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Potential Treatment of Chronic Obstructive Pulmonary Disease with Allogeneic and Autologous Telomerase-Positive Stem Cells

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ABSTRACT

The major risk factor for chronic obstructive pulmonary disease (COPD) is cigarette smoking which drives an inflammatory process that causes loss of function. Cessation of smoking, oxygen therapy, various inhalers, medications to improve vasodilation, antibiotics to combat the frequent lung infections, fluid replacement, exercise rehabilitation programs, and early detection and treatment of comorbidities are the options available to patients with COPD to reduce the burden of the disease, but do not alter the underlying progression of COPD. Lung transplantation is an increasingly discussed therapeutic option, but its use remains limited. The median survival time after lung transplant is 4.5 years. Currently, regenerative therapies are divided into extrinsic (stem cells) and intrinsic (small molecules) therapeutic strategies. The immunomodulatory effects of mesenchymal stem cells were tested in clinical trials. Systemic infusion was safe in moderate to severe COPD, but no improvement in lung function was noted. Infusion of small molecules failed in preliminary clinical trials, possibly due to the severity of the COPD. In culture, telomerase-positive stem cells were shown to differentiate into cells of the lung and in a pre-clinical animal model of lung fibrosis infusion of these same stem cells demonstrated a regeneration of bronchioles, alveolar ducts, and alveolar sacs. We hypothesized that telomerase-positive stem cells would increase lung function in individuals with COPD. In a phase-I safety study, individuals (n=51) with very severe (GOLD-4) and severe (GOLD-3) COPD were treated with autologous and/or allogeneic telomerase-positive stem cells. Results from an eight-year follow-up study demonstrated an increase in their FEV_1's from 0% to 16% above pre-treatment baseline values, with no adverse side effects. These results suggest that telomerase-positive stem cells are both safe and efficacious for the treatment of COPD.

Keywords

Adult Stem Cells, Telomerase-positive, COPD, Regenerative Medicine, Pulmonary Disease, Interstitial Lung Diseases.

Introduction

Pulmonary disease constitutes a broad range of illnesses. Significant morbidity and mortality can be attributed to both neoplastic (e.g., squamous cell cancer, non-small cell carcinoma) and infectious (i.e. tuberculosis, pneumonia) causes of lung disease. Chronic lung diseases, e.g., chronic obstructive pulmonary disease, emphysema, idiopathic pulmonary fibrosis, and interstitial lung diseases are particularly destructive and account for the highest mortality rates within the pulmonary disease category. Chronic obstructive pulmonary disease is responsible for early mortality, high

death rates and significant cost to health systems [1-4]. Chronic Obstructive Pulmonary Disease (COPD) alone is the fourth leading cause of death in the USA, averaging 126,000 claimed lives per year [5,6]. Projections for 2020 indicate that COPD will be the third leading cause of death in the world [1].

The diagnosis for lung diseases is based on spirometry, i.e., lung function tests and the saturation of oxygen within the arterial blood [6]. Spirometry measures the amount of airflow passing through the respiratory passages of the lungs during inspiration and expiration [7,8]. Two major components of spirometry are measured to make the diagnosis for lung disease: 1) the greatest volume of air that can be breathed out in the first second of a breath, the forced expiratory volume in one second (FEV_1) and 2) the greatest volume of air that can be breathed out in a single large breath, the forced vital capacity (FVC) [9,10].

Normally, 75-80% of the FVC comes out in the first second [11]. A ratio of FEV_1/FVC of less than 80% of predicted value for age of the individual in someone with associated symptoms defines a person as having COPD [9,11]. Oxygen saturation analysis is used to determine the need for long-term oxygen therapy. This therapy is recommended for those with an FEV_1 less than 35% of age-adjusted predicted value and/or those with a peripheral oxygen saturation of less than 92% (GOLD Staging System) (Table 2) [9] [GOLD].

Based on the natural history of tobacco smoke-related chronic airflow obstruction, COPD decline in the forced expiratory volume in the first second (FEV_1) appears slow at the beginning, becoming faster as the disease advances [13]. While active smoking remains the major risk factor, responsible for 80-90 % of the deaths that occur as a result of COPD, other factors are being recognized as contributors to COPD pathologies, e.g., reactive airway disease, air pollution, alpha-1-antitrypsin enzyme deficiency, occupational factors, infections, heredity, male sex, and increased age endothelial cell dysfunction [1,14-16].

The term COPD describes slowly progressive and irreversible airway obstruction. Four pathological processes are involved in the etiology of COPD: chronic bronchitis, obstruction of respiratory bronchioles, obstruction of alveoli, and emphysema [17]. Chronic bronchitis involves inflammation of the bronchi leading to bronchial and pulmonary scarring and fibrosis. The clinical picture can also involve infection of the bronchi and lungs. With this condition, less air is able to flow to and from the lungs and heavy mucus or phlegm is coughed up. Emphysema occurs when the tissues of the lungs are damaged, usually as a result of smoking, and is an irreversible and chronic disease that causes difficulty breathing and shortness of breath. The lung tissue destroyed includes the alveoli, terminal bronchioles, and respiratory bronchioles [17].

Comorbidities are frequent in COPD and significantly impact patients' quality of life, exacerbation frequency, bronchiectasis, fibrosis, obstructive sleep apnea, and increased mortality versus survival, e.g., severe alpha-1 antitrypsin deficiency, hypoxia, asthma, oxidative stress, systemic inflammation, low BMI, cachexia, pulmonary and hypertension. Cardiovascular disease, e.g., coronary artery disease, chronic heart failure, dysrhythmia, can also complicate the clinical picture [18-25]. Due to the consequence of COPD overlapping with its comorbidities, a change is necessary from the previous 'one size fits all' approach of clinical medicine to individualized patient care [22].

Patients with COPD are still diagnosed based on the presence of persistent airflow limitation measured using spirometry [6,26]. The value of the forced expiratory volume in one second (FEV_1) is useful in the diagnosis and prognosis of COPD. Previous studies on lung function in individuals with severe alpha-1 antitrypsin deficiency have shown a variable annual decline in FEV_1. It was found that active smoking, age, respiratory symptoms at baseline and repeated severe exacerbations of COPD are factors associated with an accelerated decline of lung function in individuals with severe alpha-1 antitrypsin deficiency [27].

Advances in the use of thoracic X-ray computed tomography (CT), magnetic resonance imaging (MRI) and ventilation-perfusion scintigraphy, lung structure and function abnormalities may be regionally identified and measured. These imaging endpoints may serve as biomarkers of COPD function [26,28]. Computed tomography scan images have been used to identify different radiographical COPD phenotypes based on the presence and severity of bronchiectasis, exacerbation, systemic inflammation, bronchial wall thickening, and emphysema.

Bronchiectasis is characterized by abnormal dilation of the bronchi. This is typically the consequence of chronic airway inflammation and/or infection. The prevalence of bronchiectasis in patients with COPD is high, especially in advanced stages [29,30].

Exacerbations of COPD are defined as sustained worsening of a patient's condition beyond normal day-to-day variations that is acute in onset, and that may also require a change in medication and/or hospitalization. Exacerbations have a significant and prolonged impact on health status and outcomes, and negative effects on pulmonary function [31].Treatment should be guided by the severity of lung impairment, symptoms such as dyspnea, the amount of cough and sputum production, and how often a patient experiences an exacerbation [6].

The major characteristic of COPD is systemic inflammation. Biomedical markers for inflammation are the neutrophil/lymphocyte ratio and the eosinophil/basophil ratio. In a clinical trial, patients with COPD in stable stage were enrolled. A number of clinical markers, including RBT, procalcitonin BODE index; CRP and ESR were performed as they entered the study and every three months for the next 24 months. Meanwhile, exacerbation count and mortality incidence were recorded. The correlation between the prognostic biomarkers and the prognosis of patients was analyzed. As an inexpensive, accessible, and convenient assay, RBT may be used as a practical means in the prediction of prognosis of patients with COPD in future clinical settings

[32]. The most severe pulmonary damage occurs during the emphysematous phase. Bronchial and alveolar walls are damaged and destroyed [6]. Once lung tissue is irreversibly lost, the lungs lose their elastic quality and, therefore, their intrinsic ability to obtain oxygen and eliminate carbon dioxide.

Endothelial cell dysfunction within the pulmonary vasculature is characterized by a list of alterations of endothelium towards reduced vasodilation, proinflammatory state with release of inflammatory mediators (e.g., chemokines, cytokines, and cellular proteases), detachment and apoptosis of endothelial cells, and development of atherosclerosis. COPD-induced endothelial dysfunction is associated with an elevated cardiovascular risk. Vascular endothelial cell dysfunction is a significant prognostic factor of COPD [16]. When autophagy is dysregulated by factors such as cigarette smoking, environmental insults and ageing, it can lead to formation of aggresome-bodies and enhanced production of reactive oxygen species (ROS), which contribute to the pathogenesis of chronic obstructive pulmonary disease (COPD) [33]. The inflammatory effects of cigarette smoking are a major risk factor driving the inflammatory destruction of alveolar tissue.

Current drug treatments improve symptoms but do not alter the underlying progression of this disease. The failure of anti-inflammatory drugs, like glucocorticosteroids, to have a major impact in COPD and idiopathic pulmonary fibrosis (IPF) has hastened the need to develop novel therapeutic strategies [34]. Selective phosphodiesterase-4 (PDE4) inhibitors have major anti-inflammatory effects. Cilomast and roflumilast are selective phosphodiesterase-4 inhibitors that are being studied in clinical trials, where they have been shown to improve asthma and COPD. They have recently been shown clinical efficacy in these diseases, although their utility is hampered by class related side-effects of nausea, vomiting, and diarrhea. The effectiveness of these PDE4 inhibitors may be limited by their clinical potency using doses that have minimal effects on nausea and vomiting [35-37].

None of the pharmacological treatments for COPD halt the progression of the disease [6], but pharmacologic treatment is the most important for COPD. Therapy for COPD is directed towards relief of symptoms, prevention of complications, and minimization of side effects while disease progression occurs [38]. When dyspnea limits activity or quality of life, COPD should be treated with once- or twice-daily maintenance long-acting anticholinergic and β-agonist bronchodilators; COPD-related proinflammatory mediators and signaling pathways; targeting of mucus hyper secretion and pulmonary hypertension; inhibitors of the CXCR2 receptor; activation of certain transcription factors; and PDE4 inhibitors. Patients with acute exacerbations may benefit from the addition of inhaled corticosteroids, particularly those with elevated peripheral eosinophil levels [6,21,31,39-41]. However, when COPD is combined with comorbidity, such as chronic heart failure, it presents many therapeutic challenges to treatment, including beta-blockers (BBs) and beta-agonists, for patient care [21].

Bronchodilators are the first line treatment for COPD and asthma. They cause relaxation of the smooth muscle of the airways. At present, three major classes of bronchodilators, β (2)-adrenoceptor (AR) agonists, muscarinic receptor antagonists, and xanthine's are available and can be used individually or in combination. Inhalation therapies with long and short-acting bronchiolar dilators are important in pharmacological therapy. The short-acting drugs are used for acute symptoms. The long-acting drugs are used for maintenance therapy, in conjunction with an inhaled corticosteroid. In addition, some new potentially long-acting antimuscarinic agents, such as glycopyrronium bromide, aclidinium bromide, and umeclidinium bromide, are under development, as well as combinations of several classes of long-acting bronchodilator drugs that can be used in therapy [41].

Current therapeutic approaches fail to halt disease progression. A variety of potential therapeutic targets are currently being investigated, including COPD-related proinflammatory mediators and signaling pathways. Mucous secretion and pulmonary hypertension are also an important concern in asthma and COPD. The CXCR2 receptor, the myristoylated alanine-rich C kinase substrate, selectins, the mitogen-activated protein kinase/Src kinase endothelin receptor are important targets. The peroxisome proliferator-activated receptor agonist's rosiglitazone and pioglitazone were effective in the treatment of COPD. Some therapies had an effect only on a particular subgroup of patients [42].

Supplemental oxygen for patients with resting hypoxemia (defined as SpO_2<89%) improves survival [6]. Cessation of smoking, oxygen therapy, various inhalers, medications to improve vasodilation, antibiotics to combat the frequent lung infections, fluid replacement, exercise rehabilitation programs, and early detection and treatment of comorbidities are the options available to patients with COPD to reduce the burden of the disease [9,16,17,43].

Patients with COPD suffer from refractory breathlessness, unrecognized anxiety and depression, and decreased quality of life. Palliative care improves symptom management, patient reported health-related quality of life, cost savings, and mortality though the majority of patients with COPD die without access to palliative care. There are many barriers to providing palliative care to patients with COPD including the difficulty in prognosticating a patient's course causing referrals to occur late in a patient's disease. Additionally, physicians avoid conversations about advance care planning due to unique communication barriers present with patients with COPD. Lastly, many health systems are not set up to provide trained palliative care physicians to patients with chronic disease including COPD. Palliative care in COPD is an unmet area in desperate need of quality improvement [3,44].

Avoidance of cigarettes and oxygen therapy has proven effective in altering deleterious effects of COPD. Pharmacological therapies do not seem to alter the course of the disease. Lung transplantation has been shown to be effective as well. Strict selection criteria limit

transplant to highly compliant candidates with advanced disease but preserved functional status who are capable of successfully undergoing the operation [44,45]. Surgical therapies, including single- and double-lung transplantation and lung volume reduction surgery (LVRS), show some promise but have their flaws as well. In patients with COPD, there was no survival difference between single- and double-lung transplant recipients at five years [17,46,47]. LVRS has demonstrated improvements in patients with severe emphysema involving the upper lobe, compared to patients receiving medical therapy alone [48]. The basal metabolic index (BMI), obstruction, dyspnea, and exercise capacity (BODE) score are used to inform prognostic considerations for lung transplantation for COPD. Median survival in the fourth quartile of BODE score was 4.9 years (95% CI, 4.2 - 6.4 years) in the UNOS cohort and 3.1 years (95% CI, 2.4 - 3.5 years) in the BODE validation cohort [49,50]. A Cox regression model showed that the BODE score, age and diffusing capacity of the lungs for carbon monoxide were independently related to survival, as opposed to smoking status. Median survival for patients aged less than 65 in the fourth BODE quartile was 4.6 years [51].

Studies have found that patients with severe emphysema and more homogeneous patterns of airway destruction (not limited to just the upper lobes) have a high associated mortality rate with LVRS [45,48]. In oxygen-dependent COPD, patients with severe alpha-1 antitrypsin deficiency have a longer survival time on long term oxygen therapy, but they have a similar prognosis after lung transplantation compared with patients without severe alpha-1 antitrypsin deficiency [52,53]. Lung transplantation is an increasingly discussed therapeutic option, but its use remains limited primarily due to the widespread shortage of donor lungs and the stringent requirements for qualification for this therapy [45]. Thus, despite the positive aspects of these current and developing therapies, they remain only provisional and palliative treatments at best.

Current treatments for patients ease discomfort and help decrease disease progression; however, none improve lung function or change mortality. Thus, while the conventional therapies remain palliative at best, regenerative approaches for COPD management are still in the experimental stage [2,54]. Because the disease pathogenesis of COPD includes both chronic pulmonary and systemic inflammation, the anti-inflammatory immunomodulatory effects of systemically administered mesenchymal stem cells (MSCs) may decrease inflammation, resulting in improved lung function and quality of life. The results from this clinical study showed no infusion toxicities and no deaths or serious adverse events deemed related to MSC administration. There were no significant differences in the overall number of adverse events, in the frequency of COPD exacerbations, PFTs or quality-of-life indicators or worsening of COPD in patients treated with MSCs. However, an early significant decrease in levels of circulating C-reactive protein (CRP) was observed in patients treated with MSCs who had elevated CRP levels at study entry. Therefore, the systemic MSC administration appears to be safe in patients with moderate to severe COPD and provides a basis for subsequent cell therapy investigations [55].

Stem cells have been touted as a potential treatment option for chronic diseases [56,57]. Stem cells can differentiate into several different lung cell types such as the alveolar epithelial cells [5,58] that are destroyed by cigarette smoke leading to emphysematous changes and reduced tethering of small airways causing hyperinflation and gas exchange abnormalities [59]. Pre-clinical trials in animal models have suggested reduction of inflammatory responses, repair of emphysematous lungs, and regeneration of alveolar-like structures [5]. The greatest success has been in acute lung injury models. Currently, regenerative therapies are divided into extrinsic cell therapy methods and intrinsic therapeutic strategies [59]. Extrinsic cell therapy refers to infusing (or endotracheal installation) of stem cells including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), bone marrow mesenchymal stem cells, adipose-derived mesenchymal stem cells, Wharton's jelly mesenchymal stem cells, and human lung stem cells (hLSCs) [59-61]. Intrinsic therapy refers to the delivery of small molecules (retinoid compounds have been the most studied) that can stimulate the endogenous lung stem/progenitor cells to regenerate and replace damaged structures [59,62].

Studies that used MSCs in patients with moderate-to-severe COPD patients did not lead to clear respiratory functional improvements [61]. Use of retinoic acid and similar compounds in mouse models has demonstrated regeneration of pulmonary tissue, probably due to pulmonary/progenitor stem cell activation. However, retinoic acid or agonists of retinoic acid receptor administered to moderate or severe COPD patients did not improve the density and function of the damaged lung. These novel regenerative approaches have failed in preliminary clinical trials, possibly due to the advanced severity of the disease [61]. It is possible that stem cell activation may produce better results in acute rather than chronic disease [59]. Intrinsic therapeutic studies, based on the mobilization of endogenous stem cells or progenitor cells, have largely emphasized retinoic acid compounds. This included two phase II clinical trials. These trials failed to demonstrate improved lung function. More preclinical research is required to show if retinoid compounds can activate endogenous human stem progenitor cells [59].

Stem cell therapy offers a promise in a variety of pulmonary diseases. Published results of three clinical trials indicate that administering bone marrow mononuclear cells (BMMCs) or mesenchymal stem/stromal cells (MSCs) in the setting of degenerative lung disease is safe [63]. There were concerns that the therapeutic efficacy of autologous bone marrow mesenchymal stem cells (BM-MSCs) and the adipose-derived mesenchymal stem cells (AD-MSCs) in older patients may be compromised by several age-related factors including oxidative stress, DNA damage, and pharmacological agents. Telomere length may also be an important consideration. MSCs from Wharton's jelly may have better anti-inflammatory and immunomodulatory actions [60], due to the younger age from which the stem cells were obtained.

Studies were performed to ascertain the utility of using allogeneic adipose-derived stroma stem cells (ASCs) for the treatment of COPD. Sixty-nine studies were performed in clinical models of disease. The clinical use of ASCs in severe inflammatory bowel disease produced amelioration of symptoms, and led to healing of fistulas and lower rates of recurrence. ASCs and stromal vascular fraction (SVF) cells have been shown to be effective in a wide variety of cardiovascular and pulmonary diseases, as well as in multiple sclerosis and chronic skin wounds. These effects were due to reduction of inflammation and increased tissue repair. In most cases the autologous transplant cells were derived from liposuction. Very few serious, treatment-related adverse events were reported. The main adverse event was postprocedural pain. It was noted that allogeneic adipose-derived stromal/stem cells could be given to human subjects safely [64,65]. The safety of MSC treatment has been demonstrated in the treatment of COPD, but not its efficacy [63,66,67]. These novel regenerative approaches have failed in preliminary clinical trials, possibly due to the advanced severity of the disease; source of stem cells (bone marrow, adipose tissue), route, dosage, frequency of administration, and delivery (lack of a bioactive scaffold) [61,68]. Therefore, it was proposed that the MSCs would fare better if given during the early rather than late stages of the disease [69].

Although mesenchymal stem cells and stromal vascular fraction stem cells likely represent a heterogeneous population of cells, the different cell subsets and their importance in the pathogenesis of the different clinical phenotypes requires further study. The side effects of stem cell therapy need to be determined. We should not ignore that some of the most-deadly neoplasms are arising from the implantation of naïve embryonic stem cells and induced pluripotent stem cells to treat various disease modalities [58,70].

New therapeutic strategies continue to be developed. Presently, much of the promise in treating chronic and incurable diseases of any organ system is centered on stem cell biology and the "holy grail" of regenerative medicine [56,57]. Despite the optimism and explosion of interest surrounding this new field, knowledge of the scientific basis for stem therapy for COPD is still at the experimental phase. Problems include bioethical issues, safety of cell transplantation, routes of delivery, dose, timing of administration, and outcome versus placebo control [71]. And while there have been promising results from early phase I safety trials, there remain multiple reasons that "stem cells" are not ready for clinical application, starting from a gap in understanding at the bench research level, all the way to optimal clinical application in order to provide effective therapy [72]. This has been proved especially daunting when mesenchymal stem cells have been utilized as a potential treatment modality [59-61,63,66-70, 73-77].

We propose the use of an additional set of endogenous adult stem cells for repairing damaged lung tissues following COPD, i.e., autologous and/or allogeneic connective tissue-resident telomerase-positive stem cells. Our characterization studies [78] identified three populations of endogenous telomerase-positive stem cells with the capabilities of forming various tissues of the lung, e.g., structures of the respiratory tree (TSCs and PSCs), type-I and type-II pneumocytes (TSCs and PSCs), connective tissue stroma (TSCs, PSCs, and MesoSCs), and vasculature (TSCs, PSCs, and MesoSCs) [5].

The first population of healing cells, i.e., totipotent stem cells (TSCs), were isolated and characterized from adult mammalian skeletal muscle and blood. These diploid stem cells were cloned from a single cell. The size of this novel cell population was ultra-small (0.1 to 2 μm). Carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) was expressed on the surface of non-human TSCs and CEA-CAM-1 and CD66e on the surface of human TSCs. The TSCs expressed telomerase-enzyme in the undifferentiated state. Three derived stem cell clones, each individually revealed phenotypic expression markers for 66 discrete types of ectodermal, mesodermal, and endodermal origin, spermatogonia, and notochord, when exposed to general and specific induction agents and exosomes derived from differentiated cells and tissues. Phenotypic differentiation analyses were performed using an antibody-micro array-enzyme-linked immuno-culture assay [78,79]. The clones maintained their differentiation capabilities following more than 300 population doublings. Studies have shown that these stem cells may be useful in the treatment of neurodegenerative disease, including Parkinson's disease, myocardial infarction (MI), autoimmune diseases, orthopedic repair, and type-I diabetes mellitus [56,78,80-87].

The second population of healing cells, i.e., pluripotent stem cells (PSCs), were isolated and characterized from adult mammalian skeletal muscle and blood [78]. These diploid healing cells were intermediate in size (>2 to <10 μm) and expressed stage-specific embryonic antigen-4 (SSEA-4) on the surface of non-human cells and SSEA-4 and CD10 on the surface of human cells. The PSCs expressed telomerase-enzyme in the undifferentiated state. A clone derived from a single cell revealed the presence of phenotypic expression markers for 60 discrete cell types of ectodermal, mesodermal, and endodermal origin, but not germ cells or notochord, when exposed to general and specific induction agents and exosomes derived from differentiated cells and tissues. Phenotypic differentiation analyses were performed using an antibody-micro array-enzyme-linked immuno-culture assay [78,79]. The clone maintained its differentiation capabilities in over 400 population doublings. Studies have shown that these cells may be useful in the treatment of neurodegenerative disease, including Parkinson's disease, myocardial infarction (MI), autoimmune diseases, orthopedic repair, and type-I diabetes mellitus (Table 1) [56,78,80-86,88].

The third population of healing cells, i.e., mesodermal stem cells (MesoSCs), were isolated and characterized from adult mammalian skeletal muscle and blood [78]. These diploid healing cells were larger in size (10-12 μm) and expressed Thy-1 on the surface of non-human cells and CD13, CD90, and MHC Class-1 on the surface of human MesoSCs. The MesoSCs expressed the telomerase-enzyme in the undifferentiated state. A clone derived from a single cell revealed phenotypic expression markers for

37 discrete cell types of mesodermal origin, when exposed to general and specific induction agents and exosomes derived from differentiated cells and tissues. Phenotypic differentiation analyses were performed using an antibody-micro array-enzyme-linked immuno-culture assay [69,78]. The clone maintained its differentiation capabilities following more than 690 population doublings. Studies have shown that these cells may be useful in the treatment of myocardial infarction (MI), autoimmune diseases, and orthopedic repair (Table 1) [56,78,82-84,89].

GOLD Staging System

Severity	FEV_1 % of Predicted Value
GOLD-1 (Mild)	≥ 80
GOLD-2 (Moderate)	50 – 79
GOLD-3 (Severe)	30 – 49
GOLD-4 (Very Severe)	<30 or chronic respiratory failure

Table 1: Legend. Gold Staging System for lung function based on the greatest volume of air that can be breathed out in the first second of a breath, i.e., the forced expiratory volume in one second (FEV_1) [9].

Materials and Methods

Telomerase-positive adult-derived stem cells were tested as a treatment modality in an IRB-approved study protocol for individuals with COPD. Participating individuals had been previously diagnosed with COPD by either their primary care physicians and/or pulmonologists. We utilized the individual's FEV_1's (defined as the forced volume of air that can be exhaled from the lungs in one second) as a measure of lung function. We chose to study individuals with very severe COPD (GOLD-4) and severe COPD (GOLD-3) (Table 1) rather than mild (GOLD-1) or moderate (GOLD-2) COPD that could be modulated with pharmacological agents. We hypothesized that if telomerase-positive stem cells could contribute to either stasis or reversal of symptoms, then it would be readily apparent in individuals with severe or very severe COPD. Inclusion criteria was any male or female; age range between 18 and 120; diagnosis of COPD; with a FEV_1 of less than 49% (GOLD-3 or GOLD-4); and on supplemental oxygen. Third party investigators, primary care physicians, and/or pulmonologists, provided the respective FEV_1's for the participants in the study pre- and post-treatment. A total of 51 individuals with Gold-4 or Glod-3 COPD have been treated thus far, i.e., patient (1) in (Figure 1), individuals (3) with no response to treatment, and individuals (47) that dropped out of the study for various reasons.

Our study protocol consisted of ingestion of the combinatorial nutraceuticals (CN) (DFRD, Macon, GA) daily for a minimum of 30 days prior to initial harvest and then throughout subsequent treatments. This was done to increase proliferation of telomerase-positive stem cells within the person's own connective tissues throughout their body. The participants (recipients and donors) were required to follow the informed consent compliance guidelines for telomerase-positive stem cell therapy to maximize the number of telomerase-positive stem cells for harvest and subsequent repair of their tissues. These guidelines included the following. Abstain from alcohol, tobacco products, vaping, recreational drugs, lidocaine, and chemotherapeutic agents because these agents kill telomerase-positive stem cells. Limit use of corticosteroids and caffeine because these agents alter the differentiative capabilities of telomerase-positive stem cells. Refrain from moderate to excessive physical activity for two weeks before to two weeks after stem cell treatment to maximize site-directed repair of tissue.

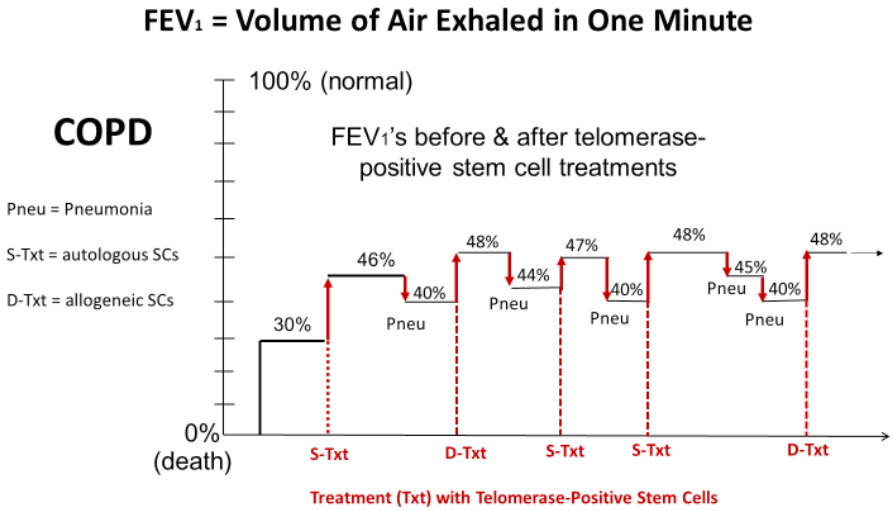

Figure 1: COPD participant, with a baseline FEV_1 of 30% (GOLD-3), treated with multiple autologous and allogeneic telomerase-positive stem cell transplants over an eight-year time frame. Within one month following their initial autologous stem cell treatment (TSCs and PSCs nebulized, followed by MesoSCs by regular intravenous infusion into median cubital vein), their FEV_1 increased to 46%, approximating a 50% increase in lung capacity. During the ensuing eight-year time frame their FEV_1's fluctuated from 40% to 48%, due to pneumonia followed by stem cell transplant, followed by pneumonia, followed by stem cell transplant, and so on and so forth. After their initial stem cell transplant the individual was able to reduce supplemental oxygen from 4-L per minute to 2-L per minute for the ensuring eight years and still maintain a greater than 98% oxygen saturation of their blood. The individual succumbed to a severe case of pneumonia eight years after initial telomerase-positive stem cell treatment.

Ingest glacial caps (GC) (DFRD) 18 hours before stem cell harvest to mobilize connective tissue-resident telomerase-positive stem cells into the blood stream. Donors were matched to recipients based on gender, ABO-blood group (or O-negative blood group), absence of infectious diseases, and absent of genes for any deleterious genetic mutations. MesoSCs express MHC Class-1 self-recognition molecules on their cell surface, and there is the chance that donor MesoSCs might induce a graft versus host response in the recipient, i.e., either the recipient rejects the donor cells or the donor cells reject the recipient [90,91]. Therefore, donor MesoSCs were not utilized for transplanting into the recipient. Donors were given the option to have their activated MesoSCs returned to them by systemic intravenous (IV) infusion [10,83,84].

The harvesting of the telomerase-positive stem cells occurred using venipuncture, removing 210-420cc's of blood by venipuncture. The telomerase-positive stem cells were separated from the blood cells using 'FDA-mandated minimal manipulative procedures', segregated into individual populations of TSCs, PSCs, and MesoSCs, and activated. Autologous and/or allogeneic TSCs and PSCs were pooled in 2-3-ml of 0.9% sterile saline for nebulization and the autologous MesoSCs diluted in 250-ml 0.9% sterile heparin/saline for IV infusion into an accessible vein, usually the median cubital vein [5,10,83,84].

Results

Multiple individuals (n=51), both male and female, age range from 40 to 90, with severe COPD and on supplemental oxygen were treated in this study. The telomerase-positive stem cells, administered by nebulization and IV infusion, were well-tolerated and no adverse side effects were noted. Most individuals treated with autologous endogenous adult-derived telomerase-positive stem cells saw a modest increase in their FEV_1 in the range of 5-10% above their baseline value for each treatment received. Three individuals, stabilized at their baseline value, but saw no subsequent increase in FEV_1 after stem cell nebulization / infusion. However, the best response to a single dose of autologous telomerase-positive stem cells engendered a 16% increase in their FEV_1 from 30% to 46%, thereby increasing their lung capacity by slightly over 50% (Figure 1). They maintained a range of FEV_1 from 40-48% during the ensuing eight years. Unfortunately, as with anyone with severe lung disease they were prone to multiple respiratory infections, with each one eliciting a decline of their FEV_1, but never below 40%. A subsequent treatment, with either autologous and/or allogeneic stem cells, would reverse the decline of their FEV_1 and raising it to at or near 48%. Throughout the eight-year fluctuations in their FEV_1 (40-48%), the drop in FEV_1 was due to sickness followed by treatment, and so on and so forth. They received a total of 16 autologous and/or allogeneic telomerase-positive stem cell treatments during this time period, 15 of which occurred after severe respiratory infections (pneumonia). They succumbed to a severe bout of pneumonia eight years after their initial telomerase-positive stem cell treatment.

Discussion

The inflammatory effects of cigarette smoking as a major cause of COPD. Further education of the public could help ameliorate the incidence of COPD. Current drug treatments improve symptoms but do not alter the underlying progression of this disease. Despite their anti-inflammatory effects, use of glucocorticosteroids has not led to a major improvement of COPD, thus requiring new approaches. New pharmacological approaches include second generation PDE4 inhibitors (cilomilast and roflumilast); once- or twice-daily maintenance long-acting anticholinergic and β-agonist bronchodilators; three major classes of bronchodilators: β(2)-adrenoceptor (AR) agonists, muscarinic receptor blockers, and xanthines. Pharmacological agents that affect signaling pathways that promote COPD such as CXCR2, mitogen-activated protein kinase/Src kinase, selectins, myristoylated alanine-rich C kinase substrate, and the endothelin receptor. In addition, improvements in the efficacy of pharmacological glucocorticoid inhalants and the development of long-activating muscarinic receptor blockers, such as glycopyrronium bromide, aclidinium bromide, and umeclidinium bromide, and long-acting bronchodilator drugs can improve the clinical situation. However, compliance of the patients is questioned because of adverse side effects of these agents [6,21,31,35-37,39-42].

Supplemental oxygen for patients with resting hypoxemia (defined as SpO_2<89%) improves survival [6]. Cessation of smoking, oxygen therapy, various inhalers, medications to improve vasodilation, antibiotics to combat the frequent lung infections, fluid replacement, exercise rehabilitation programs, and early detection and treatment of comorbidities are the options available to patients with COPD to reduce the burden of the disease [11,14,16,43].

Except for smoking cessation and oxygen therapy, medical therapy does not improve the progression of COPD. In contrast, when performed in carefully selected candidates, single or double lung transplantation can provide substantial benefits in physiology, function, quality of life, and survival. Strict selection criteria limit transplants to highly compliant candidates with advanced disease but preserved functional status who are capable of successfully undergoing the operation [44,45]. Pulmonary transplantation, although effective, has severe limitations due to the lack of an adequate supply of donor lungs [48]. Unfortunately, the median survival time after lung transplant is 4.5 years. Thus, despite the positive aspects of these current and developing therapies, they remain only provisional and palliative treatments at best.

Currently, regenerative therapies are divided into extrinsic cell therapy methods and intrinsic therapeutic strategies [59]. Extrinsic cell therapy refers to infusing (or endotracheal installation) of stem cells including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), bone marrow mesenchymal stem cells, adipose-derived mesenchymal stem cells, Wharton's jelly mesenchymal stem cells, and human lung stem cells (hLSCs) [59-61]. Intrinsic therapy involves administration of

small molecules such as retinoic acid that stimulate endogenous pulmonary stem/progenitor cells to repair or replace damaged pulmonary tissues [59,62].

Since the disease pathogenesis of COPD includes both chronic pulmonary and systemic inflammation, it has been proposed that the anti-inflammatory effects of systemically administered mesenchymal stem cells (MSCs) may decrease inflammation in order to ameliorate the effects of COPD [55]. Systemic infusion of MSCs is safe in patients with COPD, but it needs further investigation. Unfortunately, further clinical studies that used MSCs in patients with moderate-to-severe COPD patients did not lead to clear respiratory functional improvements [61].

Murine models have shown that small molecules such as agonists targeted on the retinoic acid receptor can ameliorate the effects of COPD. However, these results have not been replicated in human patients in clinical trials [61].

In a preclinical animal model of induced lung fibrosis, telomerase-positive stem cells were shown to regenerate bronchioles, alveolar ducts and alveolar sacs [5] and differentiate into lung associated cell types in vitro [56,58]. These pre-clinical studies suggested the potential for telomerase-positive stem cells to increase lung function in individuals with chronic pulmonary diseases. In a phase-I safety study, individuals with COPD and IPF were treated with autologous telomerase-positive stem cells. Both individuals showed an increase in their FEV_1's after stem cell treatment that was significantly above their respective baseline FEV_1 values before treatment [5]. The current paper is an eight-year follow-up study of those individuals, plus other individuals with COPD that were enrolled in the original study [5,10].

Post-transplant interviews, with the three individuals that showed stasis, but no increase in their FEV_1's following a telomerase-positive stem cell transplant, noted that they did not follow informed consent compliance guidelines, which stated in their informed consent document "no moderate to excessive physical activity within two weeks before to two weeks after treatment". One individual, who was an avid jogger until being diagnosed with severe COPD 10 years previously felt so great after his stem cell treatment that he jogged ten miles the same day after receiving his stem cell transplant. He could not understand why the stem cell transplant 'did not work', i.e., why his FEV_1 did not increase above his pre-treatment baseline value. Another individual, an avid golfer before his severe COPD diagnosis, returned home and in the same week of his transplant felt so great that he played three 18-hole rounds of golf back-to-back-to-back on the same day. He also could not understand why he did not see an increase in his FEV_1 above his pre-treatment baseline value. The last individual returned home, felt great, and spent two days cleaning out an aviary of bird feces (without a mask), which she had not done since her COPD diagnosis. She also could not understand why her stem cell transplant 'did not work'. We attributed their responses to the "Superman Syndrome", the feeling after infusion of activated telomerase-positive stem cells of being able to 'leap tall buildings at a single bound', 'out-run a speeding bullet', 'a fog being lifted', 'mental acuity', 'acute visual clarity', etc. We heard of this "Superman Syndrome" on multiple occasions from participants in our clinical studies (both recipients and donors receiving their activated telomerase-positive stem cells) being treated for neurogenic, cardiovascular, pulmonary, orthopedic, and autoimmune issues [5,10,80-84]. The "Superman Syndrome" can last up to two weeks post-transplant in a majority, depending on the individual, of their disease(s) being treated and their respective comorbidities.

While we initially thought it was a placebo effect of knowing one is going to receive activated stem cells that did not appear to be the case. Several of our Alzheimer's and Dementia patients had no clue they were going to be receiving stem cells of any kind. Before treatment they did not know the day, month or year; who they were with; where they were; why they were there; who the current sitting President of the United States was; and other current events. The day after treatment and for the next month or two they knew the day, month, and year; could identify the spouses or children they were with; the clinic they were being treated in; why they were at the clinic; who the current sitting President of the United States was; and other current events. The results with these patients suggested that the metal acuity portion of the Superman syndrome was not just a placebo event, but an actual unexpected phenomenon of treatment.

One of our informed consent protocol guidelines is 'not to indulge in moderate to excessive physical activity for at least two weeks before to two weeks after a stem cell transplant'. This is because the body will direct activated telomerase-positive stem cells to the most recent tissue damage, if the disease, trauma, etc. we are trying to treat is NOT life threatening. If a condition is life threatening, the activated telomerase-positive stem cells will repair that area first above all others [82-84]. We would hypothesize that in the first and second instances (jogger and golfer) the activated telomerase-positive stem cells were directed to repair damage to the muscles, tendons, and repair tissue in the lungs due to their excessive physical exertions, and restoring their FEV_1's to their pre-treatment baseline FEV_1 lung function values. In the third instance (aviary feces cleaner) we would hypothesize that the excessive damage to their lungs from breathing unfiltered bird feces overwhelmed the regenerative capabilities of their telomerase-positive stem cells to a point that it just returned their FEV_1 to their pre-treatment baseline value.

The majority of the individuals in the COPD study that dropped out before termination had modest increases in their FEV_1's of 5-10% above their initial baseline values. The self-removal from the study occurred for various reasons, i.e., they were happy with their results and did not want to return for follow-up (n=12), travel to and from and room/board on site was too expensive (n=4), they wanted to get on with their lives (n=7), they did not want to follow on-going informed consent compliance guidelines for follow-up assessments (n=12), they became too sick to travel for treatment (n=3), they passed away from acute respiratory illnesses (n=3),

they passed away from other illnesses (n=5), or they passed away in a fatal car wreck (n=1).

In contrast, our 'best' COPD participant demonstrated a 16% increase in their FEV_1 after their first autologous telomerase-positive stem cells treatment. Their baseline FEV_1 before treatment was 30% (GOLD-3) and their FEV_1 increased to 46% after their initial treatment, approximating a 50% increase in their lung capacity [5]. In addition, they were able to reduce their supplemental oxygen from 4-L per minute to 2-L per minute and still maintain a greater than 98% oxygen saturation of their blood.

During an eight-year time frame [this study] they were treated with 16 autologous and/or allogeneic telomerase-positive stem cell transplants, during which their FEV_1's fluctuated from 40% to 48% (Figure 1). This fluctuation in FEV_1's was due to respiratory infections (pneumonia) lowering their FEV_1's followed by stem cell transplants, raising their FEV_1's. Unfortunately, as with any individual with compromised lung function, they were susceptible to continued lung infections. The individual succumbed to a very severe case of pneumonia eight years after initial treatment.

It was noted that the eight-year results from this phase I study demonstrated three sets of possibilities, i.e., pre-treatment baseline values, resulting in no overt increase in function (probably by not following informed consent protocol guidelines), a moderate 5-10% increase in FEV_1's above baseline, and a best response of 16% FEV_1 above pre-treatment baseline values. All autologous and allogeneic activated stem cell treatments that were given demonstrated no adverse side effects to any of the participants (recipients or donors). These results suggested both the safety and efficacy of using endogenous telomerase-positive stem cells for the treatment of COPD.

To further our understanding of the ability of autologous and/or allogeneic telomerase-positive stem cells to treat chronic obstructive pulmonary disease, we propose a Phase II randomized double-blinded placebo-controlled studies, using a dedicated population diagnosed with very severe (GOLD-4, less than 30%) and severe (GOLD-3, less than 49%) chronic obstructive pulmonary disease with telomerase-positive stem cells and other stem cells postulated to restore lung function in individuals with severe and very severe COPD.

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