Clinical Research Protocol

A PHASE I, SINGLE CENTER, OPEN LABEL, SINGLE DOSE, DOSE ESCALATION STUDY ASSESSING THE SAFETY AND TOLERABILITY OF ALLOGENEIC MESENCHYMAL STEM CELL INFUSION IN ADULTS WITH CYSTIC FIBROSIS-CEASE CF

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Approval:

Erica A. Roesch, M.D. *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Cystic Fibrosis Foundation with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: CF-MSC-01

Protocol Title: A Phase I, Single Center, Open Label, Single Dose, Dose Escalation Study Assessing the Safety and Tolerability of Allogenei**C** M**E**senchym**A**l **S**tem C**E**ll Infusion in Adults with **C**ystic **F**ibrosis-CEASE CF

Protocol Version: Version 1.13

Protocol Date: December 17, 2019

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Investigator Signature *Date*

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Print Name and Title

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LIST OF ABBREVIATIONS

ADL	Activities of daily living
AE	Adverse experience
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAL	Bronchoalveolar lavage
BM	Bone marrow
BMM	Bone marrow macrophages
BPD	Bronchopulmonary dysplasia
BUN	Blood urea nitrogen
CBC	Complete blood count
CCC	Comprehensive Cancer Center
CF	Cystic fibrosis
CFR	Code of Federal Regulations
CFRSD	Cystic Fibrosis Respiratory Symptom Diary
CFTR	Cystic fibrosis transmembrane conductance regulator
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CRP	C-reactive protein
CTCAE	Common terminology criteria for adverse events
CWRU	Case Western Reserve University
DCRU	Dahms Clinical Research Unit
DHQ	Donor History Questionnaire
DLT	Dose limiting toxicity
DMC	Data Monitoring Committee
DMSO	Dimethylsulfoxide
DSMB	Data Safety Monitoring Board
ESR	Erythrocyte sedimentation rate
FBS	Fetal bovine serum
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	Forced expiratory flow
FEV_1	Forced expiratory volume over one second
FVC	Forced vital capacity
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GM-CSF	Granulocyte-macrophage colony-stimulating factor

GVHD	Graft versus host disease
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human immunodeficiency virus
hMSC	Human mesenchymal stem cell
hs-CRP	High sensitivity C-reactive protein
ICF	Informed consent form
ICH	International Conference on Harmonization
IL	Interleukin
IRB	Institutional Review Board
IV	Intravenous
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
mEq	Milliequivalent
MHC	Major histocompatability complex
MIP-3α	Macrophage inflammatory protein-3 alpha
MMP-9	Matrix metalloproteinase-9
MPO	Myeloperoxidase
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSC	Mesenchymal stem cell
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NCRM	National Center for Regenerative Medicine
PBSC	Peripheral blood stem cell
PI	Principal Investigator
QPIT	Quantitative pilocarpine iontophoresis test
RC	Research Coordinator
RSSQ	Respiratory and Systemic Symptoms Questionnaire
SAE	Serious adverse experience
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamate pyruvate transaminase
SOP	Standard operating procedure
TDNCC	Therapeutics Development Network Coordinating Center
TGF-β	Transforming growth factor-beta
TNF-α	Tumor necrosis factor-alpha

PROTOCOL SYNOPSIS

TITLE	A Phase I, Single Center, Open Label, Single Dose, Dose Escalation Study Assessing the Safety and Tolerability of Allogenei**C** M**E**senchym**A**l **S**tem C**E**ll Infusion in Adults with **C**ystic **F**ibrosis-CEASE CF
SPONSOR-INVESTIGATOR	Erica A. Roesch, M.D.
NUMBER OF SITES	1
RATIONALE	Cystic fibrosis (CF) is a genetically inherited disease which impacts sodium and chloride transport across the epithelium leading to viscous mucus retention, chronic bacterial infection and relentless inflammation in the lung; the main cause of morbidity and mortality in this disease. Although new therapeutics have been promising, new and innovative approaches to controlling inflammation and bacterial burden in the lungs of these patients is important towards minimizing the consequences of the disease and improving survival. We have published pre-clinical studies demonstrating that human allogeneic bone marrow derived human mesenchymal stem cells (hMSCs) may provide a new therapeutic treatment for CF lung disease, attenuating pulmonary inflammation while decreasing bacterial growth and enhancing antibiotic efficiency.
STUDY DESIGN	This will be a prospective, single-center, dose-escalation, open-label interventional study to evaluate the safety and tolerability of allogeneic hMSCs in 15 clinically stable subjects with CF age $\geq$ 18 years. After a two to six week screening period, subjects will have a Baseline visit (Days 1-2) where they will undergo a single intravenous infusion of up to 5 x 10^6 allogeneic hMSCs/kg of body weight. Infusions will be performed in the Dahms Clinical Research Unit (DCRU) of University Hospitals Cleveland Medical Center. Subjects will be monitored for any infusion related toxicities for 24 hours after the infusion. Subsequent study visits will occur on Days 7, 14, 28, Months 3 and 6 and telephone calls will occur on Days 4 (or 5), 21, 56 and Month 12. Subject safety and tolerability of a single dose of hMSCs will be evaluated at study visits by review of subject diaries, interval history, pulmonary exacerbations (standardized definition in Appendix I), physical examination, spirometry, and analysis of safety laboratories. Special attention will be placed upon detecting pulmonary exacerbations because anti-inflammatory therapies theoretically could suppress the immune system to the point where it leads to increased infectious complications, although MSC therapeutics are proposed to be antimicrobial. In addition to evaluating safety, this study will also explore efficacy end-points for future clinical trials of MSCs in CF including inflammatory biomarkers from blood and sputum. Serum markers (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL8, IL-17, and TNF-α) and sputum markers (white cell counts and differentials, IL-1β,

	IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, and active proteases including neutrophil elastase, α_1-anti-trypsin, and MMP-9) will be determined at Baseline and on Days 7 and 28 for with-in subject comparison. All subject samples will be archived for future projects. Finally, a diagnostic bone marrow exam will be performed on subjects with CF who consent to undergo the procedure. Bone marrow samples will be banked and used for future translational studies. Study-related procedures are outlined in Appendix II.
PRIMARY OBJECTIVE	To evaluate the infusion-related safety and tolerability of a single allogeneic hMSC infusion in adults with CF.
SECONDARY OBJECTIVES	• To explore preliminary evidence for the potential efficacy of MSCs as a new therapeutic to treat CF pulmonary infection and inflammation as measured by o Change in sputum inflammatory markers o Change in serum inflammatory markers • To compare allogeneic MSCs to autologous MSCs in a preclinical model. The potential efficacy of MSCs obtained from CF subjects who agree to undergo the optional bone marrow aspiration will be evaluated in validated animal models.
NUMBER OF SUBJECTS	15 subjects with cystic fibrosis for main study 1-2 healthy volunteer subjects for bone marrow donation only Throughout protocol, "subject" refers to subject with cystic fibrosis unless otherwise specified.
SUBJECT SELECTION CRITERIA	<u>Inclusion Criteria:</u> 1. Male or female 18 years of age and above 2. Confirmed diagnosis of CF as evidenced by 1 or more clinical features consistent with the CF phenotype and 1 or more of the following criteria: a. Sweat chloride equal to or greater than 60 mEq/L by quantitative pilocarpine iontophoresis test (QPIT) b. 2 well-characterized, disease causing mutations in the CFTR gene 3. Clinically stable with no significant changes in health status within 2 weeks prior to screening 4. $FEV_1 \geq 40\%$ predicted for age based on the global lung function initiative equations at the screening visit 5. Weight ≥ 40 kg at the screening visit 6. Able to perform repeatable, consistent efforts in pulmonary function testing 7. Written informed consent obtained from the subject

	Exclusion Criteria: 1. Use of an investigational agent within the 4-week period prior to Visit 1 (Day -42 to -10) 2. Chronic daily (>10 mg) or alternate daily (>20 mg on alternate days) use of systemic corticosteroids within the 4 weeks prior to Visit 1 (Day -42 to -10) or initiation of any dosage of systemic corticosteroids within 72 hours prior to Visit 2 (Day 1) 3. Use of hydroxychloroquine or immunosuppressants 4. Initiation of a new antibiotic (oral, IV, and/or inhaled) that is not part of the subject's maintenance regimen for treatment of acute respiratory symptoms within 2 weeks prior to screening through Visit 2 (Day 1) 5. Initiation of any new chronic therapy (e.g., Pulmozyme®, hypertonic saline, Kalydeco®, Orkambi®, high-dose ibuprofen, azithromycin, TOBI®, Cayston®, nebulized colistin, bronchodilators, inhaled corticosteroids, etc.) within 4 weeks prior to screening 6. Active treatment for non-tuberculous *Mycobacteria* 7. History of a sputum culture positive for a *Burkholderia cepacia* complex organism in the previous 12 months 8. Current tobacco smoker 9. Oxygen saturation < 92% on room air at Visit 1 (Day -42 to -10) 10. History of pulmonary hypertension 11. SGOT (ALT) or SGPT (AST) > 2.5 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension 12. Total bilirubin concentration > 1.2 mg/dL at screening 13. Creatinine > 1.8 mg/dL at screening 14. Pregnant, breastfeeding, or unwilling to practice birth control between Visit 2 (Day 1) and Telephone Call 3 (Day 56) (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal 15. Screening hematology with white blood cell count < 4.5 x 10^9 cells/L, hematocrit < 30%, and platelets < 150 x 10^9 platelets/L 16. History of invasive cancer requiring systemic therapy 17. History of organ transplantation 18. Currently listed for lung transplantation or having potential to be listed for lung transplantation in the succeeding 12 calendar months from screening 19. Subject unlikely to complete the study as determined by the Investigator
TEST PRODUCT,	Single Infusion of up to 5 x 10^6 allogeneic MSCs/kg of body weight. A

DOSE AND MODE OF ADMINISTRATION	dose escalation using the "3+3" design[1] will be employed. The three doses are 1 x 10^6, 3 x 10^6, and 5 x 10^6 hMSCs/kg. Allogeneic MSCs will be derived from bone marrow aspirates from a healthy donor whose serum tests negative for cytomegalovirus (CMV) antibodies. Healthy donors will undergo tests for infectious disease and screening for 41 common CFTR mutations. In addition, the MSCs will be validated for *in vitro* and *in vivo* efficacy and potency using the *in vivo* murine pre-clinical model of CF lung infection and inflammation.
CONTROL PRODUCT, DOSE AND MODE OF ADMINISTRATION	*NA*
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be studied for approximately 13 months **Screening:** between 10 and 42 days **Treatment:** 1 day **Follow-up:** 12 months We anticipate 15 months to recruit subjects (due to the 3+3 safety study design). The last subject in each cohort must finish Visit 5 before enrolling the first subject in the next cohort.
CONCOMITANT MEDICATIONS	For those on alternate month TOBI® or Cayston® therapy, on/off cycles must be timed such that a subject is at the same phase of a cycle at Visits 2-5. Allowed: All routine chronic CF medications. • Prohibited: Anti-inflammatory medications (hydroxychloroquine, corticosteroids >10mg daily or >20 mg on alternate days) and immunosuppressants between Visits 1 and 5. Changes in chronic treatment of CF (e.g., introduction, dose escalation, or elimination of chronic therapies) between Visits 1 and 5.
SAFETY EVALUATIONS	Dose-limiting toxicities, adverse events, including pulmonary exacerbations; Cystic Fibrosis Respiratory Symptom Diary (CFRSD); Respiratory and Systemic Symptoms Questionnaire (RSSQ); physical examination; assessment of vital signs, including oxygen saturation; spirometry, sputum bacterial culture (quantitative and qualitative); hematology, comprehensive chemistry, erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (hs-CRP), and urinalysis
PRIMARY ENDPOINT	To evaluate safety and tolerability of a single infusion of allogeneic hMSCs as determined by: • Dose limiting toxicity (DLT): A DLT is triggered by occurrence in the first 24 hours after hMSC infusion of grade ≥3 infusion-related allergic toxicities, which include rash, flushing, urticaria, dyspnea, fever >40°C (>104.0°F) as scored according to the National Cancer Institute (NCI) Common Terminology Criteria

	for Adverse Events (CTCAE) version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). • Incidence and severity of adverse events • Number of pulmonary exacerbations (modified Fuch's criteria[95]) • Diary reports (Cystic Fibrosis Respiratory Symptom Diary (CFRSD)) • Changes in subject reported symptoms as captured by the Respiratory and Systemic Symptoms Questionnaire (RSSQ) from Baseline (Visit 2) Day 1 to Baseline (Visit 2) Day 2, Visits 3, 4, 5, 6 and 7 and at times when a pulmonary exacerbation is being considered • Changes in physical examination • Changes in vital signs including oxygen saturation checked throughout infusion • Changes in spirometry ($FEV_{1.}$ %, $FEV_{1.}$ (Liters), $FEF_{25-75.}$) determined 30 minutes, 4 hours, and 24 hours after completion of infusion and from Baseline (Visit 2) Day 1 to Visits 3, 4, 5, 6 and 7 • Change in sputum quantitative microbiology (bacterial colony forming units between Baseline to Day 7 and Day 28) • Changes in hematology, comprehensive chemistry, ESR, hs-CRP, and urinalysis results
SECONDARY ENDPOINTS	• Change in blood inflammatory biomarkers from Baseline to Day 7 and Day 28 • Change in sputum inflammatory biomarkers from Baseline to Day 7 and Day 28
OTHER EVALUATIONS	Bone marrow aspiration is optional. CF bone marrow aspirates will be used to assess autologous approaches to MSC therapeutics. Any sputum or serum remaining after the study will be archived for future translational projects.
SAFETY MONITORING	The Therapeutics Development Network Coordinating Center (TDNCC) Medical Monitoring Group will serve as the medical monitor for this clinical trial. In addition, the Cystic Fibrosis Foundation Data Safety Monitoring Board (DSMB) will establish a Data Monitoring Committee (DMC) to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study, according to the Cystic Fibrosis Foundation Data Safety Monitoring Board Operations Manual and a DMC Charter established for this protocol. There will be 3 interim review(s) conducted by the DMC for the purpose of monitoring study conduct and assessing subject safety.

RATIONALE FOR NUMBER OF SUBJECTS	This is a single center Phase I study. Enrollment of 15 subjects will be feasible and allow us to ascertain if there are any clinically meaningful infusion-related adverse effects.

1 BACKGROUND

1.1 Introduction

Cystic fibrosis (CF) lung disease begins at birth and continues throughout life[2]. While the pathogenesis of CF lung disease is complex, excessive inflammation is a key component of disease pathology[2]. The vicious cycle of airway obstruction, chronic bacterial infection, and excessive inflammation eventually leads to irreversible lung damage[3]. It is possible that gene modifying agents will eliminate the downstream pathophysiologic consequences of defective cystic fibrosis transmembrane conductance regulator (CFTR). However, it is more likely that gene modifying agents will reduce the pathophysiologic consequences in the airway, particularly the exaggerated inflammatory response and decrease the frequency of pulmonary exacerbations, but not eliminate them completely. Therefore, future treatment regimens for CF lung disease will likely employ the administration of one or more CFTR disease modifying drugs in combination with an anti-inflammatory drug. Unfortunately, previously evaluated anti-inflammatory therapeutics, such as systemic corticosteroids and BIIL 284 BS, have been associated with side effects or have not shown to be efficacious, and dosing and monitoring of high-dose ibuprofen has precluded its widespread use[4,5].

Mesenchymal stem cell (MSC) based therapies have generated much interest due to their anti-inflammatory, anti-microbial, immunomodulatory, and regenerative properties[6]. MSCs are multipotent cells that secrete a variety of bioactive factors, which actively contribute favorably to their environment within damaged tissues. Furthermore, MSCs exert immunomodulatory effects by direct cell to cell contact[7,8]. Finally, these cells are also capable of changing in response to cues from their environment. MSCs have been evaluated in clinical trials of diseases involving multiple organ systems including bone, cartilage, lungs, pancreas, neurons, intestines, and the heart and blood vessels[6]. The medicinal potency and efficacy of the MSCs are particularly useful in scenarios of tissue damage, inflammation, and infection in the lung and gut; implicating the power of MSCs therapeutic potential in CF.

1.2 Specific Aim

To determine the safety and tolerability of a single infusion of human MSCs (hMSCs) and to explore secondary efficacy endpoints to determine if hMSCs reduce inflammation in subjects with CF. Markers of inflammation will be measured in blood and sputum before and 7 and 28 days after a single infusion of hMSCs in clinically stable adults with CF.

1.3 Significance

1.3.1 Mesenchymal stem cells mechanism of action: Anti-inflammatory, immunomodulatory, and anti-bacterial

The immunomodulatory properties of MSCs and their ability to secrete anti-inflammatory cytokines make them ideal for allogeneic infusion because immunosuppressants are not required. For this study, allogeneic hMSCs are being utilized because autologous MSCs will contain the subject's CFTR mutations, which would offset any potential benefit. MSCs are relatively immunoevasive; they express low levels of major histocompatibility complex I (MHC-I) molecules and do not express MHC-II molecules or costimulatory molecules such as CD80, CD86, or CD40[9], suggesting that allogeneic MSCs can be used in the clinical setting. MSCs home specifically to injured tissue and exert their immunomodulatory activity through the secretion of angiogenic (e.g., vascular endothelial growth factor)[10], anti-apoptotic (Bcl-2)[11], and inflammatory factors [tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-1β, -6, and -10, VEGF, hepatocyte

growth factor][10,12-15], stimulation of angiogenesis, promotion of a secure environment for host cell recovery, and repair and regeneration of injured tissue. Exogenously administered MSCs modulate the function of host cells at the site of injury, both by cell contact-dependent and paracrine mechanisms which involve secretion of specific mediators or transfer of cellular materials such as proteins, nucleic acids, and cell organelles (including mitochondria) to injured host cells via microvesicles[16,17]. MSCs also have antibacterial properties and inhibit bacterial growth by secretion of the antimicrobial peptide LL-37[18,19].

1.3.2 Mesenchymal stem cells are safe

Autologous MSCs cannot be used in this clinical trial because the MSCs will contain two mutations in CFTR. Therefore subjects with CF who will be enrolled in this trial will receive infusions of allogeneic MSCs. Infusions with allogeneic hMSCs has been shown to be safe in follow-up periods lasting several years in patients with pulmonary disease and patients with other inflammatory diseases including Crohn's disease, graft-vs.-host disease, multiple sclerosis, and type 1 diabetes[20]. Up to 1 x 10^7 allogeneic hMSCs/kg (twice the maximum dose that will be used in this study) have been infused safely. There have not been any issues with infusion-related toxicities or tumor development. Autopsy studies reveal limited residual MSCs up to 18 months after administration[21]. A meta-analysis of clinical trials of MSC infusions to evaluate safety identified 1012 participants with varying clinical conditions and identified eight randomized controlled trials with 321 subjects[22]. The authors did not detect an association between acute infusion-related toxicity, organ system complications, infection, death or malignancy, but there was a significant association between MSCs and transient fever[22].

A significant concern in CF patients receiving strong anti-inflammatory therapies is the increased risk of pulmonary exacerbations. Weiss and colleagues performed a placebo-controlled, randomized trial of mesenchymal stem cells in chronic obstructive pulmonary disease (COPD) where 31 subjects received four monthly stem cell infusions and there was no difference in hospitalizations for COPD exacerbations during the study (six in the MSC group and five in the placebo group)[23]. A recent meta-analysis did not detect an association between MSC treatment and the development of infection[22]. Preliminary data in CF animals suggests improvement in infection related outcomes, not worsening[18,19].

Another concern is the potential for increased risk of cancer. Again, this has not been detected in clinical trials[22,24]. However, the potential for malignant transformation would not be unexpected given some of the biologic similarities between stem cells and cancer cells. However, the risk of tumorigenesis is likely to be an extremely uncommon event with current culture conditions where cells are harvested for therapy well before the cultures reach senescence[20]. Another potential risk would be if hMSCs engraft for long periods of time which would result in ectopic tissue formation and increase the risk of malignant transformation Autopsy studies have not detected hMSCs in MSC recipients, thus suggesting that hMSCs appear to work through a "hit and run" mechanism[21]. There have been no reports of *de novo* tumor formation complicating MSC infusion in humans[22,24]. Nevertheless, in our protocol, cells will be passaged the minimum number of times to obtain sufficient cell yield, thereby lessening the potential for cytogenetic abnormalities. In addition, CF subjects with a history of invasive cancer requiring systemic therapy will be excluded from the study.

There have been no published clinical trials of MSCs in CF. A search of PubMed "Mesenchymal Stem Cells" limited to humans and clinical trials returns over 200 citations, including several pulmonary studies. Chang and colleagues recently published a phase 1 dose-escalation study in 9 preterm infants at risk for bronchopulmonary dysplasia and found MSC infusion to be safe and without associated serious adverse events (SAEs) or dose limiting toxicities[25]. A human trial of a commercial hUCB-MSC product (PNEUMOSTEM™) for neonatal bronchopulmonary dysplasia has been performed in South Korea and demonstrated decreased

levels of inflammatory markers in tracheal secretions and less severe respiratory disease; no long-term data are available yet[25]. A Phase I/II clinical trial of PNEUMOSTEM™ is currently enrolling in the United States.

Stem cell infusion has also been shown to be safe in 30 adults with multidrug-resistant tuberculosis[26], 87 subjects with drug refractory systemic lupus erythematosus[27], and 15 subjects with Crohn's disease[28] and has been found to have clinical benefit in pediatric patients with other diseases[29-31].

1.3.3 Overview of pre-clinical data of MSCs in cystic fibrosis

Dr. Bonfield's (Co-I) laboratory carries out studies using *in vivo* modeling of acute and chronic *Pseudomonas aeruginosa* (clinical isolate: M5715) infection in mice deficient in *Cftr*[32-34]. This murine model was used to evaluate hMSCs. Importantly, hMSCs attenuated weight loss, clinical score and lung pathology associated with chronic infection with *P. aeruginosa* in comparison to CF mice not treated with hMSCs[19]. In addition to benefits in weight gain and overall health of the animals, there was a significant decrease in lung inflammation (Figure 1A). CF mice treated with hMSCs had a significant reduction in BAL neutrophils (Figure 1A) and bacterial burden (Figure 1B). MSC administration also was associated with decreased KC (a neutrophil chemoattractant), IL-6 and IL-1β concentrations ($p<.05$). These studies also demonstrated that MSC infusion was not associated with increased infectious complications despite attenuating inflammation.

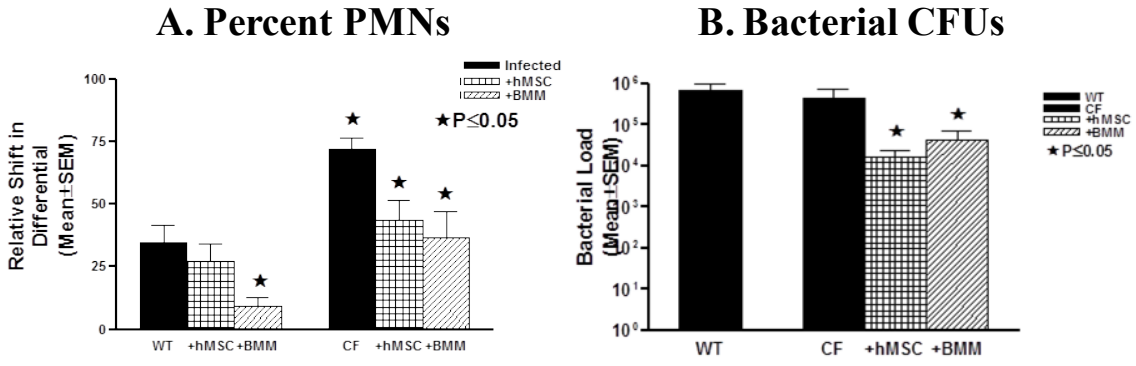

Figure 1: hMSCs are Anti-inflammatory and Antimicrobial *in vivo*. *Cftr* deficient mice and WT controls were infected with *P aeruginosa* and followed for up to 10 days. Mice received hMSCs one day after infection. Animals were euthanized and evaluated for BAL neutrophils (A), and *P. aeruginosa* CFUs (B). Treatment was associated with a decrease in neutrophils (A, $P<0.05$) and bacterial CFUs (B, $P<0.05$).

Bacterial infection with microorganisms, like *P. aeruginosa* contribute to the lung pathophysiology in CF. The challenge clinically, is to attenuate the inflammatory response that is responsible for damaging the lung architecture while at the same time not increasing infectious complications. **Figure 2** shows an example of how hMSCs impact bacterial growth and kinetics over time. These studies demonstrate that hMSC have antimicrobial potential by decreasing the overall growth of the bacteria. Interestingly, hMSCs in combination with gentamicin was even more efficient, thus suggesting the potential to use MSC to enhance antibiotic regimens for acute and/or chronic infections.

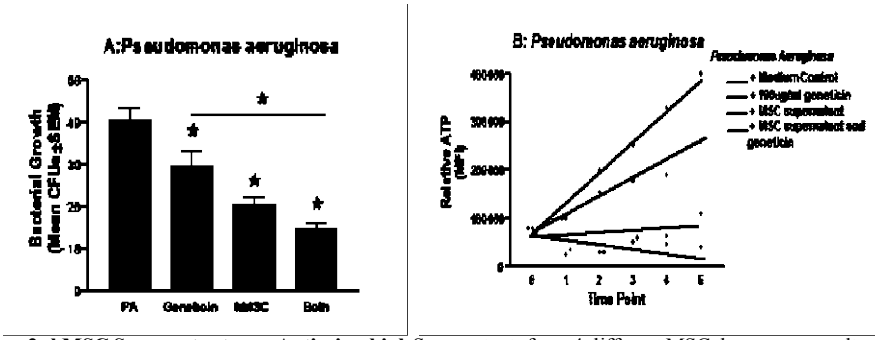

Figure 2: hMSC Supernatants are Antimicrobial. Supernatants from 4 different MSC donors were cultured with a clinical strain of *P. aeruginosa* for 5 minutes. Bacteria were then streaked onto TSA plates and cultured overnight and analyzed for CFUs (A) or ATP for determination of growth kinetics (B). Gentamicin was used as a positive control. Both gentamicin and MSC supernatants decreased CFUs ($P<0.05$) and rate of growth ($P<0.05$).The largest effect was seen with the combination of supernatants from hMSCs and gentamicin.

In addition to *P. aeruginosa*, Dr. Bonfield's lab has also shown that hMSCs have antibacterial effects on *Staphylococcus aureus*. Given that almost 25% of CF patients are infected with methicillin-resistant *S. aureus* (MRSA), which is associated with worse survival[35], these findings are particularly noteworthy. Dr. Bonfield began by evaluating the ability of hMSC supernatants to change the growth potential of *S. aureus*. Supernatants harvested from hMSCs in culture were associated with significantly fewer *S. aureus* CFUs. Bacterial CFUs were decreased further when the hMSC supernatants were given in combination with antibiotics. Our laboratory now consistently screens different donor preparations for their antimicrobial potency. These potency assays are unique and were developed here at Case Western Reserve University by Dr. Bonfield. In these *in vitro* potency and efficacy assays, MSC preparations are cultured with pathogens such as *P. aeruginosa*, *S. aureus* (including MRSA), and *Streptococcus pneumoniae*.

Cytokines are essential in the process of leukocyte recruitment and define the cell type and inflammatory response. Wild-type and *Cftr* deficient epithelial cell lines were stimulated to produce pro-inflammatory cytokines and co-cultured with human MSCs and then stimulated with lipopolysaccharide (LPS). **Figure 3** shows the results obtained when hMSCs were cultured in cell culture conditions with and without stimulation with *E. coli* LPS. In both model systems the hMSC suppressed the production of LPS induced pro-inflammatory mediators. These cell lines are now part of our *in vitro* system for monitoring MSC donor efficacy and potency in terms of inflammation and leukocyte recruitment.

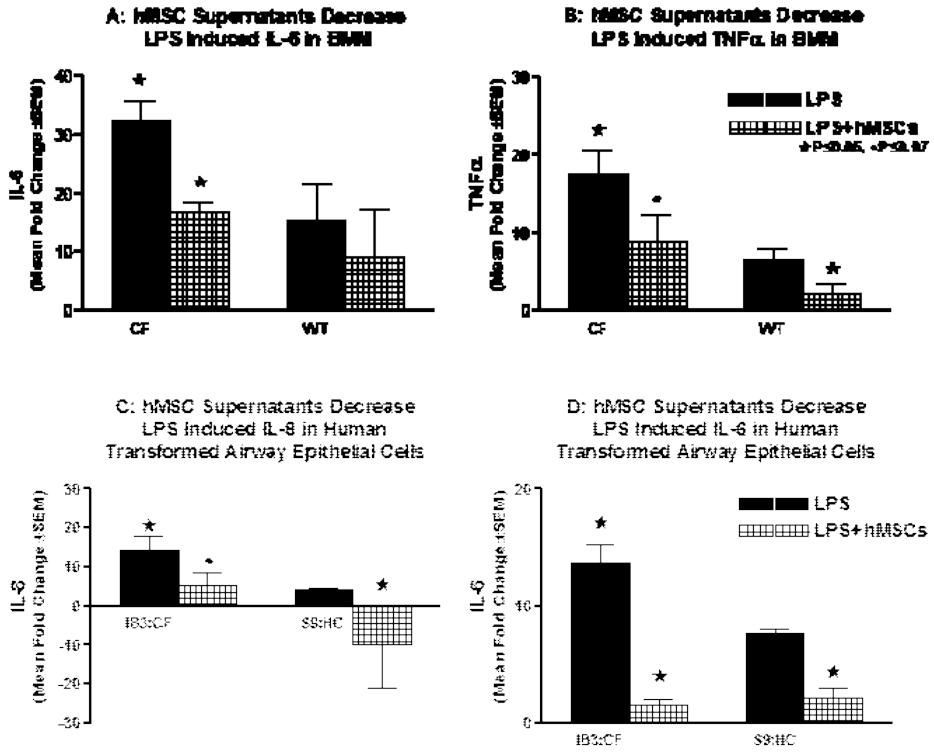

Figure 3: hMSCs are Anti-Inflammatory. MSCs from 4 different bone marrow donors were cultured with either LPS, stimulated macrophages (A and B), or epithelial cells (C and D) from either CFTR-deficient or WT sources. After 24 hours, cells were harvested and processed for cDNA and real-time PCR. LPS-induced IL-6 and TNF-a gene expression in the in macrophages was suppressed by the addition of hMSC supernatants ($P<0.05$). LPS-induced IL-6 and IL-8 gene expression in the epithelial cells was also suppressed by the addition of hMSC ($P<0.05$). BMM=bone marrow macrophages

1.3.4 Overview of Clinical Studies

1.3.4.1 Mesenchymal stem cells in pulmonary disease

COPD is a heterogeneous systemic disease that is characterized by significant inflammation. The only published study of hMSC infusion in COPD is the study by Weiss and colleagues in which the infusions were well tolerated[23]. The design was a placebo-controlled, randomized trial of mesenchymal stem cells in COPD where 30 subjects received four monthly stem cell infusions (100 x 10^6 cells) and 32 subjects received placebo. We propose using a weight based approach rather than administering a fixed dose of hMSCs. The dose of MSCs that will be used in this trial, 1 to 5 x 10^6 hMSCs/kg, would be approximately 70 to 350 x 10^6 hMSCs. It is also important to emphasize that these COPD subjects had more fixed lung disease compared to CF subjects with mild to moderate CF lung disease, the target population that will be recruited into this clinical trial. In addition, subjects with COPD were older (average age 68), had moderate to severe COPD, and had multiple co-morbidities. In this population of subjects with COPD, the infusion of hMSCs was well tolerated with no differences between the groups in adverse events (AEs). No infusion-related toxicities and no SAEs occurred that were related to study drug. The study did not detect any differences in pulmonary function test results or quality of life indicators. The subjects with the highest levels of inflammation at baseline had significant improvements in inflammatory markers compared to the placebo group, and the authors hypothesize that chronic immune based inflammatory diseases, such as CF, may be more likely to respond than what was seen in this COPD study. In addition, the dosing was the same for every subject, regardless of weight. In another clinical trial of hMSC infusion in pulmonary disease, there was significant efficacy seen in the phase 1 dose-

escalation study of preterm infants with bronchopulmonary dysplasia (BPD)[25]. In this study, concentrations of IL-6, IL-8, MMP-9, TNF-α, and transforming growth factor (TGF)-β1 in tracheal aspirates at day 7 were significantly decreased compared to baseline and 3 days post-infusion. Furthermore, the severity of BPD was reduced in MSC recipients, while AEs did not differ between the study groups. Human studies are ongoing in acute respiratory distress syndrome and idiopathic pulmonary fibrosis, but have not been completed or published.

1.3.4.2 Infusion of Mesenchymal Stem Cells in Humans

MSCs have been tested as treatment for a variety of clinical indications, and in some settings, dramatic benefit has been reported. Hillard Lazarus, M.D. and Arnold Caplan, Ph.D. designed and executed the very first in-human MSC trial, which was conducted here at Case Western Reserve University [36]. Since that very first-in-human MSC study, it is estimated that tens of thousands of subjects have received MSCs in over 500 trials[22].

1.3.4.3 Mesenchymal Stem Cell Trials at University Hospitals Cleveland Medical Center

1.3.4.3.1 CWRU 1494 Autologous Mesenchymal Stem Cell Infusion at the Time of Autologous Peripheral Blood Stem Cell Transplantation

We evaluated the feasibility and safety of autologous MSC infusion in breast cancer patients undergoing high dose chemotherapy and autologous peripheral blood stem cell (PBSC) transplantation[37]. We also investigated whether autologous MSC infusion at the time of PBSC transplantation facilitated hematopoietic engraftment, particularly megakaryopoiesis. In this study, the bone marrow (BM) aspirate from 28 enrolled subjects was successfully cultured in excess of 1 x 10^6 cells/kg (mean expansion potential 19 x 10^6 cells/kg). Subjects were re-infused with 1-2.2 x 10^6 cells/kg autologous culture-expanded MSCs, either 24 or 1 hour after PBSC infusion. Intravenous administration of MSCs was very well tolerated with no toxicity. Neutrophil and platelet count recovery was rapid (median days to ANC>500/μl=8 days and median days to platelets>20,000/μl=8.5 days) with none of the patients experiencing engraftment delays. In addition, we were able to isolate circulating clonogenic MSCs from the venous blood of subjects receiving intravenous infusion of MSCs either immediately at the end of infusion (3 out of 15 patients), 15 minutes after infusion (8 out of 15 patients), and 60 minutes after infusion (2 out of 15 patients). Clonogenic MSCs were not detected 6 and 18 hours after infusion. These data indicate that MSCs circulate for a short period of time following intravenous infusion and suggest that they distribute widely in the body. Whether these cells home to specific locations or distribute in tissues in a non-specific manner is unknown.

1.3.4.3.2 OSIR 1Y98 Allogeneic Donor Mesenchymal Stem Cell Infusion at the Time of Allogeneic Peripheral Blood Stem Cell or Bone Marrow Transplant

Our group led a multi-center clinical trial with MHC-identical donor allogeneic MSCs to promote faster hematopoietic engraftment and to limit graft versus host disease (GVHD) after allogeneic BM or PBSC transplantation in patients with hematological malignancies[38]. Forty-three subjects received myeloablative chemo-radiation therapy, MHC-identical sibling marrow (N=17) or mobilized peripheral blood (N=26) stem cells, and escalating doses of culture-expanded MSCs from a hematopoietic stem cell donor (1-5 x 10^6 cells/kg). There were no instances of MSC infusion-related toxicities. Median (range) time to neutrophil recovery >500/μl was 14 (11-22) days and platelets >20,000/μl (not transfused) was 21 (15-78) days for all subjects. Grade 0-I acute GVHD occurred in 29 subjects and grade II-III in 10 subjects; none had grade IV. Of 36 subjects at-risk, chronic GVHD did not develop in 17 participants, was of limited extent in 13 subjects, and extensive in 6 participants. The expected incidence of acute and chronic GVHD in sibling-matched allogeneic stem cell transplantation varies. In the two largest prospective studies, the incidence for grade II-IV and III-IV

acute GVHD ranged 44% to 64%[39]and 12% to 26%[40]. Extensive chronic GVHD rates in these two reports ranged from 30% to 46%. Our results compare favorably with these historic data. Despite the fact that severe acute and chronic GVHD appeared to be reduced by the co-infusion of MSCs, we did not observe an excessive number of subjects developing tumor progression adding confidence to the use of MSCs in the context of non-malignant diseases such as CF.

1.3.4.3.3 CWRU 1Y98 Late Allogeneic Donor Mesenchymal Stem Cell Infusion for Lysomsomal or Peroxisomal Disorders

Allogeneic BM transplantation can provide donor cells to correct inherited enzymatic deficiencies as well as skeletal disorders in non-malignant disorders[41]. Many of these disorders are only partially corrected by an allogeneic transplantation. We hypothesized that marrow derived stromal cells could have further beneficial effect in these patients by providing enzyme replacement to the tissues to which they distribute. We previously determined that human MSCs express high levels of arylsulfatase A and αL-Iduronidase. Arylsulfatase A deficiency results in metachromatic leukodystrophy, which is manifested during the first decade of life with progressive dementia, convulsions and cranial nerve abnormalities. αL-Iduronidase deficiency results in Hurler disease that is manifested by hepatosplenomegaly, dysostosis multiplex, and neuropsychological decline.

To correct the enzyme deficiency and provide normal progenitor/stem cells to patients with metachromatic leukodystrophy and Hurler disease, we infused allogeneic MSCs into these patients[37]. These patients previously underwent allogeneic BM transplantation from the same donor and were fully engrafted by donor hematopoietic cells. Since these patients already had donor type immune systems, infusion of donor-derived cells of mesenchymal lineage was expected to be safe and possibly provide a therapeutic benefit. Subjects were not given any myelosuppressive chemo- or radiotherapy. Between March 1999 and July 2000, 12 donors were enrolled and MSC cultures established in all cases. Eleven subjects were enrolled and infused with donor MSCs. One subject dropped out for personal reasons during the course of the investigation, after collection and cryopreservation of donor MSCs. MSCs were culture-expanded in the CWRU Cell and Gene Therapy Facility. Four subjects received 2 x 10^6 donor MSCs/kg, five subjects received 3.4 to 4.6 x 10^6 MSCs/kg, and three subjects received 10 x 10^6 MSCs/kg. Target donor MSCs were culture expanded in 8 subjects. One subject in the 4 x 10^6 MSCs/kg cohort and 2 subjects in 10 x 10^6 MSCs/kg cohort received fewer MSCs than planned. This was mainly due to the excessive time and passage numbers that would have been required to achieve the target doses. A decision was made to limit the time and the passage number in these subjects. Relatively small amounts of starting BM aspirate and our culture conditions were adequate to grow 10 x 10^6 cells/kg of human MSCs for small children (<40kg).

There were no acute or chronic toxicities related to donor MSC infusion with no significant toxicity with 15-31 months of follow-up. There was grade 1 fever in 3 subjects that was self-limited in all cases. We have clinical follow-up as well as laboratory follow-up in all subjects. Thus far, we have been able to re-culture donor MSCs from 2 recipients (0.2-2%) using the existing culture assay technique. We have not detected any immune response against donor MSCs by ELISPOT assay using recipient lymphocytes and donor MSCs. Clinical outcome was evaluated by physical examination including neurological examination, psychosocial assessments, nerve conduction velocity, and bone mineral density measurements. There was no dramatic change in subjects overall health and no readily detectable change in mental and physical development. Following allogeneic MSC infusion, bone mineral density was either maintained or slightly improved in all subjects. Five of 6 metachromatic leukodystrophy patients had electrophysiological assessment, and in four there was a clear evidence of improvement in nerve conduction velocity.

1.3.5 Factors Affecting the Characteristics of Isolated Mesenchymal Stem Cells

The factors that affect the characteristics of isolated MSCs have not been extensively studied. MSC numbers appear to decrease with age[42], and MSCs from elderly donors have decreased life span and proliferation rate[43]. There appears to be no age effect on differentiation potential[43]. There was little variability between MSCs isolated from 50 healthy donors in proteomic profile or ability to inhibit proliferation in mixed lymphocyte reaction[44].

It is uncertain whether the functional properties of MSCs are altered in human diseases. MSCs isolated from patients with advanced osteoarthritis had normal cell yield but reduced proliferation, chondrogenic and adipogenic capacity[45]. There are conflicting reports on whether MSC properties are altered in immune-mediated disease. MSCs isolated from patients with severe aplastic anemia were deficient in their ability to inhibit T-cell proliferation and cytokine release, including T-cell proliferation in allogeneic mixed lymphocyte reactions, expression of the activation marker CD38 and IFN-γ production by phytohemagglutinin-stimulated cells, production of cyclic ADP-ribose, and T-cell-mediated inhibition of hematopoietic colony formation[46]. In 1 study, stromal progenitors isolated from subjects with systemic sclerosis were functionally impaired with reduced proliferation rate and capacity for adipogenic and osteogenic differentiation[47]. A similar decrease in proliferative potential was reported in MSCs isolated from 26 subjects with rheumatoid arthritis[48]. In contrast, in 2 other studies, MSCs isolated from patients with autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjogrens syndrome, polymyalgia rheumatica, or diabetes) had similar surface phenotype, plaque-forming ability, differentiation capacity, ability to support hematopoiesis, and immunomodulatory properties as MSCs from controls, with no apparent effect of disease activity or immunosuppressant therapy[49,50].

BM stromal cells isolated from 15 MS patients had normal capacity to support hematopoiesis, with no apparent effect of recent IFN-γ treatment[51]. MSCs isolated from 10 multiple sclerosis (MS) patients had similar proliferation, differentiation capacity, toll-like receptor expression, immunomodulatory actions, ability to inhibit dendritic cell differentiation and activation, but significantly greater LPS-stimulated IP10 production compared to 6 healthy controls[52]. Thus, it is conceivable that MSCs from MS patients may have reduced immunomodulatory and/or repair capacity as a manifestation of the underlying disease or its treatment. Therefore, the planned studies of the phenotype and function of MSCs from CF subjects as part of this trial will be of great interest.

It is important to note that there can be substantial variability between different MSC preparations either due to donor or specific culture conditions[53]. Key factors include freshness of the starting material, starting cell number, culture medium, serum or serum substitute, the specific plasticware used, culture density, timing of passages, etc. Experience in our facility and others, confirms that it is possible to cryopreserve cells and that frozen MSCs have preserved viability, proliferation, differentiation, and surface marker expression and functionality[54-56]. We plan to cryopreserve our cells after primary culture only, and once thawed for culture they will be expanded and infused fresh. In our studies, specific attention will be given to identifying a donor MSC preparation with the highest degree of efficacy and potency using our *in vitro* and *in vivo* pre-clinical diagnostic models. Our assays evaluate the anti-inflammatory, antimicrobial and leukocyte recruitment potential of the MSCs *in vitro* followed by validation in our murine pre-clinical model of CF lung infection and inflammation.

In terms of MSC preparations, we will use our standardized protocols for culture and expansion of the identified donor cells, with the plan to utilize the same donor preparation for all infusions. These specifics will minimize variability in the MSC donor preparation as well as minimize any development of alloantibodies to the allogeneic MSCs. This clinical trial will utilize the standard protocols for collecting, expanding, cryopreserving, and infusing hMSCs from the Comprehensive Cancer Center (CCC) and the National Center

for Regenerative Medicine (NCRM) at Case Western Reserve University. At the time of infusion, an aliquot of MSCs that will be used in the infusion will be assessed for pre-clinical efficacy to track any potential changes in clinical potency using our *in vitro* pre-clinical models. During the infusions, attention to several factors will be important including minimization of prolonged storage in the syringe and rapid injection through a small-bore needle both of which would decrease cell viability[57].

1.3.6 Potential Safety Concerns

In general, MSC infusion in humans, including allogeneic MSCs, has been very well tolerated. Autologous MSC infusion is not an option for this clinical trial due to the underlying genetic defect. Neither acute nor long-term clinically significant AEs attributable to allogeneic MSCs have been reported. Up to 1 x 10^7 cells/kg can be infused safely (our study will infuse up to a maximum of 5 x 10^6 cells/kg)[37]. There have been no reports of immunosuppression attributed to MSC infusion leading to opportunistic infection or autoimmunity reaction. In one clinical trial a subject developed urticaria with the second infusion of allogeneic MSCs, thought to be due to development of antibodies to the fetal bovine serum (FBS) used to culture MSCs[58]. FBS will not be used in this protocol. There have been no reports of *de novo* neoplasm in recipients, either potentially derived from MSCs or due to a permissive effect of MSCs. MSCs are immune privileged and therefore infusion of allogeneic cells is possible. There have been no pulmonary complications. Nevertheless, several potential adverse effects will require close attention.

1.3.6.1 Infusion-Related Toxicity

During this study, we plan to use freshly expanded hMSCs. By using freshly expanded hMSCs for the infusions, possible infusion-related toxicities will be reduced. However, there is a possibility that a subject may be unable to get fresh hMSCs on the day of the infusion. In this instance, we may infuse expanded hMSCs that have been frozen with dimethylsulfoxide (DMSO). Most studies have reported no infusion-related AEs. A patient with encephalopathy, stroke, and myocardial infarction most likely related to DMSO used in freezing medium has been reported[59]. Anti-FBS antibodies are common but usually not clinically significant[60]. One study reported a single participant who developed an urticarial rash following a second allogeneic MSC infusion[58]. That participant had a 150-fold increase in anti-FBS antibodies after the second infusion. Because MSCs are relatively large cells (20-60 μm)[61] and cell clumping is possible, pulmonary embolism is a potential concern. No study has reported pulmonary symptoms, change in oxygen-saturation, or change in chest radiograph. Adherence to established procedures for thawing and infusing cells should minimize the potential for these AEs.

1.3.6.2 Infection

MSC infusion *per se* could be a source of infection. MSCs could be contaminated during harvest, manipulation, or infusion. One case series reported a subject with GVHD who developed central line infection and cellulitis[62], not uncommon in critically ill patients. Aseptic technique during BM aspiration and infusion, strict adherence to culture protocols, and stringent microbiologic screening are intended to minimize the risk of administering a contaminated cell product. Furthermore, MSCs are potently antimicrobial as previously discussed. Therefore, using our established good laboratory practices and extensive experience in infection control and conduct of clinical trials will minimize the potential risk of infection.

MSCs express CD13 (the cytomegalovirus (CMV) receptor) and to a lesser extent CD21 (the receptor for EBV)[63]. MSCs can be infected *in vitro* with CMV and HSV-1 but not EBV. Reassuringly, CMV, HSV-1, HSV-2, EBV, VZV RNAs were not detected by PCR in MSCs from seropositive donors. Nevertheless, careful donor screening for herpes virus, cytomegalovirus and other infectious agents in potential future studies of allogeneic MSC infusion will remain important.

Finally, because of the immunosuppressive actions of MSCs, recipients potentially are at increased risk for more severe or opportunistic infections. MSCs inhibit lymphocyte proliferative responses to herpes viruses, candida, and *S. aureus* Protein A[63]. MSC effects on virus-specific T-cell responses appear to be less than on other immune responses, e.g. to alloantigens[64]. Infections have occurred in a significant proportion participants receiving MSC infusion in some series. However, all of the reported cases have involved already immunocompromised patients with cancer and/or GVHD following HSC infusion[65-67]. Therefore, it is difficult to discern whether MSC infusion played a role in the infections. Furthermore, MSCs themselves are antimicrobial, producing peptides which aide in clearance and managing infections.

1.3.6.3 Cancer

The potential for malignant transformation would not be unexpected given some of the biologic similarities between stem cells and cancer cells. MSCs in adults may become tumorigenic[68,69] and have been implicated in several human or experimental tumors, including childhood leukemia[70], gastric epithelial cancers[71] and osteogenic sarcoma[72] In the study by Tolar et al.[72], the high frequency of osteogenic sarcoma in lung after MSC infusion in mice appeared to be related to the propensity of mouse cells to develop frequent karyotypic abnormalities even with short-term culture. Karytotypic abnormalities were not seen in some studies of human MSCs[73] but were reported in others, predominantly with prolonged culture[74-76]. An important factor appears to be continuing cultures following senescent crisis[77,78].

A number of studies demonstrate homing of MSCs to primary and metastatic cancers where they may form tumor stroma[79]. In addition, trophic or immunosuppressive effects of MSCs could create a permissive environment for cancer development. For example, MSC-derived adipocytes reduced apoptosis of acute promyelocytic leukemia cells in culture[80]. MSCs promote experimental breast cancer metastasis in mice[81]. Human MSCs mixed with human breast cancer cells injected SC as xenograft in mice led to marked increase in metastatic potential. MSCs prolonged B16 melanoma tumor cell survival when co-injected subcutaneously in mice[82]. In a randomized trial in hematologic malignancy of HLA-identical sibling matched HSC +/- MSC, there was decreased GVHD in MSC recipients (11.1% vs. 53.3%) but increased leukemia relapse (6/10 (60.0%) vs. 3/15 (20.0%))[66].

Despite these theoretical concerns, there have been no reports of *de novo* tumor formation complicating MSC infusion in humans in the more than 500 MSC clinical trials documented in Clinicatrials.gov. Nevertheless, cells will be passaged the minimum number of times to obtain sufficient cell yield to lessen potential for cytogenetic abnormalities. Also, in this trial participants with a history of invasive cancer requiring systemic therapy will be excluded.

1.3.6.4 Rejection

MSCs are immunoevasive and non-immunogenic, which allows them to be used in allogeneic and xenogeneic transplantation. Humans have received well over 20,000 infusions of MSC, both autogeneic and allogeneic, and there have been no episodes of rejection.

1.3.6.5 Ectopic Tissue Formation

MSCs have the capability to differentiate into a number of mesodermal tissues and, possibly, tissues derived from other germ layers. Ectopic calcification and/or ossification were observed with direct injection of MSCs into infarcted mouse heart[74]. Ectopic tissue formation has not been reported in any human studies. Despite being specifically sought, ectopic tissue formation was not seen in 3+ years of follow-up in 7 subjects who underwent MSC/HSC co-infusion[62].

1.3.6.6 Graft vs. Host Disease

GVHD should not be an issue with removal of lymphocytes during culture expansion. In addition, allogeneic MSCs are now standard of care in pediatric patients requiring allogeneic bone marrow transplantations for the prevention of GVHD.

1.3.7 Experience in Isolation and Culture Expansion of Mesenchymal Stem Cells

MSCs have been isolated and cultured in large numbers in the Cellular Therapy Laboratory of the Case Western Reserve University Center for Stem Cell and Regenerative Medicine under several INDs, including IND 11176 and IND 13917. MSCs are isolated from a BM aspirate (50-100 ml using a brisk pull) and culture-expanded according to procedures outlined in existing SOPs. Culture expanded MSCs are tested for morphology, surface antigen expression, potential microbiological contamination, and viability prior to release for infusion.

Human MSCs have been generated from approximately 3000 individual donor marrows over the past 25 years at Case Western Reserve University. Typically, >250 x 10^6 MSCs can be generated from a 20-mL BM aspirate. Human MSCs propagated in culture are ≥95% pure as judged by reactivity to MSC-specific monoclonal antibodies to CD105 and CD73[83]. Culture conditions are specific for growth of MSCs. Adherent $CD14^+$ or $CD45^+$ hematopoietic cells are eliminated from the culture after the first passage. The techniques for propagating MSCs in tissue culture have been highly successful, and cultures remain free of contaminating bacteria, fungus, mycoplasma or endotoxin for at least 7 weeks (personal communication J. Reese).

1.3.8 Cystic Fibrosis Clinical Trial Experience at Rainbow Babies and Children's Hospital

The Cystic Fibrosis Clinical Research Center located at Rainbow Babies and Children's Hospitals/Case Western Reserve University has extensive experience in conducting clinical trials in CF. We have a long standing tradition of research since the Clinical Care Center's inception over 50 years ago. Our Research Center was one of the original 7 Centers of the Cystic Fibrosis Foundation's Therapeutics Development Network, which was established in 1998. We have participated in nearly every clinical trial in CF since that time. This Center consistently has 30 or more active CF clinical research trials approved by the Institutional Review Board (IRB). Dr. Erica Roesch is the PI on this clinical trial. Dr. Roesch has been site PI on 4 clinical trials and site Co-I on several other CF clinical trials since joining the CF clinical research team over two years ago. Dr. Roesch will be assisted in this study by Dr. Ross Myers and Dr. Michael Konstan, both of whom are experienced clinical investigators. Dr. Konstan has published well over 100 peer reviewed manuscripts on CF clinical trials and served as site PI, site Co-I and Lead PI on dozens of multicenter clinical trials in CF. He has been instrumental in bringing several approved therapies for CF to market. In addition, Dr. James Chmiel, the previous PI on this study, will serve as a collaborator from his new institution. This Center is well-known for its excellence in clinical research.

2 STUDY RATIONALE

In CF, the current estimated median age of survival is 37 years[84]. The leading cause of shortened survival is progressive lung disease[85]. Major accomplishments have been made in developing small molecule correctors and potentiators of CFTR, but a cure remains elusive. One would predict that intervening at the gene level with gene replacement therapy or at the protein level with CFTR potentiators and/or correctors early in that pathophysiologic cascade would result in improvements in downstream consequences, particularly at the level of the exuberant inflammatory response. However, recent clinical trials with drugs to improve CFTR function have not produced measurable improvement in airway inflammation despite improving lung function

and, at times, decreasing bacterial infection[86]. As the pursuit for a cure continues, new and innovative therapeutic approaches are desperately needed to provide improved resolution of the on-going damaging infection and inflammation in the CF lung[87].

Bone marrow-derived hMSCs, which may ameliorate the consequences of CF, possess several advantageous properties [88]. First, hMSCs can be isolated from non-CF subjects by simple bone marrow aspiration and expanded in culture up to a billion fold within 8 weeks. This allows for potential wide-spread use of this therapy if hMSCs are found to be safe and effective. Second, hMSCs can differentiate into gut and respiratory epithelial cells[89,90], suggesting not just pulmonary, but systemic benefit. Third, hMSCs are immunoevasive because they lack major histocompatibility complex and co-stimulatory cell-surface antigens. Thus infusion of hMSCs from other humans is well tolerated[91]. Humans have received well over 20,000 infusions of hMSCs, both autogeneic and allogeneic, and there have been no episodes of tissue rejection or adverse reactions. Fourth, hMSCs have both anti-inflammatory and antimicrobial properties [88]. Our preclinical data suggest that hMSCs decrease bacterial burden and inflammation in a murine model of CF lung infection, and we have been able to demonstrate *in vitro* that the hMSCs have the capacity to enhance the effectiveness of antibiotics often utilized to treat the infections associated with CF[19]. Fifth, our studies also showed the unique capacity of the MSCs to cross species from humans into mice to demonstrate a beneficial effect without any major adverse effect.

The overall goal of this project is to determine the safety and tolerability of hMSCs and to explore efficacy endpoints for a subsequent clinical trial. Baseline studies for inflammatory markers in blood and sputum will be determined. This will be followed by a single infusion of hMSCs. These studies will be repeated at 7 and 28 days and compared to Baseline values (with-in subject comparison). If hMSC infusion is determined to be safe in this study, it could then be advanced to a Phase II double-blind, placebo-controlled clinical trial. If successful, hMSC therapy eventually could become part of a patient's therapeutic maintenance regimen. Such alternatives are sorely needed, because the data show that anti-inflammatory therapy is likely to ameliorate disease and prolong life, yet it is not applied to most patients due to concerns about adverse effects.

2.1 Risk / Benefit Assessment

While hMSCs are highly promising and supported by pre-clinical studies, they must be safe. The current data support their safety. While our proposal is the first for hMSCs in CF, there have been several other studies suggesting hMSCs are safe in COPD, idiopathic pulmonary fibrosis, bronchopulmonary dysplasia, acute respiratory distress syndrome, as well as many non-pulmonary diseases[23,91]. There are 500 completed or on-going clinical trials using hMSCs, and a recent meta-analysis did not reveal any association between hMSC treatment and the development of acute infusion-related toxicity, organ system complications, infection (important for CF subjects), death, or malignancy [22,92].

Our multidisciplinary team has extensive experience in isolating and expanding hMSCs. We are fortunate to collaborate with world-renown investigators from the NCRM, a multi-institutional, state-sponsored organization composed of investigators and clinicians from University Hospitals Cleveland Medical Center and Case Western Reserve University. Our collaborators have a 30 year history of adult stem cell research including the first in man studies of hMSCs and experience with studies similar to ours in patients with other chronic inflammatory diseases[93,94]. We will be utilizing the NCRM Cellular Therapies Integrated Service to help with the preparation of cells and scale up from research to clinical operations.

Infusion with allogeneic hMSCs has been shown to be safe in follow-up periods lasting up to several years in patients with pulmonary disease and other inflammatory disorders including Crohn's disease, graft-vs.-host disease, multiple sclerosis, and type 1 diabetes[20]. Up to 1 x 10^7 cells/kg have been infused safely. There have not been any issues with infusion-related toxicities or tumor development. Autopsy studies reveal limited

residual MSCs up to 18 months after administration[21]. A meta-analysis of clinical trials of MSC infusions to evaluate safety identified 1012 participants with varying clinical conditions[22]. The authors did not detect any association between hMSC infusion and acute infusion-related toxicities, organ system complications, infection, death or malignancy. However there was a significant association between MSCs and transient fever[22].

A significant concern in CF patients receiving potent anti-inflammatory drugs is the increased risk of pulmonary exacerbations. Weiss and colleagues performed a placebo-controlled, randomized trial of mesenchymal stem cells in COPD where 31 subjects received four monthly stem cell infusions and there was no difference in hospitalizations for COPD exacerbations during the study (six in the MSC group and five in the placebo group)[23]. A recent meta-analysis did not detect an association between MSC treatment and the development of infection[22]. Preliminary data in CF animals suggest improvement in infection related outcomes, not worsening[18,19]. In our studies of CF mice infected with *P. aeruginosa*, there was a decrease in the bacterial burden in the animals that received hMSC infusions (Figure 1). No secondary spread or infectious complications were noted.

Another potential concern is the possible increased risk of cancer. Again, this has not been detected in clinical trials[22,24]. However, the potential for malignant transformation would not be unexpected given some of the biologic similarities between stem cells and cancer cells. The risk of tumorigenesis is likely to be an extremely uncommon event with current culture conditions where cells are harvested for therapy well before the cultures reach senescence[20]. Another potential risk could result if hMSCs engrafted for long periods of time leading to ectopic tissue formation and increased risk of malignant transformation. Autopsy studies did not detect hMSCs in follow-up studies. It appears that hMSCs work through a "hit and run" mechanism[21]. Despite these theoretical concerns, there have been no reports of *de novo* tumor formation complicating MSC infusion in humans[22,24]. Nevertheless, in our protocol, cells will be passaged the minimum number of times to obtain sufficient cell yield to lessen potential for cytogenetic abnormalities, and any potential CF subjects with a history of invasive cancer requiring systemic therapy will be excluded from the study.

3 STUDY OBJECTIVES

3.1 Primary Objective

To evaluate the infusion-related safety and tolerability of a single allogeneic hMSC infusion in adults with CF.

3.2 Secondary Objectives

To explore preliminary evidence for the potential efficacy of hMSCs as a new therapeutic to treat CF pulmonary infection and inflammation as measured by

- Change in sputum inflammatory markers
- Change in serum inflammatory markers

To compare allogeneic hMSCs to autologous hMSCs in a preclinical model. The potential efficacy of MSCs obtained from CF subjects who agree to undergo the optional bone marrow aspiration will be evaluated in validated animal models.

3.3 Implications for Future Studies

This study is designed to address the safety and tolerability of a single hMSC infusion in subjects with CF. While in the background section we have focused primarily on the impact of MSCs on the pulmonary inflammatory response, hMSCs have potential to impact multiple other aspects of CF lung pathophysiology. MSCs also likely possess antibacterial properties. In our mouse models, hMSCs in combination with

gentamicin was more efficient in clearing bacteria than either therapy alone. This suggests the potential to use MSCs to enhance antibiotic regimens for acute pulmonary exacerbations in CF. In addition, because study subjects will be receiving hMSCs that do not possess a mutation in *cftr*, it is possible that hMSC infusion will provide a form of gene therapy. In future Phase III studies, we will evaluate the effects of hMSC infusion on outcome measures related to infection, inflammation and CFTR correction.

4 STUDY DESIGN

4.1 Study Overview

Overview of Study Design A prospective, single-center, single-dose, dose-escalation, open-label interventional study to evaluate the safety and tolerability of allogeneic hMSCs in 15 clinically stable adult subjects with CF. Because the subject's own cells will contain two CFTR mutations, allogeneic hMSC infusion is being employed in this study. Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study. The Tables in Appendix II review the schedule of study visits. After a two to six week screening period, subjects will have the Baseline visit (Days 1-2) where they will undergo a single infusion of up to 5 x 10^6 allogeneic hMSCs/kg. The CCC and NCRM at Case Western Reserve University have a long-standing approach to identifying, screening and using donors to provide hMSCs. Donors are screened per FDA guidelines for cells and tissues (21 CFR 1271). The FDA guidelines are similar to those used for blood donation by Red Cross. The allogeneic hMSCs used in this study will come from one or two healthy donors who have been well-characterized by the CCC and whose sera test negative for CMV antibodies. Healthy donors will be followed by the PI or designee for any procedure-related AEs. Infusions will be performed in the Dahms Clinical Research Unit at University Hospitals Cleveland Medical Center. Study participants will be monitored for the development of infusion-related toxicities for 24 hours. Subjects will have subsequent study visits on Days 7, 14, 28, Months 3 and 6 and telephone calls on Days 4 (or 5), 21, 56 and Month 12. Subject safety and tolerability of the single dose of hMSCs will be evaluated at the study visits by reviewing subject diaries, interval histories, and subject questionnaires, performing physical examinations and spirometry, and obtaining safety laboratories. In addition to infusion related toxicities, special focus will also be placed upon detecting pulmonary exacerbations since an anti-inflammatory therapy theoretically could suppress the immune system to the point where it leads to increased infectious complications. Appendix I lists the criteria for a pulmonary exacerbation. This study will also explore efficacy endpoints that previously have been shown to be key regulators in CF and/or hMSCs: Serum inflammatory biomarkers (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL8, IL-17, and TNF-α), and sputum inflammatory biomarkers (white cell counts with differentials, IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, and active proteases including neutrophil elastase, α_1-anti-trypsin, and MMP-9). These studies will be repeated on Days 7 and 28 and compared to Baseline values (with-in subject comparison). All subject samples will be archived for future studies to explore the impact on the microbiome and for future translational projects. Finally, an optional diagnostic bone marrow exam will be performed between screening and Day 1 on CF subjects willing to undergo the procedure, banked, and used for future translational studies.

The following treatment regimens will be used:

- Experimental treatment hMSCs at the following doses: 1 x 10^6, 3 x 10^6 or 5 x 10^6 hMSCs/kg body weight

All subjects who begin an infusion of the investigational product will be considered evaluable for safety and efficacy analyses. Incidence of AEs will be monitored during the trial.

Safety assessments will be based on the incidence of AEs including protocol defined pulmonary exacerbations and changes in lung function, sputum microbiology, safety labs, subject questionnaire, and physical examination. An Acute Illness Visit will be arranged for any subject who experiences any abrupt change in baseline clinical status, between Visit 2 and Visit 5 (the 28 days following infusion) that in the estimation of an investigator, research coordinator, other health care provider or the subject necessitates an in-person office visit.

Total duration of subject participation in the study will be approximately 13 months. Long-term post-treatment follow-up will continue for 12 months after the infusion of the investigational product for each subject. Data on subject survival and on the occurrence of new health problems will be collected from all subjects who receive the investigational product infusion. The information will be gathered during a study visit, a routine clinic visit or via telephone. These data will be collected in the source documents (e.g., subject medical record) and transcribed into an electronic CRF. Any unsuccessful efforts to contact the subject (e.g., dates of unanswered phone calls, failure to show for a clinic visit, etc.) will be documented in the subject's source documents.

Total duration of conducting the study is expected to be approximately 24 months.

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoint

The primary outcome will be the safety and tolerability of a single infusion of allogeneic hMSCs. Study participants will be monitored for potential dose limiting toxicities. The dose limiting toxicity (DLT) definition consists of the emergence of infusion-related allergic AEs as well as regimen related toxicities. For this study, a DLT will be considered for the occurrence in the first 24 hours after hMSC infusion of a grade ≥ 3 infusion-related allergic toxicities, which include rash, flushing, urticaria, dyspnea, fever >40°C (>104.0°F) as scored according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Finally, the CTCAE will be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. In addition to infusion related toxicities, special focus will also be placed upon detecting pulmonary exacerbations since anti-inflammatory therapies theoretically could suppress the immune system to the point where it leads to increased infectious complications. Appendix I lists the criteria for defining a pulmonary exacerbation treated with antibiotics. All AEs encountered during the study period will be captured and graded.

In addition to DLT, safety and tolerability of a single infusion of allogeneic mesenchymal stem cells will also be determined by

- Incidence and severity of AEs
- Number of pulmonary exacerbations (modified Fuch's definition[95])
- Diary reports (Cystic Fibrosis Respiratory Symptom Diary)
- Changes in subject reported symptoms as captured by the Respiratory and Systemic Symptoms Questionnaire from Baseline (Visit 2) Day 1 to Baseline (Visit 2) Day 2, Visits 3, 4, 5, 6, and 7 and at times when a pulmonary exacerbation is being considered.
- Changes in physical examination
- Changes in vital signs including oxygen saturation checked throughout the infusion

- Changes in spirometry (FEV_1 %, FEV_1 (Liters), FEF_{25-75}) determined 30 minutes, 4 hours and 24 hours after completion of infusion and from Baseline (Visit 2) Day 1 to Baseline (Visit 2) Day 2, Visits 3, 4, 5, 6 and 7.
- Change in sputum quantitative microbiology (change in bacterial colony forming units between Baseline Day 1 and Day 7 and Day 28)
- Changes in hematology, comprehensive chemistry, erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive proteine (hs-CRP), and urinalysis results

5.2 Secondary Endpoints

Since this is a phase I study, the primary focus is on safety. However, in a 15 subject dose-escalation study, we will also explore potential efficacy endpoints. Secondary outcomes include: changes in biomarkers of inflammation in blood (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL8, IL-17, and TNF-α) between Baseline Day 1 and Day 7 and Day 28, and changes in biomarkers of inflammation in sputum (white cell counts with differentials, IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, and active proteases including neutrophil elastase, α_1-anti-trypsin, and MMP-9) between Baseline Day 1 and Day 7 and Day 28.

6 SUBJECT SELECTION

6.1 Study Population

Study Population: CF subjects will be recruited from the LeRoy W. Matthews Cystic Fibrosis Center located at Rainbow Babies and Children's Hospital in Cleveland, OH. Our Center provides care for over 200 adult patients with CF. Given the wide interest in hMSCs in CF, we do not anticipate any issues recruiting 15 subjects for this study from our Center. However, should recruitment lag behind projected timelines, we could recruit potential study subjects from nearby CF Centers in Akron, OH, Ann Arbor, MI, Buffalo, NY, Columbus, OH, Pittsburgh, PA, and Toledo, OH. Subjects will be studied for up to 13 months. Screening will be between 10 and 42 days. Study treatment will be Day 1 with a 12-month follow-up. We anticipate 15 months to recruit subjects (due to the 3+3 safety study design, the last subject in a given cohort must complete Visit 5 before the first subject in the next cohort can be enrolled). Subjects who fail screening or who withdraw prior to completing Visit 5 will be replaced. Subjects with a diagnosis of CF who meet the inclusion and exclusion criteria will be eligible for participation in this study. Subjects who fail screening may be re-screened one additional time.

6.2 Inclusion Criteria

1. Male or female 18 years of age and above
2. Confirmed diagnosis of CF as evidenced by 1 or more clinical features consistent with the CF phenotype and 1 or more of the following criteria:
    a. Sweat chloride equal to or greater than 60 mEq/L by quantitative pilocarpine iontophoresis test (QPIT)
    b. 2 well-characterized, disease causing mutations in the CFTR gene
3. Clinically stable with no significant changes in health status within 2 weeks prior to screening
4. $FEV_1 \geq 40\%$ predicted for age based on the global lung function initiative equations at the screening visit

5. Weight ≥ 40 kg at the screening visit
6. Able to perform repeatable, consistent efforts in pulmonary function testing
7. Written informed consent obtained from the subject

6.3 Exclusion Criteria

1. Use of an investigational agent within the 4-week period prior to Visit 1 (Day -42 to -10)
2. Chronic daily (>10 mg) or alternate daily (>20 mg on alternate days) use of systemic corticosteroids within the 4 weeks prior to Visit 1 (Day -42 to -10) or initiation of any dosage of systemic corticosteroids within 72 hours prior to Visit 2 (Day 1)
3. Use of hydroxychloroquine or immunosuppressants
4. Initiation of a new antibiotic (oral, IV, and/or inhaled) that is not part of the subject's maintenance regimen for treatment of acute respiratory symptoms within 2 weeks prior to screening through Visit 2 (Day 1)
5. Initiation of any new chronic therapy (e.g., Pulmozyme®, hypertonic saline, Kalydeco®, Orkambi®, high-dose ibuprofen azithromycin, TOBI®, Cayston®, nebulized colistiin, bronchodilators, inhaled corticosteroids, etc.) within 4 weeks prior to screening
6. Active treatment for non-tuberculous *Mycobacteria*
7. History of a sputum culture positive for a *Burkholderia cepacia* complex organism in the previous 12 months
8. Current tobacco smoker
9. Oxygen saturation < 92% on room air at Visit 1 (Day -42 to -10)
10. History of pulmonary hypertension
11. SGOT (ALT) or SGPT (AST) > 2.5 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension
12. Total bilirubin concentration >1.2 mg/dL at screening
13. Creatinine > 1.8 mg/dL at screening
14. Pregnant, breastfeeding, or unwilling to practice birth control between Visit 2 (Day 1) and Telephone Call 3 (Day 56) (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal
15. Screening hematology with white blood cell count < 4.5 x 10^9 cells/L, hematocrit < 30%, and platelets < 150 x 10^9 platelets/L
16. History of invasive cancer requiring systemic therapy
17. History of organ transplantation
18. Currently listed for lung transplantation or having potential to be listed for lung transplantation in the succeeding 12 calendar months from screening
19. Subject unlikely to complete the study as determined by the Investigator

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications from screening through Visit 5 (Day 28), as medically feasible, with no introduction of new chronic therapies. Medication history will be collected for all drugs for the 30 days prior to screening. If any changes in concomitant medications are required due to AEs (i.e. illness, laboratory abnormalities, surgical procedures, etc.), the reason(s) for the change(s) must be documented. Subjects who require treatment for a pulmonary exacerbation during the course of the trial will remain in the study.

Subjects who have routinely taken more than 10 mg/day (or >20 mg on alternate days) of prednisone or other systemic corticosteroids 4 weeks prior to Visit 1 (Day -42 to -10) or who have taken these medications as needed within 72 hours prior to Visit 1 or 2 will be excluded from participation. Subjects may be re-screened once. For those subjects taking alternate month TOBI® or Cayston® therapy, on/off cycles must be timed such that a subject is at the same phase of a cycle at Visits 2, 3, 4, and 5.

7.1 Allowed

Except as noted in the prohibited medications section below or in the exclusion criteria described above, all usual CF medications and treatments are allowed. A stable therapeutic regimen (including physiotherapy) between screening and Visit 5 (Day 28) is the goal. Ongoing ***chronic*** treatment (> 4 weeks prior to screening) with Pulmozyme®, Cayston®, TOBI®, nebulized colistin, Kalydeco®, Orkambi®, high-dose ibuprofen, hypertonic saline, azithromycin, systemic or inhaled corticosteroids, short and long acting bronchodilators and airway clearance is allowed. Unless otherwise medically indicated, subjects not using these therapies should not be started on them from screening through Visit 5 (Day 28) and subjects that have been using them chronically should be encouraged to continue them through Visit 5 (Day 28). Limited use of over-the-counter anti-inflammatory drugs (acetaminophen, aspirin, ibuprofen, or naproxen) from screening through Visit 5 (Day 28) is acceptable.

7.2 Prohibited

- Intravenous antibiotics from 14 days prior to the screening visit through Visit 2 (Day 1)
- Newly prescribed oral or inhaled antibiotics prescribed for acute symptoms from 14 days prior to the screening visit through Visit 2 (Day 1)
- Systemic corticosteroids prescribed for an acute issue or for chronic therapy if the dose exceeds a prednisone equivalent of 10 mg every day or 20 mg on alternate days (maximum allowable dose for chronic systemic corticosteroids is 20 mg of prednisone or its equivalent over a two day period) from screening through Visit 5 (Day 28)
- Hydroxychloroquine from screening through Visit 5 (Day 28)
- Other immunosuppressants from screening through Visit 5 (Day 28)
- The use of an investigational agent within 4 weeks prior to Visit 1 (Day -42 to -10)

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

In this Phase I open-label study, there is no placebo group. All subjects will receive a single infusion of hMSCs. 15 eligible subjects will be recruited from the adult CF Center located at Rainbow Babies and

Children's Hospital and will receive a single dose of one of the following doses of hMSCs: 1 x 10^6, 3 x 10^6 or 5 x 10^6 hMSCs/kg body weight. A traditional 3+3 design will be utilized (Figure 4). If a maximum tolerated dose (MTD) is identified among the 3 doses, the next 3 subjects enrolled will receive the identified MTD. If there are no DLTs or one DLT, the next 3 subjects enrolled will receive same dose. If there are two or more DLTs, the next lower dose will be used as the MTD for the final 3 enrolled study subjects.

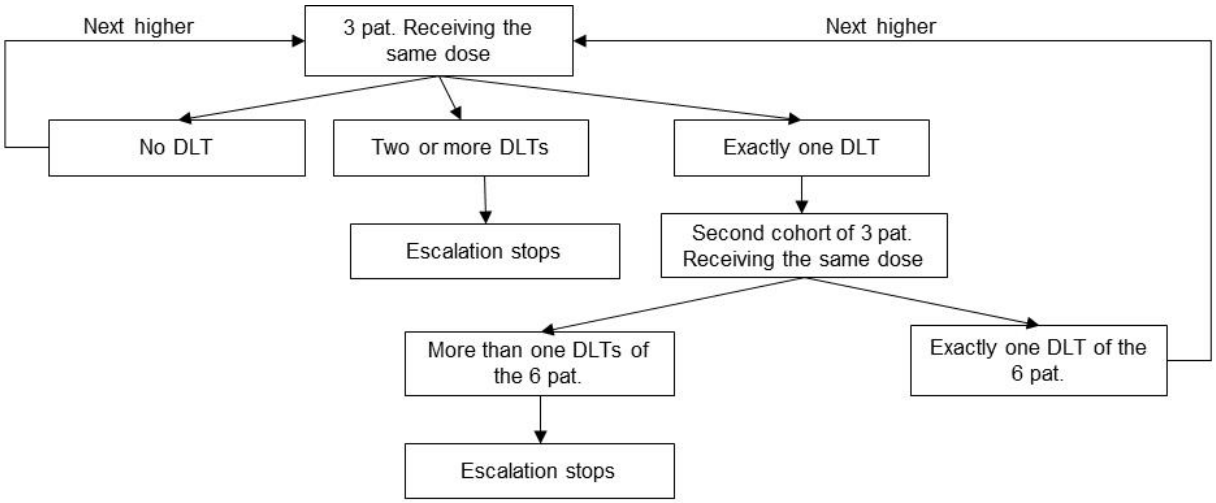

Figure 4. Study design for determining dose-limiting toxicity

8.2 Blinding

Because this is an open-label, safety and tolerability study in which all enrolled subjects will receive hMSCs, this study is unblinded.

8.3 Test and Control Formulation

The biological product (hMSCs) is manufactured in the Case Western Reserve University NCRM Cellular Therapy Laboratory under GMP-compliant regulations. This laboratory is located on the campus of Case Western Reserve University/University Hospitals Cleveland Medical Center.

Address:
WRB 6-303
2103 Cornell Road
Cleveland, Ohio 44106
(216) 368-1007

The cells obtained from the donor(s) will be cryopreserved at primary culture. There will be no passages involved. Only plated and adherent cells will be harvested. This occurs at approximately 14 days. The number of passages after thaw (prior to infusion) is dependent on the target dose (recipient receives a dose based upon weight). For most participants, we will likely reach our target dose after 2 passages and no greater than 3 passages. This translates into approximately 14 days.

The final hMSC formulation is a fresh cell suspension of 4 x 10^6 cells/ml with ≥70% viability in Plasma-Lyte A + 2.5% HSA. This product is distributed in a syringe for immediate infusion.

8.3.1 Packaging and Labeling

The biologic product is packaged and labeled for infusion according to FDA guidelines by Cell Processing Technologists employed by the CWRU NCRM Cellular Therapy Lab. Blinding is not applicable to this study. The infusion product will be labeled as follows:

Investigational Drug (Study Name or #)
FOR INFUSION
Subject Unique Identifier:

Cellular Therapy Laboratory
WRB 6-303
2103 Cornell Road
Cleveland, Ohio 44106 (216) 368-1007

Date: Total Cells:
Volume: X ml Expiration: dd/mm/yyyy Time hh:mm

CAUTION: NEW DRUG-LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE

8.3.2 Handling/Dispensing

Human MSCs will be dispensed directly from manufacturing personnel to clinical personnel per conditions noted in the IND application and the clinical protocol. Chain of custody will be tracked in writing and documents filed in both the subject study record and manufacturing record.

8.3.3 Dosage/Dosage Regimen

The first cohort of 3 subjects enrolled will receive a single infusion of 1 x 10^6 hMSCs/kg body weight, The second cohort of three subjects enrolled will receive a single infusion of 3 x 10^6 hMSCs/kg, and the third cohort of three subjects enrolled will receive a single infusion of 5 x 10^6 hMSCs/kg. The first subject in the subsequent cohort will not be enrolled until the last subject in the preceding cohort has completed Visit 5 (Day 28). There will be a minimum of 1 week between the dosing of each study subject in the first three cohorts. The final six subjects will receive a single infusion of 5 x 10^6 hMSCs/kg if that dose was well tolerated. If the highest dose was not well tolerated, then the final six subjects will receive the maximum tolerated dose (either 1 x 10^6 or 3 x 10^6 hMSCs/kg body weight).

Table 1: hMSC dose cohorts

Cohort 1	1 x 10^6 hMSCs/kg	3 subjects

Cohort 2	3 x 10^6 hMSCs/kg	3 subjects
Cohort 3	5 x 10^6 hMSCs/kg	3 subjects
Cohort 4	MTD	6 subjects

8.3.4 Administration Instructions

The person infusing the cells should wear sterile gloves.

Fresh hMSC will be administered as follows:

hMSCs are infused through a peripheral venous catheter by directly connecting the syringe of cells to the IV tubing via Y-connector.

1. Start infusing IV fluids (normal saline) at the maximal rate (250 ml/hr.).
2. Clean the port of the Y-connector as per hospital protocol.
3. Remove needle from syringe containing the hMSCs.
4. Attach the syringe with hMSCs directly to the in-line Y-connector.
5. Manually clamp the IV tubing leading back to the bag of saline behind the syringe by pinching the tubing between two fingers.
6. Administer a small aliquot (2-3 ml) of the cells from the syringe by slow IV push.
7. Release the IV tubing and allow the normal saline to flush the line for 1-2 minutes.
8. Repeat steps 5 through 7 until all the cells have been infused, completing the infusion over 10-15 minutes.
9. The first post cell infusion rinse of the syringe must now be performed.
10. Manually clamp the IV tubing between the syringe and the study participant by bending the tubing and pinching it between two fingers to draw saline fluid into the syringe, complete filling the syringe.
11. Release the IV tubing leading between the syringe and the study participant.
12. Manually clamp the IV tubing between the bag of saline and the syringe by bending the tubing and pinching it between two fingers.
13. Administer an aliquot (3-5 ml) of fluid from the syringe by IV push.
14. Release the IV tubing and allow the normal saline to flush the line for 30 seconds to 1 minute.
15. Repeat steps 12 through 14 until the syringe is empty. Completing the saline rinse should take approximately 2 minutes.
16. Perform a second post cell infusion rinse of the syringe.
17. Repeat steps 10 through 15.
18. Remove the syringe from the Y-connector port.

19. Administer normal saline through the running IV at a rate of 250 ml/hr. for 4 hours while the study participant is being monitored after the infusion.
20. Document the infusion in a procedure note in the electronic medical record (EMR).

Infusion reactions are documented in the subject's study chart and the principal investigator is notified in the event of severe reactions.

An infusion side-effect checklist is provided to the subject's nurse (a modified version of the standard form used by University Hospitals Cleveland Medical Center Seidman Cancer Center).

Side effects are monitored for 24 hours after the completion of the infusion.

8.4 Supply of Investigational Product at the Site

The cell product will be expanded to the clinical dose in culture upon receipt of a physician investigator's order. Duration of culture expansion is approximately 15-20 days after which cells will be infused once release tests are complete.

8.4.1 Storage

Stock vials of primary culture hMSCs will be secured in liquid nitrogen vapor phase until indicated for expansion per physician investigator's order. Liquid nitrogen freezers are remote alarm monitored 24/7. The expanded hMSCs for infusion will be used fresh without further frozen storage.

8.5 Investigational Product Accountability

An accurate and current accounting of the amount of hMSCs administered to each subject will be maintained by research staff. Research staff will document the total dosage of hMSCs administered to each subject. If the infusion is stopped for any reason, including adverse reaction or technical difficulty, research staff will determine how much of the infusion the subject received and record the administered dose in the study records.

8.6 Measures of Treatment Compliance

In this Phase I study, subjects will receive a single infusion of hMSCs that will be administered by research staff in the Dahms Clinical Research Unit of University Hospitals Cleveland Medical Center. Therefore we do not anticipate that there will be any issues with treatment compliance during this study.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is outlined in Appendix II.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medications and concurrent therapies will be documented at all study visits including an Acute Illness/Early Termination Visit when applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured for the 30 days prior to screening.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, *cftr* genotype analysis results, sweat chloride results, sputum microbiology, spirometry results, other pertinent respiratory history, and information regarding underlying diseases will be recorded at the screening visit (Visit 1). An interval history will be obtained at all other visits.

In the event of a pregnancy between Visit 2 (Day 1) and Telephone Call 3 (Day 56), medical information pertaining to the pregnancy will be collected through the birth of the child or the termination of the pregnancy.

9.1.4 Physical Examination

A complete physical examination will be performed by a physician (either the investigator or a sub-investigator) at Visits 1, 2 and 5 and at an Acute Illness/Early Termination Visit. Qualified staff (MD/DO, NP, RN, PA) may complete the abbreviated physical exam at all other visits. The physical exam also includes height (at screening only) and weight. The complete physical examination consists of general, HEENT, neck/thyroid, lymph nodes, cardiovascular system, chest/lung, abdomen, extremities, skin, musculoskeletal, nervous system, mental status, and other. An abbreviated exam will consist of HEENT, cardiovascular system, chest/lung, abdomen, extremities, skin, and other. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at all study visits.

9.1.6 Oximetry

Oximetry will be measured on room air with the subject at rest at all study visits.

9.1.7 Spirometry

Spirometry will be performed at all study visits and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests. Spirometry will be obtained approximately 2 hours prior to the start of the hMSC infusion and 30 minutes, 4 hours and 24 hours after completion of the infusion. Subjects will perform spirometry as is customary for that subject at clinical visits. If the subject typically does not take albuterol prior to spirometry, then he/she will not take albuterol prior to spirometry during the study. If the subject typically takes albuterol prior to spirometry, they he/she will take albuterol prior to spirometry for this study. However there is an exception. In any subject in which it is anticipated that he/she might not be able to produce sputum spontaneously at any one of the study visits in which sputum will be obtained, then those subjects will undergo sputum induction at all visits regardless of his or her ability to spontaneously expectorate sputum at the current study visit. In these cases, those individuals will receive three inhalations of albuterol sulfate via metered dose inhaler prior to <u>all</u> spirometric tests obtained during the study. Approximately 15 minutes after administering albuterol sulfate, pulmonary function testing will be performed. If a study subject is unable to produce sputum at the Baseline Visit (Day 1), then sputum will not be obtained at Visits 3 (Day 7) and 5 (Day 28).

9.1.8 Acquisition of Sputum

Sputum (approximately 1.3-2.0 grams) will be obtained at Visit 2 (Day 1) prior to the infusion of stem cells and on Visits 3 (Day 7) and 5 (Day 28) via spontaneous expectoration. Sputum samples will be split according to priority for analyses. The first aliquot will be sent to the microbiology laboratory at University Hospitals Cleveland Medical Center where quantitative bacteriology will be performed, and the second aliquot will be processed and saved for research studies. Sputum will be processed according to the current standard operating procedure (SOP) developed by CF Foundation's Therapeutics Development Network. Subjects unable to spontaneously expectorate sputum will undergo sputum induction according to the current SOP developed by CF Foundation's Therapeutics Development Network. If a sputum sample cannot be produced, or the quantity of the sample is not sufficient for all the tests to be performed, this is not considered a deviation.

9.1.9 Intravenous Infusion of hMSCs

Fresh MSCs are infused at Visit 2 (Day 1) from a syringe into a venous catheter followed by a saline wash. After the infusion of cells is complete, 10-ml of a normal saline flush is given immediately and a running IV of normal saline is maintained at 250 ml/hr for 4 hours. Subjects are monitored for 24 hours for infusion-related toxicity. Temperature, blood pressure, heart rate, respiratory rate and oxygen-saturation are measured prior to infusion and at 10 (± 5), 20 (± 5), 30 (± 5), 60 (± 15), and 90 (± 15) minutes and 2 hours (± 15 minutes) and 4 (± 15 minutes) hours post-infusion and then every 4 (± 15 minutes) hours for the remainder of the 24-hour observation period. Any toxicity is treated at the discretion of the managing physician, and toxicities are reported to the Principal Investigator, IRB, and FDA as appropriate.

MSC infusion has been very well tolerated. Although theoretically of concern, there have been no reported cases of immunosuppression, autoimmunity, cancer, significant lung damage, severe infection, or clinically significant ectopic tissue formation attributable to MSC infusion. MSC infusion could cause infection if the cells are contaminated. Strict aseptic technique during bone marrow aspiration, culture, thawing, and infusion and stringent microbiologic screening minimize this risk. Because of the immunosuppressive effects of MSCs, recipients potentially are at increased risk for infection.

9.1.10 Bone Marrow Exam

Healthy volunteers who meet screening criteria and who consent to having their mesenchymal stem cells subsequently infused into study subjects with CF will undergo a bone marrow examination according to SOPs developed by the CCC well in advance of the infusion. Either a unilateral or bilateral (depending on the yield of bone marrow aspirate) posterior iliac crest bone marrow aspiration will be performed using local anesthetics 1% Xylocaine (approximately 10 ml intradermal and subcutaneous), preservative-free bupivacaine 0.25% (approximately 10-20 ml injected locally to the site), morphine sulfate (approximately 2-6 mg by IV). Lorazepam (1 mg oral or IV) may be used if needed. Approximately 60-80 ml of marrow is pulled into syringes containing preservative-free heparin (400 units/25cc marrow) using a few brisk pulls. Bone marrow samples will be processed, and MSCs will be prepared according to CCC SOPs as previously described.

If a subject with CF consents to undergoing the optional bone marrow exam, screening procedures will be performed at Visit 1 and a single bone marrow aspiration of the posterior iliac crest will be performed at Visit 1A under local anesthetic 1% Xylocaine (approximately 10 ml intradermal and subcutaneous). For CF subjects, the optional bone marrow aspiration procedure (Visit 1A) must be completed at least 14 days before Day 1 (Visit 2). Preservative-free bupivacaine 0.25% (approximately 10-20 ml injected locally to the site), morphine sulfate (approximately 2-6 mg by IV), and lorazepam (1 mg oral or IV) may be used if needed. Approximately 5-20 ml of marrow is pulled into a syringe containing preservative-free heparin (400 units/25cc

marrow) using a brisk pull. MSCs isolated from subjects with CF will be evaluated in pre-clinical models to determine how they differ from donor MSCs. The CCC and NCRM at Case Western Reserve University have emerging cell therapy assay methods so that BM samples from subjects with CF are able to be archived and used at a later time to more fully characterize the CF defect.

The risks of a bone marrow aspiration include pain at the time of the aspirate, which is common but well controlled with adequate use of Xylocaine, bupivacaine and morphine. Other risks include bleeding, infection at the site of the aspirate, and occasional lightheadedness or fainting due to a vasovagal reaction after the procedure. Healthy volunteers and subjects with CF, who undergo the optional bone marrow examination, will be followed by the study PI or designee for AEs related to the procedure.

Inclusion Criteria for Healthy Volunteer Donors:

1) Male/female age ≥ 18 years
2) Able to understand and sign ICF (a legally authorized representative will not be permitted)

Inclusion Criteria for CF Donors:

1) CF subject enrolled in the main study and consented to this optional procedure

Exclusion Criteria for both Healthy Volunteer Donors and CF Donors:

1) Fever or current illness on the day of the cell collection
2) Evidence of communicable disease.
3) Any significant change in health status within 2 weeks prior to cell collection that the PI/Sub-Investigator deems relevant to exclude participation
4) Subject-reported history of organ transplantation
5) Subject-reported history of HIV, hepatitis B or C, or syphilis
6) For HV donors only, subject-reported known history of being diagnosed with cystic fibrosis (CF) or being a CF carrier (one copy of CF gene mutation)
7) Positive screening blood test result for any infectious disease.
8) For HV donors only, positive test result for CMV or a CF gene mutation.
9) Pregnant, planning a pregnancy, or breast-feeding at screening.

9.1.11 Adverse Events

Information regarding occurrence of AEs will be captured throughout the study from the time of consent until Telephone Call 4 (Month 12 ±1). Duration (start and stop dates), severity/grade, outcome, treatment and relation to investigational product or study procedures will be recorded in the CRF.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood (5 ml) will be obtained at all study visits and sent to the clinical hematology laboratory at University Hospitals Cleveland Medical Center for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), ESR, and serum hs-CRP determinations for assessment of systemic evidence for infection and/or inflammation.

9.2.2 Blood Chemistry Profile

Blood (5 ml) will be obtained at all study visits and sent to the clinical chemistry laboratory at University Hospitals Cleveland Medical Center for determination of sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, direct bilirubin, albumin and LDH. Because vitamin D may influence hMSC function, a vitamin D concentration will be obtained at Visit 2 and analyzed *post hoc*.

9.2.3 Pregnancy Test

A pregnancy test will be performed on female subjects who are of childbearing potential prior to their participation in the study. A serum pregnancy test will be done at screening (blood volume is included in the sample collected for chemistry profile) and urine pregnancy tests will be done at Visits 2, 5 and early termination.

9.2.4 Urinalysis

Urine will be obtained at all study visits and sent to the clinical laboratory at University Hospitals Cleveland Medical Center for determination of color, specific gravity, pH, protein, glucose, ketones, and blood.

9.3 Research Assessments

9.3.1 Serum Measurements of Inflammatory Mediators

40-mL of whole blood will be collected and placed in serum separator tubes at Visits 2 (Day 1), 3 (Day 7), and 5 (Day 28). Samples will be centrifuged. The serum will be removed, aliquoted, and frozen at -80^{o}C. Specimens will be batched and analyzed for calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL8, IL-17, and TNF-α in the Bioanalyte Core of the CF Research Center at Case Western Reserve University.

9.3.2 Sputum Cell Count and Differential

Sputum (approximately 0.065 gm) will be collected at Visits 2 (Day 1), 3 (Day 7), and 5 (Day 28) for determination of white cell counts (by hand counting) and differential cell counts performed by the CF-Therapeutics Development Network (TDN) Center for Interpretive Cytology at Case Western Reserve University according to the current CF-TDN SOP.

9.3.3 Sputum Cytokine Measurements

Sputum (approximately 0.75 gm) for determination of IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, neutrophil elastase, α_1-anti-trypsin, and MMP-9 will be collected at Visits 2 (Day 1), 3 (Day 7), and 5 (Day 28). Specimens will be collected in a sterile specimen cups. Sputum will be processed according to the current CF-TDN SOP. Aliquots will be batched and analyzed in the Bioanalyte Core of the CF Research Center at Case Western Reserve University.

9.3.4 Quantitative Bacteriology

Sputum (approximately 0.5 gm) will be collected at Visits 2 (Day 1), 3 (Day 7), and 5 (Day 28) for culture. All sputum specimens will be collected in a sterile specimen cup, Quantitative culture for typical CF pathogens will be performed in the clinical microbiology laboratory at University Hospitals Cleveland Medical Center.

9.3.5 Respiratory and Systemic Symptoms Questionnaire (RSSQ)

Investigators or their designee will interview each subject to complete an RSSQ (Boehringer Ingelheim), which is designed to capture signs and symptoms, at each study visit and for any other encounter for increased respiratory symptoms. Such encounters may occur between study visits and include unscheduled visits and phone encounters. The RSSQ must be completed prior to any other study procedures.

9.3.6 Cystic Fibrosis Respiratory Symptom Diary (CFRSD)

Study participants will complete the CFRSD (University of Washington) five times, at Visits 2-5 and once at home between Visits 4 and 5. The CFRSD will also be completed if the subject has an acute illness visit between Visit 2 and Visit 5. The CFRSD is designed to capture a change in symptoms and to quantify their severity and magnitude for the previous 24 hours during periods of stability, medically treated pulmonary exacerbations, and recovery from pulmonary exacerbations. The CFRSD will be reviewed at Visits 2-5, during Phone Call #2, and at any applicable Acute Illness Visit/Early Termination Visit. The CFRSD may be reviewed after the RSSQ is performed but must be reviewed prior to any other study procedures.

9.4 Screening Assessments for Bone Marrow Collection

9.4.1 Donor History Questionnaire (DHQ) for Healthy Volunteers Participating in the Bone Marrow Collection

The DHQ and its related materials (e.g., Medication Deferral List, Donor Educational Materials) will be used as a tool to screen donors for communicable disease risk factors. The DHQ and related documents will be obtained from the American Association of Blood Banks (AABB) website.

9.4.2 Measurements of Infectious Disease Markers for Healthy Volunteers Participating in the Bone Marrow Collection

Blood (28ml) will be obtained and sent to the Indiana Blood Center for determination of the following infectious disease markers: hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), human immunodeficiency virus (HIV1,2), hepatitis B core antibody (HBcore), human T-cell lymphotropic virus types I and II (HTLVI/II), blood group and Rh antigen (ABORh), antibody screen (AbScr), Treponema pallidum; cytomegalovirus (CMV), HIV nucleic acid test (HIVNAT), hepatitis C nucleic acid test (HCVNAT), West Nile Virus nucleic acid test (WNVNAT), and Chagas. Any positive result will be reported to the subject (verbally and in writing) and to any local medical agencies as required by law, and the subject will be instructed to follow-up with a primary care physician or will be provided a referral to an appropriate physician. Bone marrow collection will occur within 7 days of communicable disease testing, per FDA guidelines stipulated in 21CFR.1271.80(b).

9.4.3 Cystic Fibrosis Genetic Screening for Healthy Volunteers Participating in the Bone Marrow Collection

Blood (2-4ml) will be obtained and sent to the Center for Human Genetics Laboratory of University Hospitals Cleveland Medical Center to determine the carrier status of an individual for the most common 41 mutations in the CFTR gene associated with cystic fibrosis. Any positive results will be reported to the subject and genetic counseling will be provided.

9.4.4 Measurements of Infectious Disease Markers for CF Subjects Participating in the Optional Bone Marrow Collection

Blood (10 ml) will be obtained and sent to the University Hospitals Cleveland Medical Center Laboratory for determination of the following infectious disease markers: hepatitis B and C, syphilis and HIV. Any positive result will be reported to the subject (verbally and in writing) and to any local medical agencies as required by law, and the subject will be instructed to follow-up with a primary care physician or will be provided a referral to an appropriate physician.

9.4.5 Pregnancy Test

A serum pregnancy test will be performed on female subjects who are of childbearing potential during the screening visit (Visit 1) for both healthy volunteer donors and CF subject donors. For healthy volunteer donors, up to 2ml of blood will be obtained for this test. For CF subject donors, the serum pregnancy test is included as part of the screening visit procedures for the main study described earlier. A urine pregnancy test will be performed on female subjects of childbearing potential at Visit 2 for heathy volunteer donors and at Visit 1A for CF subject donors.

10 EVALUATIONS BY VISIT

In general, the order of the items listed below will be the chronological order in which they will be performed at each study visit. However, this order may change once the logistics are finalized for each visit prior to the start of the clinical trial.

For CF Subjects:

10.1 Visit 1 (Screening Visit: Day -42 to -10)

1. Review the study with the subject and obtain written informed consent and HIPAA authorization
2. Review the “Consent for Specimen Banking” and the “Consent for Collection and Use of your CFF Registry ID Number” and ensure that the subject has checked one of the two options (Subjects who elect not to provide specimens for the bio-repository or to provide their CFF Registry ID number are still eligible for this study)
3. Assign the subject a unique screening number
4. Perform and record RSSQ prior to any other study procedures
5. Record demographics and other baseline data
6. Record medical and surgical history, including a history of CF, genotype, diagnosis date, and CF signs and symptoms
7. Record concomitant medications
8. Review inclusion/exclusion criteria

9. Measure and record height and weight without shoes
10. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
11. Perform and record oximetry
12. Perform a complete physical examination
13. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
14. Collect urine for clinical laboratory test (urinalysis)
15. Collect blood for serum pregnancy test
16. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum at any visit
17. Perform and record spirometry
18. Schedule subject for ***Visit 2***
19. Screening procedures for optional diagnostic bone marrow aspiration, if applicable:
    a. Ensure that the subject has checked one of the two options regarding this optional procedure on the consent form. (Subjects who elect not to complete this procedure are still eligible for this study.)
    b. Review eligibility for bone marrow aspiration
    c. Collect 10 ml blood for laboratory tests (infectious disease markers)
20. Record any AEs that may have occurred during the study visit from the time of consent

10.2 Visit 1A (if applicable, Optional Bone Marrow Aspiration) (Day -35 to -14)

1. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
2. Review and record interval history
3. Review and record any changes to concomitant medications
4. Perform urine pregnancy test if applicable
5. Perform an abbreviated physical exam
6. Review inclusion/exclusion criteria
7. Perform bone marrow aspiration
8. Subject to rest for 30 minutes post-procedure
9. Record any AEs that may have occurred during the study visit

10.3 Follow-up Phone Call (if applicable, 24 (± 12) hours after Optional Bone Marrow Aspiration)

1. Review and record interval history
2. Review and record any changes to concomitant medications
3. Record any AEs that may have occurred since bone marrow aspiration

10.4 Visit 2 (Baseline Visit: Day 1)

1. Perform and record RSSQ prior to any other study procedures
2. Administer and review CFRSD prior to any other study procedures and record any AEs that occurred since Visit 1
3. Review and record interval history
4. Review and record any changes to concomitant medications
5. Review inclusion/exclusion criteria
6. Measure and record weight without shoes
7. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
8. Perform and record oximetry
9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH) and vitamin D
10. Collect blood for inflammatory mediators (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL-8, IL-17, and TNF-α)
11. Collect urine for clinical laboratory test (urinalysis)
12. Collect urine for pregnancy test prior to infusion
13. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
14. Perform and record spirometry approximately 2 hours before hMSC infusion
15. Obtain sputum for clinical laboratory tests (quantitative culture and microbiology) and for research tests (cell count with differential, IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, neutrophil elastase, α_1-anti-trypsin, and MMP-9). Subjects will be asked to provide sputum via spontaneous expectoration. If a subject is unable to provide sputum spontaneously, then the subject may undergo sputum induction according to the current TDN SOP. Any AEs related to the sputum procedure will be recorded.
16. Temperature, blood pressure, heart rate, respiratory rate and oxygen-saturation are measured prior to the infusion. Subjects should be seated for 5 minutes prior to obtaining vital signs.
17. Perform a complete physical examination
18. Infusion of hMSCs
19. Temperature, blood pressure, heart rate, respiratory rate and oxygen-saturation are measured prior to infusion and at 10 (± 5), 20 (± 5), 30 (± 5), 60 (± 15), and 90 (± 15) minutes and 2 hours (± 15 minutes) and 4 hours (± 15 minutes) post-infusion completion and then every 4 (± 15 minutes) hours for the remainder of the 24-hour observation period. Subjects should be seated or lying down for 5 minutes prior to obtaining vital signs.
20. Administer 3 puffs of albuterol 15 minutes (± 15 minutes) after the completion of the hMSC infusion if subject is likely to require induction to obtain sputum during the visit
21. Perform and record spirometry 30 minutes and 4 hours (± 15 minutes) after the completion of the hMSC infusion

22. Monitor study participant for 24 hours (± 30 minutes) after completion of the infusion
23. Record any AEs that may have occurred during the study visit

10.5 Visit 2 (Baseline Visit: Day 2)

1. Perform and record RSSQ prior to any other study procedures
2. Review and record interval history
3. Review and record any changes to concomitant medications
4. Measure and record weight without shoes
5. Perform and record vital signs, including a blood pressure after the subject has been seated or lying down for 5 minutes
6. Perform and record oximetry
7. Perform a complete physical examination
8. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
9. Collect urine for clinical laboratory test (urinalysis)
10. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
11. Perform and record spirometry 24 hours (± 1 hour) after the completion of the hMSC infusion
12. Schedule subject for ***Telephone Call Visit 1*** and for ***Visit 3***.
13. Record any AEs that may have occurred during the study visit

10.6 Telephone Call 1 (Day 4 or 5)

1. Review and record interval history since Visit 2
2. Perform and record RSSQ

10.7 Visit 3 (Day 7 ± 2)

1. Review and record RSSQ prior to any other study procedures
2. Administer and review CFRSD prior to any other study procedures and record any AEs that occurred since Visit 2
3. Review and record interval history
4. Review and record any changes to concomitant medications
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical examination

9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
10. Collect blood for inflammatory mediators (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL-8, IL-17, and TNF-α)
11. Collect urine for clinical laboratory test (urinalysis)
12. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
13. Perform and record spirometry
14. Obtain sputum for clinical laboratory tests (quantitative culture and microbiology) and for research tests (cell count with differential, IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, neutrophil elastase, α_1-anti-trypsin, and MMP-9). Subjects will be asked to provide sputum via spontaneous expectoration. If a subject is unable to provide sputum spontaneously, then the subject may undergo sputum induction according to the current TDN SOP. Any AEs related to the sputum procedure will be recorded
15. Schedule subject for ***Visit 4***
16. Record any AEs that may have occurred during the study visit

10.8 Visit 4 (Day 14 ± 2)

1. Perform and record RSSQ prior to any other study procedures
2. Administer and review CFRSD prior to any other study procedures and record any AEs that occurred since Visit 3
3. Distribute CFRSD that the subject must complete between Visit 4 and Visit 5
4. Review and record interval history
5. Review and record any changes to concomitant medications
6. Measure and record weight without shoes
7. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes.
8. Perform and record oximetry
9. Perform abbreviated physical examination
10. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
11. Collect urine for clinical laboratory test (urinalysis)
12. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
13. Perform and record spirometry
14. Schedule Subject for ***Telephone Call 2*** and for ***Visit 5***
15. Record any AEs that may have occurred during the study visit

10.9 Telephone Call 2 (Day 21 ± 2)

1. Review and record interval history since Visit 4
2. Perform and record RSSQ
3. Review CFRSD and record any AEs that occurred since Visit 4

10.10 Visit 5 (Day 28 ± 2)

1. Review and record RSSQ prior to any other study procedures
2. Administer and review CFRSD prior to any other study procedures and record any AEs that occurred since Telephone call 2
3. Review and record interval history
4. Review and record any changes to concomitant medications
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform complete physical examination
9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
10. Collect blood for inflammatory mediators (calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL-8, IL-17, and TNF-α)
11. Collect urine for clinical laboratory test (urinalysis)
12. Collect urine for pregnancy test
13. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
14. Perform and record spirometry
15. Obtain sputum for clinical laboratory tests (quantitative culture and microbiology) and for research tests (cell count with differential, IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, neutrophil elastase, α_1-anti-trypsin, and MMP-9). Subjects will be asked to provide sputum via spontaneous expectoration. If a subject is unable to provide sputum spontaneously, then the subject may undergo sputum induction according to the current TDN SOP. Any AEs related to the sputum procedure will be recorded
16. Schedule subject for ***Telephone Call 3*** and for ***Visit 6***
17. Record any AEs that may have occurred during the study visit

10.11 Telephone Call 3 (Day 56 ± 2)

1. Review and record interval history since Visit 5
2. Perform and record RSSQ
3. Record any AEs that occurred since Visit 5

10.12 Visit 6 (Month 3 ± 14 days)

1. Perform and record RSSQ prior to any other study procedures
2. Review and record interval history
3. Review and record any changes to concomitant medications
4. Record any AEs that occurred since Telephone call 3
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical examination
9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
10. Collect urine for clinical laboratory test (urinalysis)
11. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
12. Perform and record spirometry
13. Schedule Subject for ***Visit 7***
14. Record any AEs that may have occurred during the study visit

10.13 Visit 7 (Month 6 ± 14 days)

1. Perform and record RSSQ prior to any other study procedures
2. Review and record interval history
3. Review and record any changes to concomitant medications
4. Record any AEs that occurred since Visit 6
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical examination
9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
10. Collect urine for clinical laboratory test (urinalysis)
11. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject is likely to require induction to obtain sputum during the visit
12. Perform and record spirometry
13. Schedule Subject for ***Telephone Call 4***
14. Record any AEs that may have occurred during the study visit

10.14 Telephone Call 4 (Month 12 ± 1)

1. Review and record interval history since Visit 7
2. Record any AEs that occurred since Visit 7

10.15 Acute Illness/Early Termination Visit

1. Review and record RSSQ prior to any other study procedures
2. If Acute Illness or Early Termination Visit occurs before Visit 5, administer and review CFRSD prior to any other study procedures and record any AEs that occurred since last Visit or Telephone call
3. Review and record interval history
4. Review and record any changes to concomitant medications
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform a complete physical examination
9. Collect blood for clinical laboratory tests (comprehensive chemistry panel, ESR, hs-CRP, complete blood count with differential, direct bilirubin and LDH)
10. Collect urine for clinical laboratory test (urinalysis)
11. Collect urine for pregnancy test
12. Administer 3 puffs of albuterol 15 minutes prior to spirometry if subject performed a sputum induction during the study
13. Perform and record spirometry
14. Record any AEs that may have occurred during the study visit

For Healthy Volunteer Subjects:

10.16 Visit 1 (Screening Visit: Day -30 to -7)

1. Review the study with the subject and obtain written informed consent and HIPAA authorization
2. Assign the subject a unique screening number
3. Record demographics and other baseline data
4. Record medical and surgical history
5. Record concomitant medications
6. Review inclusion/exclusion criteria
7. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
8. Complete Donor History Questionnaire (DHQ)

9. Collect 32 ml blood for laboratory tests (infectious disease markers, CMV, CF mutations). Collect 2 ml blood for serum pregnancy test if applicable.
10. Record any AEs that may have occurred during the study visit from the time of consent

10.17 Visit 2 (Bone Marrow Aspiration)

1. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
2. Review and record interval history
3. Review and record any changes to concomitant medications
4. Perform urine pregnancy test if applicable
5. Perform an abbreviated physical exam
6. Review inclusion/exclusion criteria
7. Perform bone marrow aspiration
8. Subject to rest for 30 minutes post-procedure
9. Record any AEs that may have occurred during the study visit

10.18 Follow-up Phone Call (24 (± 12) hours after Bone Marrow Aspiration)

1. Review and record interval history
2. Review and record any changes to concomitant medications
3. Record any AEs that may have occurred since bone marrow aspiration

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An AE is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment that occurs from the time of consent through Telephone Call 4 (Month 12 ±1). An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator or Research Coordinator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. AEs will be recorded in the subject CRF. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to investigational product, or if unrelated, the cause.

AE Severity

The NCI's CTCAE Version 4.0, summarized in Table 2 below, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 2. AE Severity Grading (based on NCI's CTCAE Version 4.0)

Severity (Toxicity Grade)	Description
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

A Semi-colon indicates 'or' within the description of the grade.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

AE Relationship to Investigational Product

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 3.

Table 3. AE Relationship to Investigational Product

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.

Relationship to Drug	Comment
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the investigational product.

11.2 Serious Adverse Events (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

All SAEs will be documented (whether or not related to investigational product) on a SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end at Telephone Call 4 (Month 12 ±1).

All SAEs will be reviewed by the site investigator and sent to the TDNCC, within one business day of the site learning of the event. The site will scan and email or fax the SAE report to:

Email address: cfsaesfacsys@seattlechildrens.org

Direct dial fax number: (206) 985-3278

Study staff will notify the TDNCC of additional information or follow-up to an initial SAE Report as soon as relevant information is available. Follow-up information is reported on an SAE Report Form.

In accordance with the standard operating procedures and policies of the local IRB, the site investigator will report SAEs to the IRB. SAEs will be reported to the FDA in accordance with 21 CFR 312.32.

11.3 Medical Monitoring

TDNCC Medical Monitoring Group should be contacted directly at this number to report medical concerns or questions regarding safety:

Pager: (800) 341-0961

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Withdrawal of Subjects and Drop-outs

A subject may be discontinued from the study at any time if the subject, the investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study or treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event
- Lost to follow-up
- Physician request for early termination of study

If a subject experiences an AE, the subject will be followed and treated by the Investigator or the subject's physician until the abnormal parameter or symptom has resolved or stabilized. Subjects will be encouraged to complete an Early Termination Visit.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. If the subject withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The Investigator may retain and continue to use any data collected before such withdrawal of consent. Any samples already being analyzed will be completed and any stored samples will be destroyed if still linkable.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw from the study will be replaced if they withdraw before Visit 5. Refer to Section 10 for early termination procedures. If a subject withdraws on the day of hMSC infusion, then the Early Termination Visit will be performed on Day 4-9.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator, fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol Violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure of the investigators or study staff to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The medical monitor will determine if a protocol violation will result in withdrawal of a subject. When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by the Investigator. A copy of the form will be filed in the site's regulatory binder.

14 DATA SAFETY MONITORING

The Cystic Fibrosis Foundation Data Safety Monitoring Board (DSMB) will establish a Data Monitoring Committee (DMC) to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study, according to the Cystic Fibrosis Foundation Data Safety Monitoring Board Operations Manual and a DMC Charter to be established for this protocol. There will be 3 interim review(s) conducted by the DMC for the purpose of monitoring study conduct and assessing subject safety. Enrollment for subsequent cohorts will not begin until after the DMC reviews the data for the current cohort and approves proceeding to the next cohort. Further details regarding the timing and content of the interim reviews is included in the statistical section below.

15 STATISTICAL METHODS AND CONSIDERATIONS (BASED UPON PRIMARY AND SECONDARY ENDPOINTS)

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible subjects who are enrolled into the study and receive at least one dose of the investigational product (the Safety Population) will be included in the safety analysis. The analyses will be conducted on all data available. Subjects with missing data for a particular analysis will not be included as applicable.

15.2 Demographic and Baseline Characteristics

Demographic and baseline clinical characteristics including age, gender, race, CFTR genotype, height, weight, oxygen saturation, sputum microbiology (presence of *P. aeruginosa*, methicillin-sensitive *S. aureus*, methicillin-resistant *S. aureus*, and/other Gram-negative bacteria) and pulmonary function will be reported for each subject and summarized overall using descriptive statistics such as frequency, percentage, mean or median and range. For all analyses, Baseline will be defined as the measurement prior and closest to the administration of the infusion of hMSCs.

15.3 Analysis of Primary Endpoint

The primary endpoint of this study is to determine the maximum tolerate dose among 1 x 10^6 hMSCs/kg, 3 x 10^6 hMSCs/kg, and 5 x 10^6 hMSCs/kg. Safety data will be analyzed at both the individual level and the dose cohort level. Safety will be evaluated using reports of adverse events (AE) and measurements of vital signs, oxygen saturation, pulmonary function, laboratory parameters and adverse events associated with procedures (sputum induction for those subjects unable to spontaneously expectorate sputum and optional bone marrow aspiration). All measurements will be reported at each time point, changes from Baseline and from the previous time point will be summarized at each time point. Premature withdrawals and reasons of withdrawals will be listed.

Clinically significant AEs and alterations in vital signs, oxygen saturation, and pulmonary function at baseline, 30 minutes, 4 hours and 24 hours after the infusion will be summarized by patient and by dose cohort using frequency/percentage and mean or median/range.

Overall AEs and SAEs in each dose cohort will be coded by body system and MedDRA classification term using frequency and percentage. Frequency and percentage of all grades and grades ≥ 3 will be reported.

AEs among dose cohorts will be compared using Fisher's exact test. All clinically significant vital sign and physical examination changes as well as laboratory test abnormalities will be presented in AE listings.

Mean or percentage of clinically significant AEs, alterations in vital signs (including decreases in systolic blood pressure ≥ 20 mmHg or diastolic blood pressure ≥ 10 mmHg), spirometry measurements (FEV_{1} %, FEV_{1} (Liters), FEF_{25-75}), sputum quantitative microbiology (bacterial colony forming units) and hematology, comprehensive chemistry, ESR, hs-CRP and urinalysis results measured at Baseline and at the follow-up visits will be graphically presented.

15.4 Analysis of Secondary/Exploratory Endpoints

Although efficacy is not the primary aim of this dose escalation study, changes and patterns of change between MTD and lower doses on inflammatory biomarkers in blood and sputum measured at Baseline, day 7 and day 28 will be explored using a mixed effects model, mean and standard deviation at each time point as well as each patient's case profile will be graphically presented.

15.5 Interim Analysis

No interim statistical analysis is planned.

15.6 Sample Size

This is a single center Phase I study. Enrollment of 15 subjects will be feasible and allow us to ascertain if there are any clinically meaningful infusion-related adverse effects.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database but will be identified by subject number and initials.

All clinical information requested in this protocol will be entered into the CRFs. The study data will be entered into case report forms using Medidata Rave eCRFs which will be supported by the TDN Clinical Data Management and Programming Core at Seattle Children's Hospital. Medidata Rave is certified 21 CFR Part 11 compliant. Corrections to CRFs will have an audit trail.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. All study records will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

All procedures for the handling and analysis of data will be conducted using good clinical practice (GCP) meeting FDA guidelines for the handling and analysis of data for clinical trials. All data extracted from Medidata Rave will be downloaded to a 21 CFR Part 11 compliant cloud-based space where any manipulation of the data would occur.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis.

16.4 Archival of Data

Appropriate archiving of the database will be completed according to Medidata Rave procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to authorized personnel, IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (subject files, signed informed consent forms, CRFs, Study File Notebook, etc.) must be kept secured for a period of 2 years following marketing of the investigational product or for 2 years after centers have been notified that the IND has been discontinued.

16.6 Monitoring

This is a single site investigator initiated study. This study will be monitored by representatives from the office of the University Hospitals Cleveland Medical Center for Clinical Research and Technology per the monitoring plan as described in DMC charter. By signing this protocol, the Investigator grants permission to the office of the University Hospitals Cleveland Medical Center for Clinical Research and Technology (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number and subject initials will identify all study subjects on CRFs. Laboratory reports, spirometry reports, CRU flow sheets, and CRU physician order sheets will contain the subject's name and medical registration number.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All non-electronic study records will be kept in a locked file cabinet/file room and code sheets linking a subject's name to a subject identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be the responsibility of the Principal Investigator.

A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB is notified per IRB policy.

17.2 Institutional Review Board

The protocol and consent form will be reviewed and approved by the IRB at University Hospitals Cleveland Medical Center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain a list of IRB members or other assurance of compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning subject recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB will be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the subjects of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization for submission to the IRB. The consent form generated by the Investigator must be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will retain an IRB-approved copy of the Informed Consent Form for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.3.1 Consent for Collection and Use of CFF Registry ID Number and Sample Banking

Any remaining research samples will be stored for future studies in secured -80^{o}C freezers in the laboratories of the Cystic Fibrosis Research Center at Case Western Reserve University/University Hospitals Cleveland Medical Center. Samples will be labeled with the date, the subject's unique identifying number, and visit number.

To facilitate possible future evaluation of long-term outcomes information from all patients who screen for this study, consent will also be sought to collect the subject's CFF Registry ID number at the screening visit. The CFF registry collects data on all CF patients followed at CFF-accredited care centers at each clinical encounter, at each hospitalization or course of home IV antibiotics, and via a year-end survey. If specific consent is given to collect this number, the patient's CF registry number will be recorded in the CRF.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study funding agency (Cystic Fibrosis Foundation Therapeutics) and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

1. Conduct the study in accordance with the protocol and only make changes after obtaining appropriate approval, except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the medical monitor any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection.
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

18 APPENDIX I: DEFINITION OF A PULMONARY EXACERBATION (MODIFIED FUCHS CRITERIA)[95]

An exacerbation of respiratory symptoms is defined as a subject undergoing a change in antibiotic therapy for any 4 of the following 12 signs or symptoms:

1. Change in sputum
2. New or increased hemoptysis
3. Increased cough
4. Increased dyspnea
5. Malaise, fatigue, or lethargy
6. Temperature above 38 °C
7. Anorexia or weight loss
8. Sinus pain or tenderness
9. Change in sinus discharge
10. Change in physical examination of the chest
11. Decrease in pulmonary function by 10 percent or more from a previously recorded value
12. Radiographic changes indicative of pulmonary infection

19 APPENDIX II. SCHEDULE OF STUDY VISITS

	Visit 1 Screening	**Visit 2 Baseline**		**Phone Call 1**	**Visit 3**	**Visit 4**	**Phone Call 2**	**Visit 5**	**Phone Call 3**	**Visit 6 and Visit 7**	**Acute Illness[p]/ Early Term. Visit[q]**	**Phone Call 4**
	Day -42 to -10	**Day 1**	**Day 2**	**Day 4 or 5**	**Day 7 ± 2**	**Day 14 ± 2**	**Day 21 ± 2**	**Day 28 ± 2**	**Day 56 ± 2**	**Month 3 ± 14 days and Month 6 ± 14 days**		**Month 12 ± 1**
Informed consent	X											
Medical & surgical history	X											
Demographics and baseline characteristics	X											
Interval history		X[c]	X	X	X	X	X	X	X	X	X	X
Review previous and concomitant therapy	X	X[c]	X		X	X		X		X	X	
Review inclusion/exclusion criteria	X	X[c]										
Height[a], weight, vital signs, oximetry	X	X[c]	X		X	X		X		X	X	
Complete physical exam	X	X[c]	X					X			X	
Abbreviated physical exam					X	X				X		
Blood draw-safety labs[g]	X	X[c]	X		X	X		X		X	X	
Blood draw-vitamin D		X[c]										
Blood draw inflammatory mediators[h]		X[c]			X			X				

	Visit 1 Screening	**Visit 2 Baseline**		**Phone Call 1**	**Visit 3**	**Visit 4**	**Phone Call 2**	**Visit 5**	**Phone Call 3**	**Visit 6 and Visit 7**	**Acute Illness[p]/ Early Term. Visit[q]**	**Phone Call 4**
	Day -42 to -10	**Day 1**	**Day 2**	**Day 4 or 5**	**Day 7 ± 2**	**Day 14 ± 2**	**Day 21 ± 2**	**Day 28 ± 2**	**Day 56 ± 2**	**Month 3 ± 14 days and Month 6 ± 14 days**		**Month 12 ± 1**
Serum pregnancy test (if applicable)	X											
Urinalysis	X	X[c]	X		X	X		X		X	X	
Urine pregnancy test (if applicable)		X[c]						X			X	
Spirometry	X	X[c, d, e]	X[o]		X[e]	X		X[e]		X	X	
Acquisition of sputum[f]		X[c]			X			X				
Sputum quantitative bacteriology		X[c]			X			X				
Sputum cell count		X[c]			X			X				
Sputum cytokine measurements[i]		X[c]			X			X				
RSSQ	X	X[c]	X	X[j]	X	X	X[j]	X	X[j]	X	X	
CFRSD		X			X	X	X	X			X[k]	
Diagnostic bone marrow exam (optional)[b]	X											
Investigational product (hMSC) infusion		X[l, m, n]										
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X

[a] Height will only be obtained one time in the study at the Screening Visit
[b] See Appendix IIa for Schedule of Events for Stem Cell Collection from CF Donors
[c] Other procedures and collections should be done prior to infusion of stem cells
[d] On the day of hMSC infusion, spirometry should be performed approximately 2 hours prior to infusion and 30 minutes (± 15 minutes) and 4 hours (±15 minutes) after completion of infusion
[e] At visits 2, 4, and 6, spirometry should be performed prior to the acquisition of sputum
[f] Sputum can be collected by spontaneous expectoration. In those subjects unable to spontaneously expectorate, sputum may be induced by inhalation with 3% hypertonic saline using the current TDN SOP
[g] CBC with differential, comprehensive chemistry panel, hs-CRP, ESR, direct bilirubin and LDH
[h] Calprotectin, MPO, GM-CSF, IL-1β, IL-6, IL8, IL-17, and TNF-α
[I] IL-1β, IL-6, IL-8, IL-10, IL-17, GM-CSF, MIP-3α, TNF-α, neutrophil elastase, α_1-anti-trypsin, and MMP-9
[j] RSSQ should be administered if any increased respiratory symptoms
[k] Only if Acute Illness/Early Termination Visit is prior to Visit 5
[l] MSCs will be derived from bone marrow samples obtained from healthy volunteers and processed and evaluated according to CCC SOP well in advanced of infusion. CF study staff will inform CCC collaborators when hMSCs will be needed approximately 14 days prior to infusion. CCC collaborators will prepare the proper dose to be infused based upon the weight of the subject with CF
[m] Infusion is given by slow IV push and takes less than 30 minutes depending upon the volume to be infused
[n] Subjects will be monitored in the CRU for 24 hours after the completion of the infusion. Temperature, blood pressure, heart rate, respiratory rate and oxygen-saturation are measured prior to infusion and at 10 (± 5), 20 (± 5), 30 (± 5), 60 (± 15), and 90 (± 15) minutes and 2 hours (±15 minutes) and 4 (± 15 minutes) hours post-infusion and then every 4 (± 15 minutes) hours for the remainder of the 24-hour observation period.
[o] On Day 2, spirometry should be performed at 24 (± 1) hours after completion of infusion
[p] An Acute Illness Visit will be arranged for any subject who experiences any abrupt change in baseline clinical status, between Visit 2 and Visit 5
[q] An Early Termination Visit will be completed for any subject terminating study participation between Visit 2 and Visit 7.

APPENDIX IIa. SCHEDULE OF STUDY VISITS FOR OPTIONAL STEM CELL COLLECTION FROM CF SUBJECT DONORS

	Visit 1 Screening	**Visit 1A**	**Follow-up Phone Call**
	Day -42 to -10[1]	**Day -35 to -14**	**24 (± 12) hours after bone marrow aspiration**
Informed consent	X		
Interval history		X	X
Review previous and concomitant therapy		X	X
Review inclusion/exclusion criteria	X	X	
Phlebotomy for infectious disease limited panel	X		
Vital signs		X	
Urine pregnancy test (if applicable)		X	
Abbreviated physical exam		X	
Bone marrow aspiration of the posterior iliac crest		X	
Adverse events	X	X	X

[1]The Visit 1 Screening window in this table refers to the main study (for consistency with the table in Appendix II). If a CF subject plans to participate in the optional stem cell collection procedure at Visit 1A, then the Visit 1 Screening must be completed by Day -14 or earlier in order to complete Visit 1A and Visit 1 at the appropriate times.

APPENDIX IIb. SCHEDULE OF STUDY VISITS FOR STEM CELL COLLECTION FROM HEALTHY VOLUNTEER DONORS

	Visit 1 Screening	**Visit 2**	**Follow-up Phone Call**
	Day -30 to -7	**Day 1**	**24 (± 12) hours after bone marrow aspiration**
Informed consent	X		
Medical & surgical history	X		
Demographics and baseline characteristics	X		
Interval history		X	X
Review previous and concomitant therapy	X	X	X
Review inclusion/exclusion criteria	X	X	
DHQ	X		
Serum pregnancy test	X		
Phlebotomy for infectious disease panel including CMV	X		
Phlebotomy for CF mutation screening	X		
Vital signs	X	X	
Urine pregnancy test (if applicable)		X	
Abbreviated physical exam		X	
Bone marrow aspiration of the posterior iliac crest		X	
Adverse events	X	X	X

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Revision History Log

Revision Number	Revision or Update	Date of Release (ddMMMyyyy)	Approver
1.12	Employment of Revision History Log for document control purposes. Sponsor Investigator transfer from James Chmiel to Erica Roesch. Inclusion Criteria changed (#4) FEV ≥ 50% predicted to ≥ 40% predicted.	01NOV2018	Laura Dixon
1.13	Section 9.4.2 updated to indicate bone marrow collection will take place within 7 days of infectious disease testing.	17DEC2019	Laura Dixon