The Effect of Sodium Pyruvate Nasal Spray on Coughing in Patients with Idiopathic Pulmonary Fibrosis

A double-blinded randomized placebo-controlled phase 3 clinical trial

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The Effect of Sodium Pyruvate Nasal Spray on Coughing in Patients with Idiopathic Pulmonary Fibrosis

1. A Statement of the Objectives and Purpose of the Study

1.1. Introduction

1.1.1. Idiopathic Pulmonary Fibrosis Background

Idiopathic pulmonary fibrosis (IPF) belongs to a group of conditions called interstitial lung diseases (also known as ILD), which describes lung diseases that involve inflammation or scarring in the lung. IPF is a chronic progressive lung disorder associated with excessive tissue remodeling, scarring, and fibrosis, which makes the lungs unable to effectively transport oxygen into the bloodstream resulting in decreased forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) values, decreased SaO_2, a decrease in nitric oxide associated with nasal inflammation that causes congestion and coughing (31-39).

Inflammatory pathways are upregulated in the nasal epithelium in 80% of patients with IPF which is part of the etiology of the disease that effects all lung functions (28). Nasal inflammation induces oxidative stress, decreases lung functions including FEV_1 and FVC values, increases mucus and coughing (54, 55). A decrease in total lung functions and capacity results in hypoxemia, dyspnea, and poor quality of life, especially sleep disorders (31-39).

IPF usually affects people between the ages of 30 and 70. The most common signs and symptoms of IPF are shortness of breath and persistent dry, hacking cough. Many affected individuals also experience a loss of appetite and gradual weight loss. Most affected individuals survive 3 to 5 years after their diagnosis. However, the course of the disease is highly variable; some affected people become seriously ill within a few months, while others may live with the disease for a decade or longer. In most cases, IPF is sporadic and results from environmental damage to the lungs and not genetic factors. However, a small percentage of people have genetic risk factors resulting in familial pulmonary fibrosis.

1.1.2. Sodium Pyruvate

Sodium pyruvate is a natural antioxidant of the human body and as an antioxidant it has been shown to significantly reduce inflammatory agents throughout the human body, including the lungs and nasal passages, allowing nasal nitric oxide to reach the lungs to increase bronchodilation (11-27). In addition to 8 human clinical studies conducted on the effect of inhaled sodium pyruvate on the lungs, 7 human nasal inhalation clinical studies were conducted using a sodium pyruvate nasal spray which showed decreased nasal inflammation, a reduction in inflammatory cytokines, and when measured, demonstrated an increase in lung functions, a decrease in coughing, including in patients with pulmonary fibrosis and IPF (21-27). Cellular Sciences received Orphan Drug designations for Cystic Fibrosis and Interstitial lung Disease.

Globally over 3.5 million patients have been treated in over 200 hospitals, demonstrating the ability of sodium pyruvate nasal spray to reduce nasal inflammation, erythema, and edema, and allow an increased level of nasal nitric oxide to reach the lungs and increase lung functions (21-27). Additionally, in these studies, subjects reported a high degree of satisfaction with the product, which did as well as, or better than, nasal sprays containing steroids.

1.1.3. Pre-clinical Studies (NOAEL)

As part of Cellular Science's Investigational New Drug (IND) submissions, 5 animal studies were conducted with solutions of differing concentrations of sodium pyruvate. These included a "Maximum Tolerated Nose Dose" study in rabbits (2000× the therapeutic human dose). The human therapeutic dose of a 20 mM solution that contains 0.2% of sodium pyruvate in 0.9% saline with 0.02% benzalkonium chloride (2.1 mg/mL sodium pyruvate). For humans, 12 squirts per day equals 2.5 mg/day or 0.21 mg/squirt. In all animal studies, no adverse events were reported. Gross necropsy included terminal body weight, and gross observations of external surfaces (especially nares, eyes and mouth); all orifices; and thoracic, abdominal and pelvic cavities were examined with no abnormal findings. Tissues from the respiratory tree (defined as larynx, trachea and lungs), plus nasal passage tissue, were analyzed for any histopathology. There were no significant differences in the nasal passages and respiratory tree between rabbits and no tissue or histopathological abnormalities were found after histological examination.

1.1.3.1. Rat Study: 180-Day

Sodium Pyruvate: A 90- and 180-Day Nose-Only Inhalation Study in Rats (Study 2).

In the second 180-day nose only inhalation study, the rats received a dosage of 5.76 mg sodium pyruvate/m^3, 85 minutes in which rats were exposed to 25 times the sodium pyruvate level expected to be administered to humans based on body weights. No product-related adverse reactions were observed or reported in any animal study, and the drug regimens were judged to be safe as determined by the investigators report. In the 180-day rat study, the rats were given more than 2.5× the 20 mM (5.76 mg) dosages used in human inhalation studies. If you include the difference in body weights of humans vs rats the concentrations tested were 250× the 0.2% sodium pyruvate concentration. After 180 days, the remaining rats from each group were sacrificed and gross morphological observations on lung, sinus cavities, liver, kidney, heart, and body weights were recorded. Histopathological evaluations were performed on lung, trachea, larynx, nose, liver, heart, and kidneys. No tissue or histopathological abnormalities were found. The study report concluded: "…inhalation of target concentrations of 5.76 mg sodium pyruvate/m^3, 85 minutes/day, 7 days/week for 180 days resulted in no clinical signs of toxicity attributable to administration of the test article, minimal effects on body weight in females, and no compound-related microscopic lesions."

1.1.3.2. Rat Study: Lung Injury

Effect of Multiple Intratracheal Administrations of Sodium Pyruvate on Lung Injury Caused by Bleomycin

Bleomycin administered to rats to cause lung injury is the standard model used to test drugs for idiopathic pulmonary fibrosis. Sodium pyruvate in 0.9% sodium chloride solution or 0.9% sodium chloride (normal saline vehicle) was then administered to bleomycin treated rats by intratracheal injection to see if the drug regimen had any effect on the progression of the lung injury. The rats were sacrificed 24 hours, 72 hours, 1 week, and 2 weeks post injury (8 rats per group). The rats sacrificed at 2 weeks were administered the drug on the third and seventh-day post-bleomycin insult, while the other rats received only a single administration of sodium pyruvate. Significant improvement was only observed in the rats administered the sodium pyruvate and sacrificed 2 weeks after bleomycin insult, compared to the sodium chloride control. There was a significant ($p<0.01$) reduction in total cells found in the bronchoalveolar lavage, indicating a reduction in airway inflammation. It was concluded that sodium pyruvate was

effective in reducing inflammation and lung damage in this chronic fibrotic stage of lung injury. (It should be noted that this type of injury, with subsequent fibrotic infiltration, is typical of the fibrosing group of interstitial diseases in humans including IPF.)

1.1.3.3. Dog Study

Effects on Cardiovascular and Respiratory Parameters in the Telemetered Dog (GLP) (30)
NIH Independent Studies Safety Study: RCC Study 855869 (GE Study Number B101019)

Four beagle dogs were dosed intravenously by slow bolus injection (5-10 mL/min). All treatments were given at a dose of 2.9 mL/kg. The period between each treatment was at least 3 days. The dogs were treated with control item (0.9% saline), TRIS/EDTA vehicle control solution, and test item containing 44.6 mg/mL [1-^{13}C] pyruvate and 0.85 mg/mL AH111501 and filtered test item. Animals were trained several times to accustom them to dosing procedures, as well as respiratory parameters measurements. Tracheal airflow was measured using a sealed mask (covering the animal's snout) attached to a pneumotachograph and a pulmonary monitoring system. Respiratory rate and tidal volume were monitored in the sling-restrained animals. Values were recorded for approximately 4 to 5 min before administration and at 15, 32, 62, and 122 min after the end of dosing. Mean values over 3 min were reported.

The various treatments had no marked effects on absolute and percent change values of respiratory parameters (respiratory rate, tidal volume, and minute volume) when measured at approximately 15, 32, 62, and 122 min after treatment. There were no significant differences between the treatment groups.

In conclusion, slow intravenous administration of test item containing [1-^{13}C]pyruvate and AH111501 at a dose of 2.9 mL/kg had no significant effects on the respiratory parameters monitored in dogs. When dose levels were expressed as human equivalent doses based on body surface area, they correspond to 72 mg/kg of [1-^{13}C]pyruvate and 1.4 mg/kg of AH111501.

1.1.3.4. NIH Pharmacokinetics

National Cancer Institute Nonclinical Studies: Safety and Efficacy Studies in Animals and Humans demonstrating the Pharmacodynamic, Pharmacokinetics and Noncarcinogenic Effect of Pyruvate (30)

A range of nonclinical studies have been undertaken as part of the development program which was designed to support early clinical studies. These studies used non-^{13}C enriched pyruvate and impurities, [1-^{13}C] pyruvate , hyperpolarized [1-^{13}C] pyruvate, and [1-^{14}C] pyruvate, as appropriate. [^{13}C] pyruvate and pyruvate have the same chemical characteristics and will therefore have the same safety profile, pharmacodynamics and pharmacokinetics as regular pyruvate. The program comprised:

A pharmacodynamic study in the healthy dog using hyperpolarized [1-^{13}C]pyruvate.

Two biodistribution studies in rats using [1-^{13}C]pyruvate test articles spiked with [1-^{14}C]pyruvate.

Safety pharmacology studies assessing effects on

1) The hERG channel,

2) The cardiovascular system in the conscious and anesthetized dog,

3) Central nervous system (CNS) in the conscious rat and dog, and

4) **Respiratory system in conscious dog.** In these studies, 2 [1-^{13}C]pyruvate test articles and various pyruvate test articles were used.

Toxicology studies including 1) expanded acute toxicity in the rat and dog, 2) studies of genetic toxicology (in vitro and in vivo) using [1-^{13}C]pyruvate test articles, and 3) a study of local tolerance using a pyruvate test article.

In 1 of the pharmacokinetic studies and in all GLP safety pharmacology and toxicology studies the pyruvate or [1-^{13}C]-pyruvate test articles contained AH111501. The injection dose was 0.43 mL/kg of drug product test article having a pyruvate concentration between 220 and 280 mM, no more than 3.0 μM residual AH 111501 and in a TRIS/EDTA buffer of pH 6.7 to 8.0. The recommended clinical injection rate was 5 mL/s. No adverse events or histological changes were noted.

1.1.3.5. Pharmacodynamic

Effects of Sodium Pyruvate and Mechanism of Action. NIH published clinical studies.

Sodium pyruvate is biochemically classified as an alpha-ketoacid and is part of the body's natural endogenous anti-oxidant defense system. It is found in every cell of the human body and is part of the Krebs citric acid cycle. It is transported into mitochondria and the breakdown products are acetate, CO_2 and H_2O. It is also converted to lactate and amino acids. The breakdown products and metabolism are well defined in biochemistry books and in the literature and this testing confirmed that hyperpolarized [^{13}C]pyruvate is taken up by all organs including the respiratory system and metabolized to acetate and CO_2 and H_2O and is also converted to lactate or alanine in a well-defined biochemical pathway (13,14,16,19,21-28,30). When high doses of pyruvate are injected, the half-life was determined to be 15 minutes. Every organ of the body took up pyruvate via the pyruvate/lactate transporter and it was metabolized (30). It is also secreted by cells, readily enters cells, and can directly react with toxic compounds such as H_2O_2 and peroxynitrites to "detoxify them" (13,14,16,19,21-28,30). Normal plasma levels of sodium pyruvate are approximately 0.1 mM (0.011 mg/mL) and normal intracellular levels can reach 0.5 mM (0.055 mg/mL). Sodium pyruvate has been shown to have protective anti-oxidant activity both in vitro (36-48) and in vivo (49-66). There are 3 major chemical pathways by which sodium pyruvate can either directly or indirectly generate or augment an anti-oxidant or anti-inflammatory effect to increase lung functions including FEV_1, FVC and reduce coughing:

Hydrogen Peroxide

H_2O_2 is directly toxic to cells and also is a precursor to other reactive oxygen species. Inhibition of H_2O_2 has broad anti-oxidant and anti-inflammatory effects. Sodium pyruvate directly interacts non-enzymatically with H_2O_2 to generate the non-toxic compounds acetate, CO_2, and H_2O (13-17). This effect is especially relevant to the nasal and respiratory pathway and is essential to increase lung function (21-27).

$NADPH_2$

An essential component in the reductive activity of thiol compounds like GSH is the generation of $NADPH_2$, which aids in driving the reaction to the reduced state (i.e. GSH), thus replenishing anti-oxidant activity. Sodium pyruvate has been shown to play an important role in the generation of intracellular $NADPH_2$ and therefore helps in maintaining optimal levels of thiol compounds, such as GSH (19).

Reduction of Inflammatory Cytokines

Sodium pyruvate has been shown to reduce several inflammatory cytokines (27, 30).

1.1.3.6. Pharmacokinetics: Movement of Sodium Pyruvate in the Body (NIH Research Data) (30)

Intravenous or inhalation of hyperpolarized [^{13}C]pyruvate enables real-time detection of metabolism by magnetic resonance spectroscopic imaging (MRSI). The signal from a given tissue depends on the dose administered, the perfusion of the tissue and the rate of formation of the metabolites, and the relative levels of the metabolites depend on the energy needs of the specific tissue. The breakdown products and metabolism is well defined in biochemistry books and in the literature and this testing confirmed that hyperpolarized [^{13}C]pyruvate is taken up by all organs including the respiratory system and metabolized to acetate and CO_2 and H_2O and is also converted to lactate or alanine in a well-defined biochemical pathway (28-30). When high doses of pyruvate are injected into the blood stream, the no observed adverse effect level (NOAEL) for pyruvate injection is 1.4 mL/kg (or 100 mL for a 70-kg subject) of a 250 mM formulation (equal to 31 mg/kg of a solution of [^{13}C]pyruvate which is 2.1 g/70 kg), the half-life was determined to be 15 minutes. Every organ of the body took up pyruvate via the pyruvate/lactate transport and it was metabolized (28-30). In Bleomycin injured rats, injecting 10 mL of a 32 mM [^{13}C]-pyruvate demonstrated the uptake by lung cells and the nearly 100% conversion of pyruvate to lactate (30). Based on the data provide by National Institutes of Health (NIH), delivering 1 mL of a 20 mM sodium pyruvate saline solution (2 mg/mL for a 70-kg subject) in the nasal cavity is 1000× less then delivered by injection. Pyruvate is completely metabolized in the nasal cavity and cannot enter the circulatory system and effect other organs. When high concentrations of oxygen radicals are present, sodium pyruvate directly interacts non-enzymatically with H_2O_2 to generate the non-toxic compounds acetate, CO_2 and H_2O (1-17). This effect is especially relevant to the nasal and respiratory pathway and is essential to increase lung function (21-27).

1.1.3.7. NIH Carcinogenesis, Mutagenesis, Genotoxicity Tests

The pyruvate test article has also been evaluated in 2 in vitro genetic toxicology systems, comprising the bacterial reverse mutation test (Ames test) and the chromosomal aberration assay with mouse lymphoma cells (MLA test), as well as in 2 separate mammalian in vivo assays in rats, comprising the test for induction of micronuclei in the polychromatic erythrocyte of the bone marrow, and detection of DNA damage in the blood using the Comet assay. In the tests, pyruvate has never been found to mutate DNA, is not genotoxic, mutagenic or carcinogenic (30).

1.1.4. Safety, Dosing and Efficacy of Sodium Pyruvate Inhalation Therapy

Six different nasal spray formula combinations were tested for safety and efficacy in a series of studies with subjects who normally use nasal sprays. The sodium pyruvate concentrations were 5 mM (0.55 mg/mL, 10 mM (1.1 mg/mL), 20 mM (2.2 mg/mL) and 40 mM (4.4 mg/mL). The sodium pyruvate was delivered in sterile sodium chloride solutions of 0.9%. All formulations reduced inflammation in the nasal cavities and improved the nasal cellular morphology, and increased lung functions including FEV_1, FVC, peak expiratory flow (PEF), and SaO_2 values when measured. There were not any adverse events observed. In testing 39 patients with 4 different formulations, 5 mM, 10 mM, 20 mM and 40 mM, the most efficacious was the 20 mM (0.2%, 2.2 mg/mL) sodium pyruvate nasal spray. Also in a cilia mobility test, again the formulation that produced the most effective cilia movement to remove mucus, and post nasal drip was the 20 mM (0.2%, 2.2mg/ml) sodium pyruvate formula against saline. The best formulas for cilia movement as reported in the literature were close to isotonic formulas (57,58) The 5 mM and 10 mM formulations (hypotonic) were not as effective. The 40 mM or the 50 mM formulation, when tested later, produced a hypertonic salt effect that decreases cilia movements and were irritating to the patients nasal cavities. The 20 mM (0.2%. 2.2mg/ml) sodium pyruvate nasal spray, which is slightly hypertonic but closer to isotonic produced excellent results in all the lung and sinus diseases tested, and in cilia movement, including patients with hypoxemia, where FEV_1 and SaO_2 values increased significantly (21-27).

Human Mucociliary clearance time MCT is a general measure of Sino nasal health. The MCT measures a composite of ciliary beat frequency (CBF), ciliary coordination and mucus rheology. Times longer than 20-25 minutes are generally considered abnormally delayed. Faster MCT is clinically beneficial to counteract stagnation of sinonasal secretions. Several studies have shown the effect of nasal saline on MCT. PharmaMax investigators have found isotonic saline sped clearance time by 2%, while the 20mM sodium pyruvate formula sped clearance by 17% 10-20 minutes after treatment in 32 adults. All other formulations of sodium pyruvate had clearance times of 4% or less.

1.1.5. The Nasal Mechanism of Action of Nitric Oxide in Patients with IPF

The upper and lower airways form 1 contiguous and functionally related organ that is critical to normal lung functions. The nasal cavity produces 900-1100 parts per billion of nitric oxide, which is used to kill invading bacteria, fungi, and viruses compared to the lungs which produce 4-48 parts per billion nitric oxide. Nasal nitric oxide also produces clinically useful bronchodilation and has been shown to reduce pulmonary fibrosis (43-53). Blockage of nasal nitric oxide by inflammation reduces the amount of nitric oxide reaching the lungs, which reduces critical lung functions, leading to increased lung and nasal infections, a reduced SaO_2 level, reduced FEV_1 and FVC levels also leading to mouth breathing and coughing. Nasal steroids and other over-the-counter (OTC) nasal treatments shut down the synthesis of nasal nitric oxide, which then leads to decreased lung functions and a 34% increase in infections (21-27).

1.1.6. Prior Sodium Pyruvate Treatment in Pulmonary Fibrosis and IPF Studies

A phase 2 study was designed to demonstrate safety, efficacy, and determine which medical endpoints produced clinically and statistically significant results. Inflammatory pathways are upregulated in the nasal epithelium in 80% of patients with IPF, which is part of the etiology of

the disease that effects all lung functions (28). Inflammation induced oxidative stress decreases lung functions including FEV_1 and FVC values and increases mucus and coughing. Nasal steroids and OTC products have been ineffective in treating these conditions in IPF patients, especially when these patients experience exacerbation do to stress or allergies.

Coughing frequency is high in patients with advanced IPF, with median (range) 24-h cough counts varying from 226 (36–946) to 520 (117–1493) depending on the population studied (34-41). In mild to moderate cases in IPF patients, cough counts vary from 4-57 per 24-h. Strikingly, IPF patients experience more cough symptoms during the daytime (median hourly cough rate 14.6 during the day versus 1.9 during the night) (34-41). Chronic cough in IPF is not related to age or gender and is more common in advanced disease and in “never-smokers” (34-41). There is no clear explanation why IPF patients without a history of smoking cough more.

An initial 21-day sub-chronic clinical trial was conducted that included 15 patients with pulmonary fibrosis (9 with pulmonary fibrosis and chronic obstructive pulmonary disease (COPD) and 6 with idiopathic pulmonary fibrosis without COPD) that remained on their normal medications, but were also administered the 20 mM (2.2 mg/mL) sodium pyruvate nasal spray. If the patients were using other nasal sprays as part of their normal therapy, the other nasal spray was eliminated. In all 15 patients, the test results were compared to their previous 3-week screening and baseline data (their current therapies) as the positive control for each variable including all their lung functions, FEV_1, FVC, PEF, FEV_1/FVC ratios, SaO_2, nitric oxide, coughing rates and nasal inflammation (23).

Results

Coughing

Nine patients with pulmonary fibrosis with COPD and 6 patients with idiopathic pulmonary fibrosis without COPD that remained on their normal medications, were administered the 20 mM (2.2 mg/mL) sodium pyruvate nasal spray 3 times per day for 22 days. The data from this study showed that coughing episodes per day was significantly reduced in all 15 patients on day 8 by 30% ($p = 0.007$) and continued to decrease on day 14 by 55% ($p = 0.0001$) and on the 22 day of the trial, coughing decreased by 59% ($p = 0.0001$). This correlated with a significant ($p = 0.010$) improvement in nasal irritation/erythema with most patients being free of irritation by day 22 ($p < 0.001$); and a significant ($p = 0.010$) increase in the group average expelled NO by day 8 (23).

A significant ($p = 0.010$) improvement in lung function (breathing) was observed in all patients with IPF without COPD (n6) by day 1, increasing to $p = 0.0005$ by day 22 compared to their baseline therapies, determined by changes in FVC, FEV_1, PEF, and FEV_1/FVC ratios. The improved FEV_1/FVC ratio from 52% to 86% was clinically significant and indicated that current therapies in use are inadequate to treat patient with IPF.

The screening data indicate that the 6 IPF patients were not receiving much, if any, benefit from their current therapy. However, they all had significant improvements in breathing at all time periods for FVC, FEV_1, and PEF despite the fact that they were all on concomitant standard lung function therapy. Patients #5 and #7 were taking albuterol, a β2-receptor antagonist, and Advair Diskus, a β2-receptor antagonist-steroid combination for rescue; Patient #6 was taking ipratropium-albuterol, a β2-receptor antagonist; and patient #8 was taking albuterol and budesonide/formoterol (Symbicort; a steroid). The sodium pyruvate treatment was able to significantly improve the breathing of these 6 patients, despite 5 of them being on steroid medications.

Importantly, the percentage increase in lung function during the study period for the 6 subjects with IPF, compared to the baseline (their current therapies), was also significant, with increases that ranged from 19% to 73% with $p = 0.002$. The clinical data demonstrated the ability of the sodium pyruvate nasal spray to immediately increase FEV_1 and FVC values on day 1, while the patients continuously used their other steroid inhaled medications.

	Day 1	**Day 8**	**Day 15**	**Day 22**
FVC	27%	26%	24%	19%
FEV_1	45%	56%	53%	51%
PEF	53%	68%	73%	63%

The ratio was calculated from the data obtained. Note that a ratio of 80% or higher is considered normal. Group average ratios indicate that at baseline and then again at 1-hour post-pyruvate treatment, the ratios were 52% and 66% respectively. However, during sodium pyruvate administration during the trial, the ratios increased to 87%, 87%, and 86% for the 8, 15, and 22 test days.

	Baseline	**Day 1**	**Day 8**	**Day 15**	**Day 22**
FEV_1 Average	0.80	1.40	1.81	1.78	1.64
FVC Average	1.55	2.12	2.08	2.05	1.92
FEV_1/FVC	52%	66%	87%	87%	86%

Following this, 5 new patients with pulmonary fibrosis and COPD had their medications removed and were administered a saline placebo control for 3 weeks. At the end of 3 weeks they were administered only the sodium pyruvate nasal spray solution for 3 days in order to assess its effect. The data from the 3-day trial, indicated a statistically and clinically significant immediate average improvement in lung function (breathing), compared to the saline placebo control baseline data, as determined by changes in FVC, FEV_1, PEF, and FEV_1/FVC, ratios which persisted throughout the 3-day trial. A significant immediate average improvement was also seen in SaO_2 levels, compared to the placebo control, such that all subjects had SaO_2 levels of ≥ 97, which persisted throughout the trial.

Comparison to Nintedanib

Nintedanib slows the rate of decline for FVC by 52.3% in 24 weeks compared to the non-treatment group of a 66.7% decline in FVC. It is a pill and has many side effects especially on the liver. Nintedanib also demonstrated no efficacy in reducing coughing, increasing SaO_2, decreasing nasal inflammation, increasing the synthesis of nasal nitric oxide needed for bronchial dilation or sleeping. The sodium pyruvate nasal spray demonstrated in over 1,869 patients with various lung and sinus diseases that the effect occurs in minutes and lasts for 4-6 hours allowing the patient improved breathing and sleeping. Quality of life is important.

1.1.7. Clinical Experience and Real-world Use

In China, over 3 million sodium pyruvate-based nasal spray units have been used to treat patients, which include 150,000 patients with COPD and 1,800 pulmonary fibrosis patients, in over 200 hospitals. To date, the hospitals have reported that the 0.2% pyruvate nasal spray has demonstrated both efficacy and safety, with no adverse events reported. The nasal inhalation of sodium pyruvate in clinical studies reduced the nasal inflammation while increasing lung functions, with a significant reduction in the use of steroids and over the counter medications.

1.1.8. Previous Studies

The safety and efficacy of the 0.2% sodium pyruvate nasal spray in patients with allergic rhinitis, nonallergic rhinitis, COPD, cystic fibrosis, sinusitis, and pulmonary fibrosis, including IPF, were collected from 1,869 patients over 22 years from the 23 phase 1/2 clinical trials listed in the 2022 investigator's brochure. No adverse events in clinical trials have been reported. Seven of those studies were saline placebo-controlled clinicals, 3 were randomized placebo controlled clinicals, 5 were blinded randomized placebo controlled clinicals and 8 used a positive control. The 8 positive controlled studies were a direct comparison of the sodium pyruvate nasal spray (N115) to current Rx and OTC nasal sprays. The trials were from 8 hours to 12 weeks or more. N115 produced clinically and statistically significant results in reducing lung and sinus inflammation caused by oxygen radicals, reduced coughing, and increased SaO_2 levels (decreased hypoxemia) that improved sleeping, so that the patient's quality of life increased.

We examined the effect in patients with long COVID in a phase 2 clinical trial (n22). During the first 7 days, when there was no treatment, patient data demonstrated little to no change in symptoms including body ache, headache, coughing/sneezing, and trouble breathing. However, after the inhalation of the 0.2% sodium pyruvate nasal spray for the next 7 days, patient data demonstrated clinically and statistically significant improvements in headaches ($p = 0.0373$), improvements in coughing/sneezing ($p = 0.0091$) by 60%, and improvements in trouble breathing ($p < 0.0001$) 61%. Fatigue, anxiety, loss of taste/smell, congestion and body aches also showed some improvement (23).

Clinical studies with 75 COPD patients, demonstrated that nebulized inhalation of sodium pyruvate decreased expired hydrogen peroxide by 30% after 4 hours compared to patients administered a saline control. Inhaled nebulized sodium pyruvate (N115) increases FEV_1 values and increased total lung capacity in COPD (n130) patients. N115 has been administered in single doses to subjects or up to 3 times per day for 6 weeks to subjects with chronic obstructive pulmonary disease (23-27). These patients experienced increased pulmonary function as determined by FEV_1 (12.8%) and PEF (34.5%) measurements after 4 hours (referenced in Cellular Sciences IND 50089.

Clinically, sodium pyruvate has been given to patients for a variety of disorders ranging from Friedreich's ataxia (20) to open heart operations. It has been administered via several routes including intravenous (10-20 times our inhaled therapeutic dose) (21-27,30), topical administration for hyperkeratotic disorders, and in dietary supplementation. Sodium pyruvate has been shown to protect neurons, lungs, hearts, muscles, cerebral metabolism, embryos, eyes, kidneys, cellular DNA and membranes from oxygen radical damage (13-18, 20-27). The levels of pyruvate in these studies were 2-32 mM. In human and animal studies, pyruvate was shown to facilitate wound healing, decrease inflammation, and decrease production of oxygen radicals (20-27). It is being sold as an OTC product and is a constituent in a therapeutic solution used in

open heart surgery, kidney surgery, eye surgery, for hyperkeratotic disorders, to treat mitochondrial diseases, in organ transplant media that is being used to preserve human lungs, hearts, and other organs for human transplants. These transplant media contain sodium pyruvate ranging from 10 mM to 32 mM to keep the organs safe and functional for longer periods of time. There were no safety concerns for this compound.

Based on results from clinical research, it seems reasonable to treat patients with IPF with a sodium pyruvate nasal spray, which is shown to reduce inflammation, increase FEV_1 and FVC and values, and decrease coughing and other nasal symptoms.

2. Purpose and Objectives of the Studies

The purpose of this clinical trial is to administer a sodium pyruvate nasal spray that eliminates nasal oxidative stresses, caused by oxygen radicals, and demonstrate the efficacy of sodium pyruvate to reduce coughing and increase lung functions.

This will be a 21-day double-blinded randomized placebo-controlled trial designed to determine if patients with idiopathic pulmonary fibrosis treated with 0.2% sodium pyruvate (2.1 mg/mL) in 0.9% sodium chloride nasal spray solution will:

2.1. Primary Objective

Reduce coughing episodes in IPF patients by 25%. (An episode is defined as 5 or more coughs per hour.)

2.2. Secondary Objective

Increase lung function (FEV_1, FVC endpoints of 12% or more) and Improve FEV_1/FVC ratios.

3. Product Description

The product is a 20 mM (0.2%, 2.2 mg/mL) sodium pyruvate nasal spray in 0.9% sodium chloride with 0.02% benzalkonium chloride preservative, pH 7.2 (N115). The placebo control is 0.9% saline and 0.02% benzalkonium chloride preservative, pH 7.2. Both N115 and saline controls are delivered by a Mistette Mark II (MeadWestvaco, Richmond, VA, USA) nasal spray pump, or similar device, that delivers a 0.1 mL metered dose from a 30 mL polypropylene bottle. The product is sealed in tamper evident plastic and labeled with “Caution: New Drug—Limited by Federal (or United States) law to investigational use.” 2 bottles are packaged in a white paperboard box with the expiration date of the product printed on the box. The N115 and placebo are packaged in identical packaging and are indistinguishable from each other to ensure blinding.

Good clinical practice (GCP) rules will be followed and the trial will be conducted, monitored, audited, recorded and the data reported to the Food and Drug Administration (FDA) and on clinicaltrials.gov.

4. Qualifications of the Principal Investigator

Dr. Manuel Michael Lam MD, the principal investigator for this study, is a board-certified physician who specializes in internal medicine. His research team has clinical interests in idiopathic pulmonary fibrosis, asthma and COPD. The references list shows the clinical studies in lung diseases in which Dr. Lam and his team have performed clinical research.

4.1. Research Facilities

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Hialeah Gardens, FL 33016

IRB#: 120180213

4.2. Pulmonary Testing Center and Clinical Laboratory

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New England IRB

5. Study Design Overview

5.1. Pre-study

Individuals with a clinical diagnosis of idiopathic pulmonary fibrosis (as defined by the WHO and the Thoracic Society) will be solicited for participation in this phase 3 trial. After signing the informed consent, participants will be randomly assigned to either the placebo or drug groups in a double-blinded randomized fashion. On pre-study day 1 (Screening Visit), and, after signing the informed consent, they will have their degree of nasal inflammation and erythema, nasal cellular morphology, SaO_2, and FEV_1, and FVC levels recorded (baseline). All subjects will have a blood sample taken, and women of child-bearing age will provide a urine sample. The subjects will fill out a quality of life questionnaire (chronic respiratory disease questionnaire (CRDQ), and they will be given a data logbook in which they will record the number of coughs they experience each day for 7 days until they return to the clinic to begin the study (baseline). Any subject who is not compliant ≥ 80% will not be entered into the trial.

Blood will be analyzed for CBCs, and for standard clinical variables, including urea nitrogen, serum creatinine, total protein, serum albumin, total globulin, albumin/globulin ratio, total bilirubin, alkaline phosphatase, aspartate aminotransferase (AST or SGOT), Alanine Aminotransferase (ALT or SGPT), Lactate Dehydrogenase (LDH), Gamma-glutamyl Transferase (GGT), Serum Sodium, Potassium, Chloride Serum, Carbon Dioxide, C-reactive protein, and Total Cholesterol:HDL Ratio to determine organ health. Subjects may not enter the study until the blood and urine samples are analyzed and evaluated to confirm that subjects do not have a metabolic abnormality that would prevent entry, and that women are not pregnant.

5.2. Study Days

Following the 7-day pre-study period, which includes time for analysis of the blood and urine, eligible IPF patients will return to the clinic and be admitted to the 21-day study. On the first day of the study, these patients will have a physical exam and their vital signs will be recorded. Then, their degree of nasal inflammation and erythema will be evaluated and recorded as before, as will their SaO2, FEV1 and FVC levels and frequency of coughing. Patients will be instructed on how to self-administer their first dose of the nasal spray drug (0.2% sodium pyruvate in 0.9% sodium chloride solution) by first forcefully expelling air from their lungs, and

then spraying 3 squirts of the drug into each nostril, and then forcefully inhaling the drug so that the drug is inhaled into the lungs. After 1 hour, the subjects will have their vital signs taken again. Efficacy and safety evaluations will occur at this 1-hour time period, too, and then again at 2 and 3 hours. The subjects will fill out the product evaluation questionnaire, be given a daily data log form and be given enough drug to last for 21 days (3 bottles), and released from the clinic.

For the next 21 days, subjects will self-administer 3 squirts per nostril of the nasal spray, as instructed, 3 times daily; upon wakening, noon/midday, and before bedtime, Additionally, they will fill out the Daily Data Log form each day to record the number of daily coughs. Patients will be contacted by phone every 2 days to review symptoms and protocol compliance.

On days 8, 14 and 22, they will return to the clinic and have their blood analyzed, vital signs taken, and their degree of nasal inflammation, erythema and cellular morphology recorded. As before, they will be tested for FEV_1, FVC and SaO_2 levels. The number of coughs they experienced and other information will be discussed with the clinician. They will fill out the product evaluation questionnaire and the CRDQ, and they will then be released from the study on day 22. These data will then be evaluated.

6. Criteria for Patient Selection, Exclusion of Patients, and Number of Patients

6.1. Subject Selection and Number of Patients to be Studied

The patient population is adult patients ≥40 years old with mild to moderate IPF with FVC and FEV1 values ≥50% of predicted values. IPF is defined in accordance with the most recent collaborative guidelines from the American Thoracic Society, the European Respiratory Society, the Japanese Respiratory Society, and the Latin American Thoracic Association (56) including HRCT scan and/or lung biopsy consistent with unusual interstitial pneumonia (UIP), especially honeycombing, without identifiable cause related to other ILD such as drug toxicity, occupational or environmental exposure or connective tissue disease.

The study goal will be to enroll 25 patients with confirmed IPF into the trial in the sodium pyruvate (N115) treatment group and 25 with confirmed IPF to be enrolled in the trial in the placebo group. Efforts will be made to include women and minorities. The study will be a randomized, double-blinded placebo-controlled trial to subjects and study investigators.

6.2. Inclusion Criteria

- Individuals with a clinical diagnosis of idiopathic pulmonary fibrosis as determined by clinical evaluation and lung function tests (56), with a condition-related cough.
- Mild to moderate FEV_1 and FVC at 50% or greater of standard
- Individuals who agree to abstain from sexual intercourse, or agree to use condoms or vaginal diaphragms or other devices designed to prevent contraception, during the entire course of the study.

6.3. Exclusion Criteria

- Pulmonary disease other than idiopathic pulmonary fibrosis.
- Respiratory infections in the last 2 weeks.

- Clinically significant cardiac disease including uncontrolled congestive heart failure and unstable angina.
- Pregnancy (urine pregnancy test will be performed prior to enrollment).
- Females of childbearing potential age not on adequate contraception or lactating females.
- Subjects less than 18 years of age.
- Hospitalization within last 6 months due to acute exacerbation of airway disease.
- Subjects with a clinically significant abnormal chest X-ray within past 12 months.
- Medication changes within 1 month of study entry except for antiviral, antibiotic, or antimicrobial medications as well as corticosteroids, antihistamines, or anti-inflammatory medications.
- Subjects who have participated in another drug treatment study within the last month.
- Subjects with a current history of alcohol or recreational drug abuse.
- Subjects who have taken dietary supplements containing pyruvate within 24 hours prior to the screening visit.
- Subjects with metabolic diseases (diabetes, hypoglycemia, etc.).

6.4. Inclusion of Women and Minorities

Every attempt will be made to include all genders and minorities that present with pulmonary fibrosis and are not exempted due to exclusion criteria.

7. Study Design

Assignment of Subject Number and Randomization

This is a randomized placebo-controlled double-blinded trial. Upon enrollment, each subject will be sequentially issued a unique subject number starting at 100. Once a number has been assigned to a subject, it cannot be re-assigned to another subject. Subjects will be encouraged to complete the study, although they may withdraw at any time without prejudice. Termination will be reported to the sponsor in a timely manner. Details of the reason(s) why a subject was dropped or withdrew from the study will be documented. Enrollment will continue until the number of subjects specified in the protocol has been attained. The data from all subjects that have been given a study compound will be retained for analysis.

8. Randomization

The subjects will be randomized to the placebo control or N115 drug study groups using a random number set.

9. Telephone Communication

Subjects will be instructed to call the clinic should they be experiencing any self-perceived adverse events, or if they have any study-related questions. All telephone information will be documented.

10. Products Description (How Administered)

The product is an all-natural patented 0.2% sodium pyruvate in 0.9% sodium chloride nasal spray solution, pH 7.2, with 0.02% benzalkonium chloride as a preservative. The patient will be instructed on the use of the nasal spray prior to the start of the clinical trial. The plastic bottle contains 30 mL. It is non-steroidal. Patients will forcibly expel air from their lungs and then will administer 3 sprays per nostril while inhaling strongly so that the drug is transported through the nose and into the lungs. The drug is to be administered 3 times per day (morning, mid-day, and evening). The product comes with instructions for administration (3 sprays per nostril).

11. Concomitant Therapy

The investigator is to record the use of all concomitant medications, both prescribed and over the counter (OTC) (including but not limited to natural food supplements), in the CRF. Subjects may continue to use their normal therapy as long as they list it as a current medication, and it is not prohibited by the exclusion criteria. Allowable concomitant therapy includes antiviral, antibiotic, antimicrobial, corticosteroids, antihistamines, or anti-inflammatory medications. The clinician may prescribe "rescue medication" should it be required by any of the patients. Patients may continue taking nutritional supplements as long as they do not contain pyruvate.

Subjects may not use any of the following during the trial or for 1 week before enrollment in the trial: all intranasal medications or lavages.

12. The Planned Maximum Dosage, and the Duration of Individual Patient Exposure to the Drug

This is a constant dose study. The amount of delivered sodium pyruvate was determined in prior clinical trials to be 3 sprays of 0.2% (2.2 mg/mL) sodium pyruvate in 0.9% sodium chloride solution per nostril, 3 times per day. The dose was determined by evaluating the data from both animal and human studies (cited above). Patients will forcibly expel air from their lungs and then will administer 3 sprays per nostril while inhaling strongly so that the drug is transported through the nose and into the lungs. The drug is to be administered 3 times per day (morning, mid-day, and evening). The product comes with instructions for administration (3 sprays per nostril).

13. A Description of the Observations and Measurements to be Made to Fulfill the Objectives of the Study

Observations for safety will include a pregnancy test for women of child-bearing age, blood tests for organ function, and vital signs. Adverse events will be monitored.

Observations for efficacy will include evaluation of lung function, as determined by FEV_1, FVC, nasal inflammation and erythema, and number of daily coughs.

13.1. Testing Schedule

Test	Screening	Day 1; 0,1,2,3 Hours	Day 8	Day 14	Day 22
Clinic Visit	+	+	+	+	+
Informed Consent	+				
Medical History	+				
Physical Exam/	+	+		+	+
Product Eval Q	+			+	+
CDRQ	+		+	+	+
Administer Drug		+	+	+	+
Blood Sample	+			+	+
Vital Signs	+	+	+	+	+
Pregnancy Test for Females	+				
Concurrent Medications Taken	+	+	+	+	+
SaO_2	+	+	+	+	+
Nasal Inflammation	+	+	+	+	+
FEV_1	+	+	+	+	+
FVC	+	+	+	+	+
Coughing Data Logbook*	+	+	+	+	+
Adverse Events			+	+	+
Phase Product Evaluation.	+	+	+	+	+

*The coughing data logbook will be filled out by the patient every day, starting at the pre-study visit through day 14.

13.2. Safety Testing

- Physical Exam
- Vital Signs
- Weight
- Blood pressure
- Pulse
- Temperature
- Blood analysis
- Complete blood count (CBC)
- Clinical tests, including:
    - Urea nitrogen: serum creatinine: total protein: serum albumin: total globulin: albumin/globulin ratio: total bilirubin: alkaline phosphatase: aspartate aminotransferase (AST or SGOT): alanine aminotransferase (ALT or SGPT): LDH: GGT: serum sodium: potassium: chloride serum: carbon dioxide: total cholesterol:hdl ratio:
- Urine test for women of child-bearing age

- Efficacy tests
    - Nasal examinations by a rhinoscope fitted with a camera
    - FEV_1, FVC

14. Withdrawal Criteria

The patients can withdraw from the study at any time, and for any reason. All data collected from these patients will be analyzed. Reasons for withdrawal will be recorded to insure that it was not due to an adverse event.

The subject has the right to withdraw from the study at any time.

The subject will be withdrawn from the study if:

- There is deterioration in the subject's signs and symptoms and/or the subject develops a disease or condition that, in the opinion of the investigator, would compromise the subject's safety by continuing in the study.
- In the investigator's judgment, it is in the best interest of the subject.
- There is a violation of the protocol inclusion and/or exclusion criteria, as deemed relevant by the investigator and discussed with the medical monitor.
- The subject begins to take any medication(s) that is(are) excluded by the protocol.
- Any of the conditions for stopping rules are met.

If a subject withdraws prematurely from the study due to the above criteria or any other reason, study staff should make every effort (at withdrawal) to complete the full set of evaluations listed for the early termination/end-of-study visit. The reason for subject withdrawal must be documented in the case report form (CRF).

If a subject withdraws from the study due to an adverse event (AE) (eg, clinical signs, symptoms, or clinically significant laboratory abnormality), the subject will be asked to return to the clinic, at a minimum, for the evaluations scheduled for the posttreatment follow-up period. If the AE has still not resolved, additional follow-up will be performed, as appropriate, and documented in the subject's medical records. As a minimum requirement, AEs should be followed for 30 days after the subject's last dose of study medication.

In the case of a subject lost to follow-up, attempts to contact the subject must be made and documented in the subject's medical records.

Withdrawn subjects will not be replaced.

15. A Description of Clinical Procedures, Laboratory Tests, or Other Measures to be Taken to Monitor the Effects of the Drug in Human Subjects and to Minimize Risk

Tests and analyses will be conducted 3 times during the study. If any aberrant values occur, the principal investigator will determine if that patient should continue in the study or be removed. All aberrant values that occur during the study will be considered adverse events. A summary of possible risks will be provided to the patients. Previous risks of using the product are minimal, which included irritation to the nose cavity reported by 1 patient out of 1,869.

16. Safety Precautions Included in this Study

The 0.2% nasal sodium pyruvate inhalation has been administered to both healthy humans and humans with lung diseases, without the occurrence of any SAE.

- First administration of nasal spray on the premises of a hospital/clinic.
- The initial nasal spray will be administered under the supervision of medical staff.
- If a subject develops any adverse event during the follow-up period after study drug administration, the subject will be treated to a satisfactory resolution before being discharged from the clinic.
- Subjects will remain at the test site for monitoring for 30 minutes after the initial inhalation of the nasal spray.
- Follow up telephone communications, as needed.

17. Product Evaluation Questionnaire

This self-administered questionnaire measures the patients' impressions of how the drug affected their nasal cavity, their coughing, and their breathing. It is designed to evaluate changes in a subject's quality of life over time. It will be administered during all clinic visits.

18. Daily Data Logbook

Subjects will use this diary card to record the number of their coughs per day, any self-perceived adverse events, and any medication changes during the study.

19. Urinalysis

Samples will be taken and analyzed to determine if any child-bearing females are pregnant.

20. Study Management

20.1. Case Report Forms

Case Report Forms (CRF) will be supplied by Cellular Sciences. Data will be recorded in the CRFs according to the following:

Instructions to data entry personnel and monitor:

Explain all missing data and include reference to any original data that is not contained in the study file. If a space is blank because the item was not done, mark the item “N/D”. If the item is not applicable to the individual case, mark the item "N/A”. If an item is unknown, mark the space “UNK". Legibly write or print all entries in black ink. If an entry error has been made, draw a single straight line through the wrong entry and enter the correct data above it. Initial and date all such changes. Do not erase or opaque out errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date the clarification.

20.2. Data Collection, Management, and Statistical Analysis

The data management staff at the research clinic will collect and manage the data. Data will be obtained, maintained, and transmitted securely in accordance with HIPAA guidelines. Monitoring and auditing of the data will be conducted by a staff member of Cellular Sciences.

Statistical analysis will be performed on deidentified data. The basic statistical analysis will be repeated measurements of Analysis of Variance using baseline measurements as a covariant (ANCOVA). Age, sex, time since initial diagnosis with IPF, concurrent medications and smoking status are considered potential covariates. Number of coughing episodes reported for a 24-hour pre-study period, and other pre-study data information, will be compared to the data collected during the trial. The differences between baseline data and the data collected during the trial will be compared and analyzed (Within treatment). Additionally, the data of the patients on the experimental therapy will be compared to the data generated by the patients on placebo (Between treatments). P values of <0.5 will be considered significant. FEV1 and FVC will similarly be analyzed as secondary outcome measurements. Relative changes will be analyzed by JMP, Matillion, XLSTAT or R software by Dr. Masiqués or Dr. Lupfer, who will be responsible for the statistical analysis.

Partial patient data will be included in the statistical analysis if patients withdraw from the study, but data from non-compliant patients will be excluded. Our previous research (REF) indicates that a sample size of 20 is sufficient to detect a statistically significant difference ($p<0.05$) between treatment groups. We have chosen to enroll 25 patients in each treatment group to ensure we achieve the necessary sample size, taking into account that some patients may not complete the study, and some data will be incomplete or excluded from the study.

Study Monitoring Sciences. The monitor will visit the clinical frequently during the trial to assure that all data are being collected and that the Case Report Forms are completed and correctly filled out. The CRFs and related source documents will be reviewed in detail by the study monitor at each visit, in accordance with relevant SOPs and ICH GCP regulations. This includes results of tests performed as a requirement for participation in this study and any other medical records required to confirm information contained in the CRFs, such as past medical history and secondary diagnoses. The investigator and staff will be expected to cooperate with the study monitor and provide any missing or incomplete information whenever possible.

20.3. Data from Patients who Withdraw from the Study

If a subject withdraws from a study, the data collected on the subject to the point of withdrawal will remain part of the study database and will not be removed.

The investigator may ask a subject who is withdrawing whether the subject wishes to provide continued follow-up and further data collection subsequent to their withdrawal from the interventional portion of the study. Under this circumstance, the discussion with the subject would distinguish between study-related interventions and continued follow-up of associated clinical outcome information, such as medical course or laboratory results obtained through non-invasive chart review and address the maintenance of privacy and confidentiality of the subject's information.

If a subject withdraws from the interventional portion of the study but agrees to continued follow-up of associated clinical outcome information as described in the previous paragraph, the investigator must obtain the subject's informed consent for this limited participation in the study (assuming such a situation was not described in the original informed consent form). In accordance with FDA regulations, Institutional Review Board (IRB) approval of informed consent documents would be required (21 CFR 50.25, 56.109(b), 312.60, 312.66, 812.100).

If a subject withdraws from the interventional portion of a study and does not consent to continued follow-up of associated clinical outcome information, the investigator must not access for purposes related to the study the subject's medical record or other confidential records requiring the subject's consent. However, an investigator may review study data related to the subject, collected prior to the subject's withdrawal from the study, and may consult public records, such as those establishing survival status.

21. Product Packaging

The product packaging will comply with FDA regulations. The nasal spray will be contained in a sterile plastic vial that can be squeezed to administer the correct dosage. The vials necessary for each study period (i.e. 14 days) will be placed in a paperboard box in a plastic bag with the patient's number.

22. Product Storage Requirements

At Research Center

The products will be stored at room temperature, but not above 85ºF.

In Home

The products will be stored at room temperature, but not above 85ºF.

23. Record Retention

All records will be kept by the clinic for a period of 3 years. After that they will be sent to Cellular Sciences.

24. Reports on Adverse Events

All Adverse Events, as defined below, will be recorded, evaluated and reported in accordance with 21 CFR 312.32. These Adverse Events may occur during the study or after the study has completed.

24.1. Definition of an Adverse Event

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or *life-threatening suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or *serious suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or *unexpected suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic

enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

24.2. Examples of Adverse Events

- Inter-current illnesses
- Injuries
- New symptomatology (this does not include symptoms expected as a result of the subject's condition unless it is beyond what is normally expected)
- Significant laboratory abnormalities
- New abnormal physical exam findings
- Significant exacerbation of pre-existing conditions
- Death
- A life-threatening event
- In-patient hospitalization (not required as part of the treatment) or prolongation of existing hospitalization
- Disability or permanent damage
- A congenital anomaly/birth defect
- Cancer
- Drug overdose

24.3. Recording and Documentation of Adverse Events

Any adverse event (i.e., a new adverse event or an exacerbation of a pre-existing condition) with an onset date after study enrollment should be recorded as an adverse event on the CRF. All adverse events must be recorded on the appropriate CRF regardless of the severity or relationship to study medication. An adverse event or laboratory abnormality with an onset date before study enrollment is considered to be pre-existing in nature. These pre-existing events will be noted in the CRFs.

All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be recorded on the Florida Advanced Medical Research Adverse Events form.

24.3.1 Investigator Report of a "Serious Adverse Event" to Cellular Sciences.

Cellular Sciences (Sponsor) must be notified regarding the occurrence of any SAE that occurs after enrollment in the study protocol, regardless of the causal assessment to study medication. The procedures for reporting SAEs are as follows:

- Complete the "Serious Adverse Event Report."
- Email the SAE Report to Cellular Sciences (see below) within 24 hours of the investigator's knowledge of the event.
- These preliminary reports must be followed by detailed descriptions that include copies of hospital case reports, autopsy reports, and other documents when requested and applicable. Cellular Sciences may request additional information from the Investigator to ensure the timely completion of accurate safety reports.

The contact information for reporting SAEs is as follows:

Ronald J. Amen, Ph.D.	Alain Martin, Ph.D.
Director	Director
Cellular Sciences	Cellular Sciences
18101 Catherine Circle	84 Park Ave
Villa Park, CA 92861	Flemington, NJ 08822
ronald.amen@techenterprises.org	al.martin.hq@emphycorp.com

24.3.2 Sponsor Safety Reporting

(1) IND safety reports. The sponsor must notify the FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under paragraph (c)(1)(i), (c)(1)(ii), (c)(1)(iii), or (c)(1)(iv) of 21 CFR 312.32. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

(i) ***Serious and unexpected suspected adverse reaction.*** The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

(A) A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);

(B) One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);

(C) An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

(ii) ***Findings from other studies.*** The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies (other than those reported under paragraph (c)(1)(i) of 21 CFR 312.32), whether or not conducted under an IND, and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug. Ordinarily, such a finding would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

(iii) ***Findings from animal or in vitro testing.*** The sponsor must report any findings from animal or in vitro testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure. Ordinarily, any such findings would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

(iv) ***Increased rate of occurrence of serious suspected adverse reactions.*** The sponsor must report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

(v) ***Submission of IND safety reports.*** The sponsor must submit each IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. FDA will periodically issue guidance on how to provide the electronic submission (e.g., method of transmission, media, file formats, preparation and organization of files). The sponsor may submit foreign suspected adverse reactions on a Council for International Organizations of Medical Sciences (CIOMS) I Form instead of a FDA Form 3500A. Reports of overall findings or pooled analyses from published and unpublished in vitro, animal, epidemiological, or clinical studies must be submitted in a narrative format. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division in the Center for Drug Evaluation and Research or in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. Upon request from FDA, the sponsor must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

(2) ***Unexpected fatal or life-threatening suspected adverse reaction reports.*** The sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

(3) ***Reporting format or frequency.*** FDA may require a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the FDA review division that has responsibility for review of the IND.

(4) ***Investigations of marketed drugs.*** A sponsor of a clinical study of a drug marketed or approved in the United States that is conducted under an IND is required to submit IND safety reports for suspected adverse reactions that are observed in the clinical study, at domestic or foreign study sites. The sponsor must also submit safety information from the clinical study as prescribed by the postmarketing safety reporting requirements.

(5) ***Reporting study endpoints.*** Study endpoints (e.g., mortality or major morbidity) must be reported to FDA by the sponsor as described in the protocol and ordinarily would not be reported under paragraph (c) of this section. However, if a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the drug and the event (e.g., death from anaphylaxis), the event must be reported under § 312.32(c)(1)(i) as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality).

(d) ***Followup.***

(1) The sponsor must promptly investigate all safety information it receives.

(2) Relevant followup information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Followup IND Safety Report."

(3) If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable is so reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

All AEs will be monitored by the PMT. Less than serious or non-serious expected adverse events will be reported only at the time of protocol continuation reports.

24.4. Attribution

For reporting purposes, attribution is the assessment of the likelihood that an AE is caused by the research agent or protocol intervention. The attribution is assigned by the treating physician/Principal Investigator after considering the clinical information, the medical history of the subject, and experience with the research agent/intervention. This is recorded using the Adverse Event Report (COH AER) form in 1 of 5 categories scored as the following: 5 = related, 4 = probably related, 3 = possibly related, 2 = unlikely related, and 1 = unrelated.

Disclaimer. A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse event. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse event.

24.5. Post-study Adverse Events

The Investigator will notify Cellular Sciences of any serious adverse event he/she becomes aware of that occurs at any time after a subject has completed study participation, if in the Investigator's judgment the event may be reasonably related to the medication used in the study. The Investigator will follow the subject until the adverse event resolves or becomes stable.

25. References

1. Dekhuijzen PN, Aben KK, Dekker I, et al. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1996; 154(3 Pt 1):813-816.
2. Dohlman AW, Black HR, Royall JA. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. Am Rev Respir Dis. 1993; 148(4 Pt 1):955-960.
3. Kietzmann D, Kahl R, Muller M, et al. Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS. Intensive Care Med. 1993; 19(2):78-81.
4. Sznajder JI, Fraiman A, Hall JB, et al. Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. Chest. 1989; 96(3):606-612.
5. Baldwin SR, Simon RH, Grum CM, et al. Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet. 1986; 1(8471):11-14.
6. Cross CE, Halliwell B, Borish ET, et al. Oxygen radicals and human disease. Ann Intern Med. 1987; 107(4):526-545.
7. Jamieson D. Oxygen toxicity and reactive oxygen metabolites in mammals. Free Radic Biol Med. 1989; 7(1):87-108.
8. Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. J Allergy Clin Immunol. 2002; 110(3):349-356.
9. Ogasawara H, Yoshimura S, Kumoi T. Hydrogen peroxide generation by eosinophils in allergic rhinitis. Auris Nasus Larynx. 1991; 18(2):133-143.
10. Brigham KL: Role of free radicals in lung injury. Chest 1986, 89(6):859-863.
11. Bunton CA: Oxidation of α-Diketones andα-Keto-Acids by Hydrogen Peroxide. Nature 1949, 163(4142):444-444.
12. Melzer E, Schmidt HL. Carbon isotope effects on the decarboxylation of carboxylic acids. Comparison of the lactate oxidase reaction and the degradation of pyruvate by H2O2. Biochem J. 1988; 252(3):913-915.
13. O'Donnell-Tormey J, Nathan CF, Lanks K, et al. Secretion of pyruvate. An antioxidant defense of mammalian cells. J Exp Med. 1987; 165(2):500-514.
14. Andrae U, Singh J, Ziegler-Skylakakis K. Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity. Toxicol Lett. 1985; 28(2-3):93-98.
15. Varma SD, Morris SM. Peroxide damage to the eye lens in vitro prevention by pyruvate. Free Radic Res Commun. 1988; 4(5):283- 290.
16. Nath KA, Enright H, Nutter L, et al. Effect of pyruvate on oxidant injury to isolated and cellular DNA. Kidney Int. 1994; 45(1):166- 176.

17. Brunkhorst R, Mahiout A. Pyruvate neutralizes peritoneal dialysate cytotoxicity: maintained integrity and proliferation of cultured human mesothelial cells. Kidney Int. 1995; 48(1):177-181.

18. Shah SV. Role of reactive oxygen metabolites in experimental glomerular disease. Kidney Int. 1989, 35(5):1093-1106.

19. Lehninger 1981 Biochemistry (12)

20. Dijkstra U, Gabreëls F, Joosten E, et al. Friedreich's ataxia: intravenous pyruvate load to demonstrate a defect in pyruvate metabolism. Neurol. 1984; 34(11):1493-1497.

21. Reel, J., and C. Lupfer. 2021. sodium pyruvate Ameliorates Influenza A Virus Infection In Vivo. Microbiol Res 12: 258-267.

22. Abusalamah, H., J. M. Reel, and C. R. Lupfer. 2020. Pyruvate affects inflammatory responses of macrophages during influenza A virus infection. Virus Res 286: 198088.

23. Christopher Lupfer et al. inhalation of sodium pyruvate to reduce the symptoms and severity of respiratory diseases including COVID-19, long COVID and pulmonary fibrosis. Eur j of Respir Med. 2021; 3(3): 229-237. 10.31488 EJRM.121

24. Sheridan, J., E. Kern, A. Martin, and A. Booth. 1997. Evaluation of antioxidant healing formulations in topical therapy of experimental cutaneous and genital herpes simplex virus infections. Antiviral Res 36: 157-166.

25. Martin Alain et al. Inhalation of sodium pyruvate to reduce hypoxemia and dyspnea associated with chronic respiratory diseases- a multi-study retrospective analysis. European journal of respiratory medicine. 2011; 4(1); 258-261. Doi; 10.31488/EJRM.124

26. Votto, J. J., J. B. Bowen, R. W. Barton, and R. S. Thrall. 2008. Inhaled sodium pyruvate improved FEV1 and decreased expired breath levels of nitric oxide in patients with chronic obstructive pulmonary disease. *J Aerosol Med Pulm Drug Deliv* 21: 329-334.

27. Martin Alain, et al. Inhaled sodium pyruvate reduces oxygen radical and inflammatory cytokines in patients with lung and sinus diseases. European Journal of Respiratory Medicine. 2022; 4(2): 320-326. Doi; 10.31488/EJRM.133

28. He N. Xu et al Differentiating inflamed and normal lungs by the apparent reaction rate constants of lactate dehydrogenase probed by hyperpolarized C 13 labeled pyruvate. Home vol 6 Feb 28 2016

29. Mehrdad Pourfathi et al. Metabolic Imaging Of Acute Lung Injury Using Hyperpolarized 13C pyruvate in Rodent lungs. Magnetic Resonance Imaging. Dissertation. University of Pennsylvania, 2019 NIH National cancer Institute.

30. Hyperpolarized Pyruvate (13C) Injection Investigator's Brochure

31. Lamas DJ, Kawut SM, Bagiella E, et al. Delayed access and survival in idiopathic pulmonary fibrosis: a cohort study. Am J Respir Crit Care Med 2011; 184: 842–847.

32. Mermigkis C, Stagaki E, Tryfon S, et al. How common is sleep-disordered breathing in patients with idiopathic pulmonary fibrosis? Sleep Breath 2010; 14: 387–390.

33. Kilduff CE, Counter MJ, Mermigkis C, Bouloukaki I, Antoniou K, et al. Obstructive sleep apnea should be treated in patients with idiopathic pulmonary fibrosis. Sleep Breath 2015; 19: 385–391.

34. Birring SS, Ing AJ, Chan K, et al. Obstructive sleep apnea: a cause of chronic cough. Cough 2007; 3: 7.

35. Chan KK, Ing AJ, Laks L, et al. Chronic cough in patients with sleep-disordered breathing. Eur Respir J 2010; 35: 368–372.

36. Chan K, Ing A, Birring SS. Cough in obstructive sleep apnea. Pulm Pharmacol Ther 2015; 35: 129–131.

37. Boulet LP, Coeytaux RR, McCrory DC, et al. Tools for assessing outcomes in studies of chronic cough: CHEST guideline and expert panel report. Chest 2015; 147: 804–814.

38. Lee KK, Matos S, Evans DH, et al. A longitudinal assessment of acute cough. Am J Respir Crit Care Med 2013; 187: 991–997.

39. Lechtzin N, Hilliard ME, Horton MR. Validation of the cough quality-of-life questionnaire in patients with idiopathic pulmonary fibrosis. Chest 2013; 143: 1745–1749.

40. Morice AH, Kastelik JA, Thompson R. Cough challenge in the assessment of cough reflex. Br J Clin Pharmacol 2001; 52: 365–375.

41. Spinou A, Birring SS. An update on measurement and monitoring of cough: what are the important study endpoints? J Thorac Dis 2014; 6: Suppl. 7, S728–S734.

42. Birring SS, Spinou A. How best to measure cough clinically. Curr Opin Pharmacol 2015; 22: 37–40.

43. Moncada, S. et al Nitric Oxide: physiology, pathophysiology, and pharmacology. 1991 Pharmacological Reviews Vol. 43 no2 pp109-141

44. Nathan, C. Nitric oxide as a secretory product of mammalian cells. FASEB journal vol. 6 Sept. 1992 pp3051-3064

45. Rossaint, R. et al Inhaled nitric oxide: its effect on pulmonary circulation and airway smooth muscle cells. Euro Heart Jour. 1993 vol. 14 Supp. Pp133-140

46. Roberts, J. et al. Inhaled nitric oxide and persistent pulmonary hypertension of the new borns. The new England journal of Medicine Feb 27, 1997 pp 605-610

47. Barnes, P. The effect of drugs on exhaled nitric oxide. Euro Respir. Rev. 1999; 9: 68, 231-233.

48. Baraldi, E. et al. Application of exhaled nitric oxide measurement in pediatrics. Euro Respir. Rev. 1999; 9: 68, 234-240.

49. Lundberg, J. Nitric oxide in the nasal airways. Euro Respir. Rev. 1999; 9: 68, 241-245.

50. Shingo Noguchi et al. Nitric oxide exerts protective effects against bleomycin-induced pulmonary Fibrosis in mice. Respiratory research 2014 15:92

51. M. Yoshida et al. The effect of low dose inhalation of nitric oxide in patients with pulmonary Fibrosis, Eur Respir. J 1997; 10: 2051-2054

52. Yong GL et al. Outpatient inhaled nitric oxide in a patient with idiopathic pulmonary fibrosis: a bridge to lung transplant. J Heart lung transplant 2001 Nov; 20(11): 1224 7

53. Ian M Balfour-Lynn et al Reduced upper airway nitric oxide in cystic fibrosis Archives of diseases in childhood 1996; 75: 319-322

54. Suh-Young Lee, Influence of Chronic Sinusitis and Nasal Polyp on the Lower Airway of Subjects without lower airway diseases Allergy Asthma Immunol Res. 2014 July;6(4):310-315. Doi.org/ 10.4168/air 2014.4.4.310

55. Beibei Sun et al. Associations of short-term PM2.5 exposures with nasal oxidative stress, inflammation and lung function impairment and modification by GSTT1-null genotype: A panel study of the retired adults. Environmental Pollution Volume 285, 15 September 2021, 117215

56. Athol U Wells. The revised ATS/ERS/JRS/ALAT diagnostic criteria for idiopathic pulmonary fibrosis (IPF) - practical implications. Respiratory Research, 2013;14 Suppl 1(Suppl 1):S2. doi: 10.1186/1465-9921-14-S1-S2.

57. Y G Min et al Hypertonic saline decreases ciliary movement in human nasal epithelium in vitro. Otolaryngol head and neck surgery 2001. March, 124(3): 313-6

58. Chang-noon Kim et al Effect of hypo-, iso, and hypertonic saline irrigation on secretory mucins and morphology on cultured human casal epithelial cells. Acta Itilarynogol 2005 Dec; 125 (12): 1296-300