30 **Follow-up.** Subjects will be monitored for safety for 28 days post treatment. During this time, subjects will return to standard neonatal care practices as directed by the clinical care

team. Subjects will be followed via medical chart review until discharged from the N/IICU, expired, or reached one year of age.

Efficacy Evaluations. Primary evaluation criteria for efficacy will be total days spent on mechanical ventilation, total days spent on oxygen and measurements of pulmonary physiologic change to include PaO₂, PaCO₂ and pulmonary compliance.

Safety Evaluations. This is a dose escalating study to determine the maximum dose tolerated (MDT) of PFOB in preterm infants with BPD at 30-60 chronological days.

Criteria for Holding Treatment Dose. The study team will hold a treatment dose if any one of the following parameters does not return to the pre-treatment baseline range: PCO₂ (20-point difference or above 80 for 2 hours), oxygen requirements (within 10 percentage points), PIP (within 20%) OR hypotension with hemodynamic instability, and/or significant acidosis. Infants may be treated with lower total pulmonary volumes of PFOB depending on their lung pathology and tolerance of the liquid instillation.

Criteria for Safety Analysis. Safety will be assessed using a composite of five safety endpoints during the five day period: (1) significant desaturation events (2) sustained hypotension (3) Pneumothorax/pleural effusion with PFOB (4) sustained hypercarbia (5) evidence of renal insufficiency, hyperkalemia, or metabolic acidosis.

Statistical and Analytic Plan. Descriptive statistics will be used to report the safety of PFOB in each cohort. Efficacy of PFOB will be assessed by comparing clinical variables using either parametric or non-parametric tests for continuous variable and chi-square or fisher's exact test as appropriate for categorical outcomes.

Data and Safety Monitoring Plan. A Data Safety Monitoring Board (DSMB) will be established to monitor data to ensure there are no serious safety concerns. Members of the DSMB and independent medical monitors will include non-study team neonatologists and pulmonary specialists at CHOP. Prior to study initiation, a complete data and safety monitoring plan will be designed with the assistance of the CHOP IND/IDE Support Program and the Office of Research Compliance (ORC).

Table A – Schedule of Study Procedures

Schedule of Evaluations	Days								Months	
	Baseline ¹	D 1	D 2	D 3	D 4	D 5	D 11-17 Observation ⁶	D 10	Monthly, Months 2-11	1 Year Follow-up
Acceptable window for endpoint $\pm$ Days [Dy]							± 4 Dy	± 4 Dy	± 4 Dy	± 10 Dy
Consent	X									
Medical Record Review	X									X
Vitals ²	X	X	X	X	X	X	X ³	X	X	
Vent Settings ²	X	X	X	X	X	X	X ³	X	X	
Blood Gases with Electrolytes ⁴	X	X	X	X	X	X	X ³	X ³	X	
Laboratory Tests	X	X	X	X	X	X	X ³	X ³	X	
Echocardiogram	X					X				
Concomitant Medications ⁴	X	X	X	X	X	X	X	X	X	
Chest X-Ray	X	X	X	X	X	X	X	X	X	
Tracheal Aspirate	X					X		X		
Explicit Urine Output Measurement	X	X	X	X	X	X				
PFOB Treatment Dosing ⁵		X	X	X	X	X				
Safety Monitoring		X	X	X	X	X	X	X		
Adverse Event Assessment		X	X	X	X	X	X	X		
Phone Follow-Up										X

¹ Before administration of PFOB: Within 168 to 1 hours to T=0 on Day 1

5 ² HR, BP (systolic, diastolic and mean arterial). At all treatment doses, vitals taken pre- and post-dose

³ PIP, PEEP, MAP, RR, TV, FiO₂, SpO₂. Respiratory Severity Score will be calculated by obtaining the product of MAP x FiO₂.

⁴ PaCO₂, pH, and HCO₃ (calculated). Blood gas with electrolytes will be collecting Days 1-5

⁵ Administer treatment doses in aliquots of 2.5 mL/kg according to dosing schedule

10 ⁶ The results will be obtained by chart review of medical records. Chest x-rays, laboratory tests, and blood gases are taken regularly on patients as part of clinical care

Example 3 – Clinical Study Methods

Background: Previous studies of perfluorooctylbromide (PFOB) partial liquid ventilation (PLV) in critically ill infants with acute respiratory distress found improvements in lung compliance, oxygenation, and survival. However, no studies have tested its safety or efficacy in a chronic disease such as Bronchopulmonary Dysplasia (BPD).

Objectives: To evaluate the safety and feasibility of PFOB-PLV in very preterm infants with severe BPD.

Methods: This single center, pilot randomized, non-blinded clinical trial (NCT0304170) conducted between June 2017 and September 2018 enrolled preterm infants < 32 weeks gestational age (GA) who were diagnosed with severe BPD at 36 weeks postmenstrual age (PMA) and receiving invasive mechanical ventilation at enrollment. Subjects were randomized in blocks of 4 with a 3:1 allocation to receive either PFOB-PLV instilled via side port of endotracheal tube (up to 20 mL/kg/day; n=9) or standard care (n=3) across 5 treatment days. The primary outcome was a composite of 5 safety endpoints (Table 1). Secondary efficacy variables were measures of respiratory support.

Results: Twelve subjects (mean, GA of 25.7 ± 1.2 weeks; 58% male) enrolled in this study with a mean PMA of 46.4 ± 4.78 weeks. Total volume of PFOB instilled ranged from 33 mL- 178 mL (Table 2). None of the 5 evaluated safety endpoints occurred during the study period in either group. Serious adverse events (SAE) of over-distended lungs, confirmed by radiograph, and acute renal insufficiency occurred in 2 subjects. Both events resolved within 12 hours (Table 2). Following these SAEs, the study team decreased the PFOB dose to 5 mL/kg and then to 2.5 mL/kg; no SAEs were observed using this lower dose. Measures of respiratory support increased in the PFOB group during treatment ($P = .01$), then returned to baseline. A small, statistically significant decrease in partial pressure of CO_2 (PCO_2) from 68.6 mmHg to 63.2 mmHg occurred in the PFOB group, when comparing 3 weeks pre/post treatment. Radiopaque PFOB in the lungs provided excellent imaging of lung pathology (Image 1). Residual PFOB in either right and/or left lung was between 15% -48% in 5 PFOB subjects 30 days post-treatment with no observed side effects. Extubation occurred in 2 PFOB subjects within 3 weeks.

Conclusion: Among a small group of premature infants with severe BPD, treatment with a low dose of PFOB (up to 2.5 mL/kg/day) was safe and feasible. Residual PFOB in chronic lung disease can remain 30 days post-treatment without observable side effects.

Table 1 - Primary Safety endpoints

Safety Analysis	Definition
Sustained oxygen desaturations	SpO ₂ saturations $\leq$ 80% for more than 10 minutes without response to increased supplemental O ₂
Persistent hypotension	$\geq$ 20% decrease in blood pressure without response to therapy
Major mucous plugging events	Events that are unresolved after two bronchoscopes
Pneumothoraces	Confirmed by chest x-ray
Sustained CO ₂ retention	> 95 mmHg for over 4 hours

Table 2 - Summary of Results

Subject	Dosing Regimen (mL/kg)	Total PFOB instilled (mL/kg)	Total PFOB instilled (mL)	Serious Adverse Event
1	20	40	177.8	Over-distension of lungs with PFOB, hyperkalemia
2	5	15	45	None
4	5	10	46	Acute renal insufficiency, oliguria, hyperkalemia without apparent over-distension by radiograph
5	2.5	7.5	36	None
6	2.5	7.5	36	None
7	2.5	7.5	36	None
9	2.5	7.5	33	None
11	2.5	12.5	60	None
12	2.5	5.0	13.6	None

Example 4 – Dosing Protocol

Once challenge with infants with CLD between ages 3 and 9 weeks of age is that there may be a difference in lung damage in the older infants and therefore less tolerance of PFOB.

5 It is possible that even if one sub-groups them into two groups (Group 1: 20-42 days old; Group 2: 43 to 63 days old), there may be more severely ill infants within the older group. Factors such as cystic disease, degree of inflammation or more extensive airway pathology may cause less tolerance of the Liquid because of bronchospasm or other factors.

10 The inventors have observed a high degree of tolerance previously (1992-1997) in infants up to 30 days of age, allowing total lung filling without side effects. At least 2 of these babies were 18 days old with severe lung disease and on high ventilator pressures (up to 38 cm H₂O PIP), These 2 infants were on 80-100% oxygen at time of liquid instillation. They both were on 30% O₂ after 4 days of treatment. They tolerated total lung filling. They previously treated an infant 90 days old who tolerated filling the lungs.

15 The theory is that the highest volume of liquid in the lungs will have the greatest chance to demonstrate efficacy (as has been shown previously in animal models and in infants). By highest volume, it is meant demonstration of a fluid Meniscus in the ETT. The inventors suggest an escalating dose protocol to safely advance fluid dosing to full volume in the lungs. Therefore, if the infants tolerate liquid doses in increasing volume, one can safely work to the
20 goal.

There will be evaporative loss of liquid. In five-month infants recently treated, the lung pathology was generally so severe that evaporative losses were very low in about half. However, in a larger population of 3-9 week age infants, they expect evaporative losses to be much higher, resulting in the ability to tolerate higher doses of the drug to fill the lungs to the
25 Meniscus.

As daily dose volume increases, when the inventors reach 5 ml/kg/day, if there is no evaporation, after 3 days they could be reaching full lung volume (FRC). FRC should be in the range of 14- 15 ml/kg. Therefore, on the 3rd or 4th day there is a chance that a meniscus volume of fluid will be reached. If this happens, obviously a smaller volume of fluid will be dosed. On
30 subsequent days only enough liquid will be instilled to reach a meniscus. Clearly, if the inventors are dosing at a level of 7.5 ml/kg/day, the infant may reach FRC volume (meniscus) as early as day 2.

For this safety protocol, if a certain dose level is not tolerated, it will not be administered the next day. If there is more than one day when it is not safe to give a dose, that

subject's subsequent doses will be held, the infant will be considered an escalation failure but the baby will still remain in the protocol for observation. Then, two additional infants at that dose will be entered in the study and treated.

5 The inventors predict that infants in Group 2 will be the most likely to fail dose escalation dosing. If two infants selected for the older group fail, we will stop enrolling babies in that age range group but will continue studying infants in the younger group.

The dose escalating protocol plans on daily doses of 2.5 ml/kg, 5 ml/kg, 7.5 ml/kg, 10 ml/kg, and filled to Meniscus. The safety protocol will have 1 control infant for each 3 liquid patients.

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15 All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or
20 similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

V. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED

1. A method of treating an infant subject with broncopulmonary dysplasia (BPD) patient comprising the steps of:
 - (a) administering to the lung of the patient an effective amount of a fluorocarbon liquid having an equilibrium coefficient of spreading which is a positive number, said amount of administered fluorocarbon liquid not exceeding 35% of the functional residual capacity of the lung of the patient upon exhalation taking into account any positive or negative expiratory pressure applied to said patient's lung; and
 - (b) introducing a breathing gas into the lung wherein said introduced breathing gas physically admixes with and oxygenates said fluorocarbon liquid within the lung.
2. The method of claim 1, wherein said fluorocarbon liquid comprises a mixture of two or more fluorocarbons.
3. The method of claim 2, wherein the mixture of fluorocarbons comprises at least one fluorocarbon having an equilibrium coefficient of spreading which is a negative number.
4. The method of claims 1-3, wherein said fluorocarbon liquid further comprises a surfactant.
5. The method of claims 1-4, wherein said fluorocarbon liquid comprises a fluorocarbon-hydrocarbon compound.
6. The method of claims 1-5, wherein said fluorocarbon liquid comprises a non-halogenated fluorocarbon.
7. The method of claims 1-5, wherein said fluorocarbon liquid comprises a halogenated fluorocarbon.
8. The method of claim 7, wherein said halogenated fluorocarbon is perfluoro-octyl-bromide.

9. The method of claims 1-8, wherein said fluorocarbon liquid has a surface tension value less than 20 dynes/cm.
10. The method of claims 1-4, wherein said fluorocarbon liquid comprises a cyclic fluorocarbon.
11. The method of claim 10, wherein the cyclic fluorocarbon is selected from the group consisting of perfluorodecalin, F-adamantane, F-methyladamantane, F-1, 3-dimethyladamantane, F-dimethylbicyclo[3,3,1]nonane, F-dimethylbicyclo[3,3,1]nonane and combinations thereof.
12. The method of claims 1-4, wherein said fluorocarbon liquid comprises a perfluorinated amine selected from the group consisting of F-tripropylamine, F-tri-butylamine, F-4-methyloctahydroquinoline, F-n-methyl-decahydroisoquinoline, F-n-methyldecahydroquinoline, F-n-cyclohexylpurrolidine, F-2-butyltetrahydrofuran and combinations thereof.
13. The method of claims 1-12, further comprising the step of introducing said breathing gas by spontaneous ventilation while the fluorocarbon liquid is in the lung.
14. The method of claims 1-12, further comprising the step of introducing said breathing gas using mechanical ventilation while the fluorocarbon liquid is in the lung.
15. The method of claims 1-14, wherein said fluorocarbon liquid is administered as an aerosol.
16. The method of claims 1-15, wherein the subject is an infant of 12 months corrected age or less, or is a pre-term infant, or is both.
17. The method of claims 1-16 wherein the subject suffers from mild BPD.
18. The method of claims 1-16, wherein the subject suffers from moderate BPD.

19. The method of claims 1-16, wherein the subject suffers from severe BPD.
20. The method of claims 1-19, wherein the dose is between 1.0 mg/kg/day and 5.0 mg/kg.day fluorocarbon liquid, such as 2.5 mg/kg/day.
21. The method of claim 1-19, wherein the subject is dosed twice per day.
22. The method of claims 1-20, wherein the subject is dosed up to five consecutive days or up to ten days.
23. The method of claim 22, wherein the cumulative five-day dosage is no more than 12.5 mg/kg.
24. The method of claim 22, wherein the cumulative five-day dosage is no more than 10 mg/kg or 7.5 mg/kg.
25. The method of claim 22, wherein the cumulative five-day dosage is no more than 5 mg/kg.
26. The method of claims 1-20, wherein the subject is dosed up to ten consecutive days.
27. The method of claim 26, wherein the cumulative ten-day dosage is no more than 25 mg/kg.
28. The method of claim 26, wherein the cumulative ten-day dosage is no more than 20 mg/kg or 15 mg/kg.
29. The method of claim 26, wherein the cumulative ten-day dosage is no more than 10 mg/kg.
30. The method of claim 1, wherein the subject is a pre-term infant of six months corrected age or less, diagnosed with severe BPD, is dosed twice per day to a total of 2.5 mg/kg/day and is treated for five, six, seven, eight, nine or ten consecutive days.

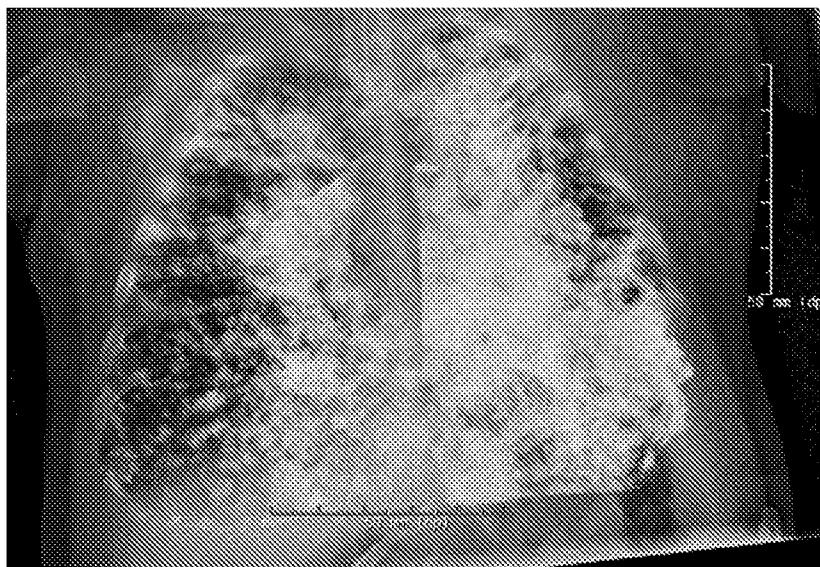


FIG. 1



FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/030418

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/02; A61K 31/00; A61P 11/00 (2020.01)

CPC - A61K 31/02; A61K 31/025; A61P 11/00 (2020.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,655,521 A (FAITHFULL et al) 12 August 1997 (12.08.1997) entire document	1-3
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Y		30
Y	WO 2017/070338 A1 (INDIANA UNIVERSITY RESEARCH & TECHNOLOGY CORPORATION) 27 April 2017 (27.04.2017) entire document	30

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 June 2020

Date of mailing of the international search report

09 JUL 2020

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/030418

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.