A PILOT STUDY: EVALUATING THE SAFETY AND FEASIBILITY OF USING AUTOLOGOUS ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (SVF) FOR THE TREATMENT OF AERO-DIGESTIVE FISTULAE IN ADULTS

Principal Investigator: Timothy A. Woodward, M.D.
Mayo Clinic Florida
4500 San Pablo Road
Jacksonville, Florida 32224

Study Product: Uncultured, autologous, adipose-derived stromal vascular fraction (SVF)

Protocol Number: (IRBe) 17-003774

IND Number: 17796

Version 1.0: [10/20/2017]
Version 1.1: [07/23/2018] Tracked Changes
Version 2.0 **[07/25/2018] Changes Accepted**
Version 2.1 **[9/17/2018] Tracked Changes**
Version 3.0 [9/18/2018] Changes Accepted

Table of Contents

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AMSC	Adipose-derived Mesenchymal Stem Cells
RNP	Registered Nurse Practitioner
CAD	Coronary Artery Disease
CFR	Code of Federal Regulations
CLD	Chronic Liver Disease
CRD	Chronic Renal Disease
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HTN	Hypertension
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
MNCs	Mononuclear Cells
MSCs	Mesenchymal Stem Cells
MADF	Malignant Aerodigestive Fistula
PHI	Protected Health Information
PI	Principal Investigator
PVD	Peripheral Vascular Disease
RTEF	Recurrent Tracheoesophageal Fistula
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
SVF	Stromal Vascular Fraction
WOC RN	Wound Ostomy Continence Registered Nurse

Study Summary

Title	A Pilot Study: Evaluating the Safety and Feasibility of Using Autologous Adipose-Derived Stromal Vascular Fraction (SVF) for the Treatment of Aerodigestive Fistulae in Adults
Running Title	Adipose Derived SVF for Aero-digestive Fistulae
Protocol Number	17-003774
Phase	Pilot Study
Methodology	Pilot of a small number of patients to test the safety and feasibility of autologous SVF administration for the treatment of aerodigestive fistulae.
Overall Study Duration	5 years
Subject Participation	Patients with tracheoesophageal, bronchoesophageal, and tracheopharyngeal fistulae.
Single or Multi-Site	Single site – Mayo Clinic in Florida
Objectives	Determine safety and feasibility of using institutionally prepared autologous, uncultured SVF on patients with aerodigestive fistulae secondary to malignancy, trauma or surgery.
Number of Subjects	10 patients.
Diagnosis and Main Inclusion/Exclusion Criteria	Patients older than 18 years old with aerodigestive fistulae. Either recurrent or de novo fistulae measuring between 2 - 15 mm Exclusion criteria: patients with severe malnutrition BMI <16 (which would interfere with the lipoaspiration procedure)
Study Product, Dose, Route, Regimen	**Study Product: Uncultured, autologous, adipose-derived stromal vascular fraction(SVF)** Two-thirds of the obtained SVF will be injected into the submucosa of the de-epithelialized fistula and one-third will be suspended without expansion in a fibrin sealant containing 5 $x10^6$ mononuclear cells per ml. The fibrin glue will be endoscopically injected inside the fistulous tract.
Duration of Administration	Permanently. Single SVF administration or until further management is warranted by either endoscopic or surgical intervention.
Statistical Methodology	There will be descriptive statistics for the pilot of 10 cases.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable regulations and Mayo Clinic research policies and procedures.

1.1 Specific Aims

The primary aim of this pilot study is to evaluate the feasibility, time, cost, safety, limitations, and efficacy of the use of institutionally processed SVF for management and closure of aero-digestive fistulae. This pilot study would help identify design issues and the potential success of fistulae closure by the means of autologous SVF administration before a full-scale trial is performed.

A secondary aim is the description of aero-digestive fistulae characteristics such as size, etiology, recurrence, localization, and the association of these factors with the outcome after SVF administration. The SVF quantification, characterization and differentiation in vitro will be described.

This process will help identify the type of fistulae that are susceptible to closure with human cell therapy.

1.2 Background

Stem Cell Therapy

Mesenchymal stem cells (MSCs) are multipotent cells capable of developing into several types of connective tissue such as muscle, fat, cartilage and bone. The use of MSCs for tissue regeneration is promising and the field of bioengineering has developed a variety of scaffolds for stem cell administration. Different biological sources and processing options are available. For instance, adipose-derived stem cells (AMSCs) can be isolated from human adipose tissue by the means of needle biopsy, excision or lipoaspiration. Subsequently, they can be transplanted to the same individual (autologous) or to a different individual (allogenic), with or without cell expansion and with or without the use of a scaffold. The scaffold may be either cellular or acellular. These variations may have an impact in the outcome of therapy and need to be evaluated for each case.

Adipose-derived stem cells have recently emerged as a more viable source for clinical applications, compared to bone-marrow mesenchymal stromal cells (BM-MSCs) because of their abundance and easy access. They have been used for the treatment of refractory and complex perianal fistulas in Crohn's disease as well as for the treatment of pressure ulcers and rectovaginal fistulas with promising outcomes. The stromal vascular fraction (SVF), isolated when fresh lipoaspirates are enzymatically digested with a collagenase, contains a heterogeneous cellular and extracellular milieu. SVF is an aqueous fraction, consisting of endothelial cells and their precursors, macrophages, smooth muscle cells, lymphocytes, pericytes, pre-adipocytes and actual AMSCs. Past studies have shown that such tissues contain progenitor cells that can release multiple angiogenic growth factors and cytokines including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and chemokine stromal cell-derived factor-1 (SDF-1).[1]

Pathology and impact of aerodigestive fistulas

An aero-digestive fistula refers to an abnormal communication between the respiratory and digestive tracts. These may be secondary to trauma, malignancy, surgery, and congenital malformations. Some examples of aero-digestive fistulae include: tracheo-esophageal, tracheo-pharyngeal, and broncho-esophageal fistulae. The esophagus has an immediate contact with the membranous portion of the trachea and the left bronchus. This predisposes to the formation of fistulous tracts between these structures.[2] Esophagorespiratory fistulae are uncommon. They usually form a communication between the esophagus and the trachea and less frequently, they enter a bronchus or the pleural cavity. They may also terminate in the mediastinum, pericardium or skin.[3]

The congenital type occurs with or without esophageal atresia or stenosis and usually communicates with the trachea. Esophagorespiratory fistulae in adults may be congenital. The etiology of the acquired fistulae, in their probable order of frequency, is as follows: 1. Carcinoma of the esophagus. 2. Trauma due to ingested foreign bodies, caustics, instrumental dilatation, and crushing injuries of the thorax. 3. Infections of the esophagus, trachea, bronchi, pleura, or mediastinum, due chiefly to tuberculosis and syphilis. Pathogenic organisms, fungi, and nonspecific infections may also be the cause. 4. Unknown etiology. 5. Esophageal traction diverticula. 6. Broncholithiasis. 7. Aneurysm. 8. Esophagomalacia. [3]

The symptoms and signs depend on the etiology, primary pathologic changes, and site of the fistula. Difficulty in swallowing of liquids is usually the initial symptom. Dysphagia increases, and later there are strangling, cough, and often dyspnea during meals. Swallowing in the dorsal recumbent position may minimize these symptoms. Sputum mixed with food is often raised in increasing amounts. It may contain purulent material and blood. Substernal or referred pain to the shoulders may be present. Food intake may be reduced to a minimum, with resulting loss of weight. A pathognomonical triad has been proposed (Saegesser et al., 1968): paroxysmal cough after liquid ingestion, episodical abdominal distension and recurrent bronchopulmonary infections .[2] Complications include chronic cough, recurring pneumonia, bronchiectasis, pulmonary abscess and gangrene, mediastinal abscess, fatal hemorrhage, and death if the trachea is suddenly blocked. [3]

Most esophagorespiratory fistulas are single; double or more complicated tracts have been recorded in cancer and after lye ingestion. The fistulous opening is located in the middle third of the esophagus in 60% of the cases and in the upper third in 25% of the cases. The remainder is located in the lower third or cervical esophagus. [2]

The pathological basis of malignant aerodigestive fistula (MADF) formation is the spreading of the esophageal cancer into the airways or lungs, or the propagation of pulmonary and mediastinal tumors into the esophagus. Primary malignancies that are most often causative are esophageal, lung and thyroid cancers, as well as, lymphoma.[4] Presumably, it occurs more frequently than the 5-10% incidence noted in literature, especially at the end stage of the malignant disease. Its early diagnosis and treatment is extremely important, because sealing the fistula can improve the survival and the quality of life of the patient.[5]

Current treatment for aerodigestive fistulas
Treatment varies according to the primary disease, complications, condition of the patient, and extent of the pathologic changes. There is rarely spontaneous closure and healing in view of the recurrent infections due to passage of food and saliva into the respiratory tract. Surgical therapy often comprises division of the fistulous tract and resection of irreversibly damaged lung tissue. Treatment of MADFs raises difficulties, especially when healing is compromised by prior irradiation. Endoluminal approaches have proven useful for these patients, especially when at time of diagnosis, the patient's performance and nutritional status precludes aggressive surgical therapy. Palliative measures have evolved over the years and have included surgical (i.e. enteric bypasses or surgical exclusion of the fistula), esophageal endoprothesis, esophageal intubation, application of self-expandable metallic stents and supportive medical therapy, with or without enterostomies.[4]

Impact of recurrent fistulae
Despite the relative success of surgical repair, recurrent tracheoesophageal fistula (RTEF) has been reported in up to 20% of patients. Unfortunately, open procedures on RTEF have been met with high levels of postoperative morbidity, mortality, and recurrence. This has led investigators to explore less invasive techniques in the surgical repair of RTEF. Endoscopic approaches using a ventilating bronchoscope were first introduced in the German literature in the mid-1970s. Since then, various endoscopic techniques in the repair of RTEF have been described and unfortunately, several attempts may be required before complete closure is achieved, which can result in repeated and potentially unnecessary, exposure to anesthetic risk.[6]

In a review of literature by Gresham et. al., a total of 15 articles were identified in the literature from 1975 to 2007 describing endoscopic techniques for the repair of RTEF. Various, but similar, techniques to endoscopic repair have been performed. These can be categorized into 3 approaches: de-epithelialization of the fistula, application of tissue adhesives, or a combination of the two. Fibrin glue and Histoacryl have been applied alone or in combination with de-epithelialization techniques to promote fibrin and scar tissue formation. The greatest chance for success, both as a first attempt and overall, was achieved in cases where a combined approach of de-epithelialization and placement of tissue adhesive was performed, 67.7% and 93.3%, respectively. Authors repeated therapy as needed to achieve successful closure. A collection of the entire population of reported cases, including all 3 approaches, illustrated a success rate of only 48.6% after one attempt at repair. Although techniques and likely surgical experience varied, the overall success rate including all techniques and despite the number of repairs was 81.1%.[6]

This data demonstrates the current need of novel therapies in order to enhance early fistulae closure and decrease the number of interventions before fistula healing is achieved.

Cell Therapy in the management of fistulas
In fistulising Crohn's disease, local injection of stem cells is considered to be beneficial with sustained efficacy based on the fact that AMSCs have the ability to inhibit surrounding inflammation by suppressing proliferation of activated lymphocytes and have some regenerative potential for replacement of the defective fistula tract. [7] Multiple trials demonstrate that AMSC treatment for patients with Crohn's fistulae (perianal[8], rectovaginal[9] and enterocutaneous[10]) was well tolerated with favorable therapeutic outcomes.

Autologous AMSC administration has been used in vivo for the closure of recurrent bronchopleural fistulae. Wigle et al reported the case of a recurrent bronchopleural fistula which after 30 bronchoscopic and surgical interventions, was finally closed by the means of a surgically implanted autologous adipose stem cell-seeded matrix.[11] Garcia-Arranz et al described a successful bronchoscopic intervention for the closure of recurrent bronchopleural fistulae in two patients using autologous, non-expanded AMSCs endoscopically injected into the fistulous de-epithelialized area.[12] Similarly, Garcia-Olmo et al treated a tracheomediastinal fistula with bronchoscopic injection of AMSCs suspended in fibrin glue with successful fistula re-epithelialization and closure.[13]It is hopeful that a heterogeneous population of stromal cells containing AMSCs would be similarly beneficial in RTEF.

Study Population
The population to be studied includes adults between 18 and 90 years old with tracheo-esophageal, tracheo-pharyngeal and broncho-esophageal fistulae measuring up to 1.5 cm. De novo and recurrent fistulas will be included in the inpatient and outpatient setting. Subjects will be identified in the inpatient or outpatient setting during consults and/or appointments with the Gastroenterology and/or Thoracic Surgery Departments. When a potential patient is identified, a research nurse (RN) consult will be initiated in order to identify if the patient meets all the inclusion criteria for the study. Baseline values, fistula assessment and a picture of the fistula will be obtained. Approximately twenty to thirty patients with aerodigestive fistulae are seen in this healthcare center per year across the departments of gastroenterology, thoracic surgery and otorhinolaryngology. In this study our goal is to have 10 subjects complete all study procedures up to at least the 3 month follow-up visit.

1.3 Investigational Agent
Adult stem cells have been found to be available in several tissues and organs such as skin, brain, liver, and bone marrow. The ability to self-renew and differentiate in multiple cell phenotypes is well established. Several research studies have used adult bone marrow derived stem cells but unfortunately, small numbers of stem cells are generated from bone marrow in the absence of culture expansion, and the amount of stem cells also decreases with age. More recently, adipose tissue has been used to isolate stem cells through a process of filtration, collagenase digestion, and centrifugation. It is more easily obtained and is found to differentiate into cells of the mesenchymal lineage (eg, bone, fat, cartilage, or muscle). Adipose-derived MSCs have a potential for angiogenesis and vasculogenesis and are able to produce cytokines in the pathologic tissue. For these reasons, they are of interest in the process of wound healing.[1]

More specifically, Mesenchymal stem cells (MSCs) are multipotent or able to differentiate into multiple tissue types. MSCs can give rise to bone, cartilage, fat, tendons, and ligaments. MSCs express the cell surface markers CD73, CD90, CD105, SCF R, STRO-1, but lack CD34, CD45, CD14, CD11b, CD19, CD79a, and HLA-DR (Kim et al., 2014). In addition to differentiating into different types of tissue to accelerate healing, MSCs secrete many different cytokines and growth factors that may aid in wound healing. These factors include: VEGF-α, G-CSF, GM-CSF, IGF-1, IL-2, IL-6, IL-7, IL-11, IL-11, LIF, bFGF, MCP-1 and SDF-1, among other factors (Blaber et al., 2012).

The stromal vascular fraction (SVF), isolated from fresh lipoaspirates and enzymatically digested results in an aqueous fraction of heterogeneous cells proteins and growth factors consisting of endothelial cells and their precursors, macrophages, smooth muscle cells, lymphocytes, pericytes, pre-adipocytes and actual AMSCs. The ultimate goal is to create a shorter healing time for the patient so immediate, same day, autologous application is a benefit. There is less manipulation of the cells in the uncultured state, possibly increasing the safety to the patient. This will also be an autologous application, making the risk of infection or rejection very low.

1.4 Clinical Data to Date

Most data to date involve the use of AMSCs. ADSC were systematically evaluated for their ability to rebuild volume in depressed scars following the subcutaneous injection of ADSC which differentiated towards adipocytes. This trial of 36 patients was completed in 2007 but has not, to our knowledge, yet been documented in any peer-reviewed international journal. Only one report relates to a field in which ADSCs have been evaluated for their reconstructive properties based on their classic mesenchymal differentiation potentials, specifically in the field of bone or cartilage reconstruction when the osteogenic or chondrogenic potentials of ADSC are well-established and widely investigated. In this case report with 36 months of follow-up, the defect was successfully reconstructed with a microvascular flap using betatricalcium phosphate, autologous ADSCs and bone morphogenetic protein-2 to trigger their osteogenesis. Although this result is encouraging, a case report cannot give prove a general effect and no conclusions can be definitively drawn until phase I and II trials have been conducted.

Most other ADSC trials concern fistula complications that result from tissue degeneration following an uncontrolled inflammatory process. In a noteworthy case report, Garcia-Olmo et al found that expanded ADSCs are more efficient than the freshly-prepared conterpart in treating Crohn's disease. The trials on fistula indicate that ADSCs are very efficient in controlling inflammation and improving the healing process. Garcia-Olmo et al published the conclusions of randomized phase I and II trials in which they compared injection of ADSC into rectal mucosa with fibrin glue to fibrin glue alone on 25 patients with complex perianal fistulas associated or not with Crohn's disease.

Following a first dose of 20 million ADSCs, a second dose of 40 million was administered 8 weeks later in cases where there was no initial healing. Patients were considered healed when a total epithelialization of the external opening was evident after 8 wk. Seventy one percent of patients treated with ADSC and fibrin glue displayed fistula healing compared to 16% observed in patients treated with fibrin glue alone ($P < 0.001$). This positive effect on healing is all the more remarkable as it is otherwise poorly documented, even in rodent models. Two further clinical trials have focused on Graft versus Host disease, an application which is not surprising given the efficiency of MSCs at the clinical level and the similarity in immunomodulation properties between MSCs and ADSCs. Multiple sclerosis can be also included in the field of the modulation of inflammation/immune response. In a rodent model, it was demonstrated that intravenously injected ADSCs can home to the lymph nodes and brain and that they act by suppressing the autoimmune response in early phases of disease as well as by inducing local neuroregeneration by endogenous progenitors in animals with established disease. The strong immunosuppressive effects of ADSC reported by various independent groups naturally led to investigation the effect of ADSC on MS.

Based on the positive and well-documented positive effects of intravenous administration of MSCs, positive effects of ADSCs are also expected in this field. Only two trials have investigated the effect of ADSCs on chronic critical limb ischemia, one after intra-muscular injections, the second with intravenous injections in diabetic patients. A further planned trial also intends to use allogenic ADSCs in the context of fistula. This trial is important as it could open up the field of ADSC-associated regenerative medicine. [14]

Table 4 Clincial trials using adipose-derived stromal cell

Clinical trials with ADSC	Design	Results	Ref
Maxillary reconstruction	Autologous ADSC case report	Success	{Mesimaki, 2009 #480}
Cryptoglandular origin fistula with or without Crohn's disease	Autologous ADSC phase Ⅰ / Ⅱ intra-tissue	ADSCs more effective (P = 0.001). Recurrence rate with ADSC = 17.6%	{Garcia-Olmo, 2008 #451; Garcia-Olmo, 2009 #449}
	Autologous ADSC Phase Ⅱ, 2 arms (fibrin glue, fibrin glue + ADSC)	Ongoing, not recruiting	NCT00115466*
Crohn's disease fistula	Autologous ADSC, phase Ⅰ and Ⅱ	Phase Ⅰ, complete Phase Ⅱ recruiting	NCT00992485* NCT01011244*
	Autologous ADSC, phase Ⅰ	Phase Ⅰ / Ⅱ recruiting	NCT01157650*
	Allogenic ADSC: phase Ⅰ / Ⅱ 20×10^6 then 40×10^6	recruiting	NCT00999115*
Complex Perianal Fistulas not associated to Crohn's disease	Autologous ADSC, phase Ⅲ three arms (fibrine, ADSC, fibrin glue + ADSC; 20×10^6 then 40×10^6 when no effect)	Completed (214 enrolled patients)	NCT00475410*
	Long term safety	Recruiting	NCT01020825*
Depressed Scar	Autologous ADSC predifferentiated towards adipocyte, phase Ⅱ, Ⅲ	Complete	NCT00992147*
Chronic critical limb Ischemia	Autologous ADSC, phase Ⅰ im 100×10^6	Recruiting	NCT01211028*
Chronic critical limb Ischemia in diabetic patients	Autologous ADSC, phase Ⅰ / Ⅱ iv administration	Recruiting	NCT01079403*
Fecal incontinence	Autologous ADSC, phase Ⅰ	Recruiting	NCT01011686*
GVHD	Autologous ADSC iv 10^6/kg	4/5 alive (after a median follow-up of 40 mo)	{Fang, 2007 #469}
	Autologous ADSC Three arms no administration, iv 10^6/kg or 3×10^6/kg	Recruiting	NCT01222039
Secondary Progressive Multiple Sclerosis	Autologous ADSC phase Ⅰ / Ⅱ 3 arms (iv 10^6 and 4×10^6/kg against no intervention)	Recruiting	NCT01056471*

The clinical trials that are indexed in this table were retrieved using adipose, derived and stem in clinicaltrial and Pubmed websites. As discussed in the text, there is some confusion about the use of the term adipose-derived stroma/stem cells that can be used for crude SVF. This term should be restricted to cultured adipose derived mesenchymal stem cells and the table lists the trials using such cells (*identifier on Clinicaltrials website: *http://clinicaltrials.gov/ct2/results?term=adipose+derived+cells). ADSC: Adipose-derived stromal cell; GVHD: Graft-versus-host disease.

Table from Casteilla et al. [14]

1.5 Dose Rationale and Risk/Benefits

From the point of view of safety and adverse-side effects, two key issues are the possibility of undesirable differentiation and the possibility of interaction between ADSCs and resident cancer cells. Concerning the first point, undesirable calcifications have been observed after the administration of bone marrow stem cells in the heart after infarction. More recently, cysts and microcalcifications were detected in 4 out of 70 patients after breast reconstruction using lipoaspirate associated with crude SVF. To our knowledge, no other cases of undesirable differentiation have been described, suggesting either that such events are rare or have not been fully and systematically evaluated.[14]

No definitive conclusion can be reached on the possible interaction between ADSCs and cancer cells as contradictory reports have been published. Immunosuppressive capacity and modulation of inflammation are shared with ADSCs, which seem very efficient both in vitro and in different in vivo situations. This immunosuppressive effect associated with the angiogenic properties of ADSC raises questions about the interactions between these cells and cancer cells. Donnerberg et al. concluded that ADSCs could trigger the growth of tumors from active cancer cells but not

from dormant cells.[14] Another systematic analysis of the issue found that malignancy occurred only in studies involving participants with ongoing or previous malignancies; no de novo malignancies were observed.[15]

In a meta-analysis of clinical trials that examined the use MSCs to evaluate their safety, the review did not detect an association between acute toxicity related to the infusion of MSCs, organ system complications, infection, death or malignancy. There was a significant association between MSCs and transient fever however no long term sequelae developed.[15] Risk is assumed to be very small for this study because of a focal injection and smaller dose versus systemic injection.

2 Study Objectives

Primary Objective
To assess the safety and tolerability of a single dose of autologous stromal vascular fraction (SVF) applied endoscopically into tracheoesophageal fistulae.

Secondary Objective
To assess the efficacy of stromal vascular fraction's (SVF) ability to improve the success of surgical repair and prevent recurrence of tracheoesophageal fistula (RTEF).

3 Study Design

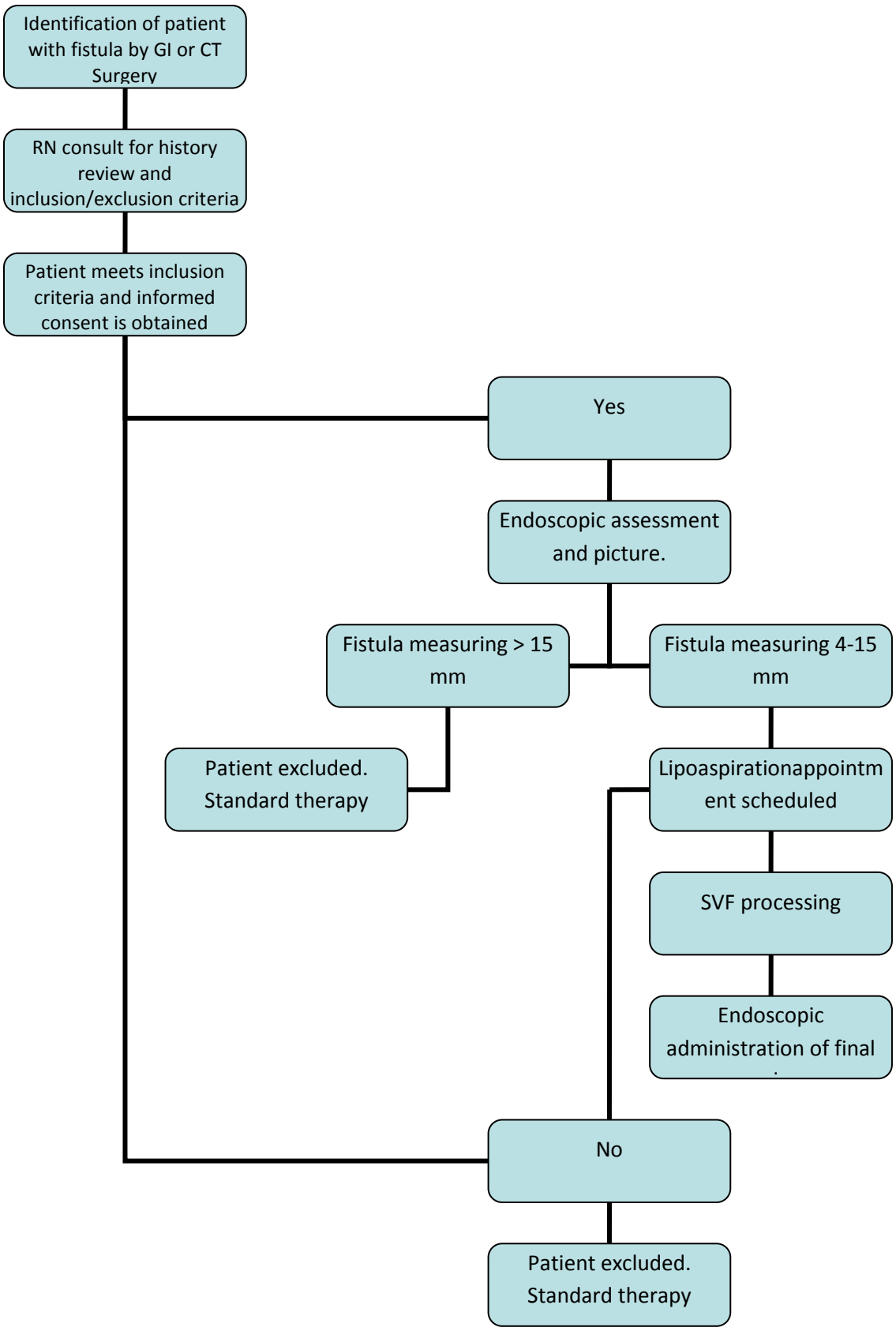
3.1 General Design

This is an open label pilot study to assess the safety and feasibility of harvesting and applying autologous adipose derived SVF and the efficacy of the SVF for closure of aero-digestive fistulas. Both the researcher and the participant in this research study will know the treatment the participant is receiving. No control group will be required as every participant will receive the same therapy. Subjects will be identified in the inpatient or outpatient setting during consults and/or appointments with Gastroenterology and/or Thoracic Surgery Departments. When a potential patient is identified, a research coordinator nurse (RN) consult will be initiated in order to identify if the patient meets all the inclusion/exclusion criteria for the study.
After signing of informed consent and successful screening, baseline values, fistula measurements and a radiographical assessment of the fistula will be obtained from the subject. The adipose tissue lipoaspiration and subsequent endoscopy will be scheduled. . The adipose tissue harvest will be performed in the Regenerative Medicine Therapy Suites by qualified providers. The SVF will be manufactured on site in the Regenerative Medicine Therapy Suites and injected into the thrombin portion of the TISSEEL syringe and labeled for the individual patient. Then the final product will be taken to the gastrointestinal/bronchoscopic laboratory for endoscopic administration.

Placement of an Alimaxx or Wallflex stent covering the fistulous tract after SVF administration procedure will be considered in order to prevent mechanical recanalization of the fistula. The patient will be allowed to eat low residue meals 8 hours after the procedure. The status of the fistulae will be assessed with contrast studies (e.g. barium swallow) or other imaging studies such as CT scan or MRI, immediately after the procedure. Other radiographic studies can be performed within 24 hours post procedure. The patient and fistula status will be reassessed with imaging studies after 1 month. If a stent was placed, the latter will be removed during this visit.

Follow up visits will take place every 3 months for the first year and then yearly for five years. Imaging studies, such as barium swallow or CT scan to assess fistula closure will be performed on every visit as clinically indicated. Evaluations will consist of an assessment of fistula healing including fistula characteristics and measurements. Clinical signs and symptoms such as cough, dysphagia, dyspnea, abdominal distention, sputum mixed with food, wheezing and lower respiratory tract infections will be documented.

The nurse will also examine the lipoaspiration site for any signs of bleeding and/or infection. The patient's temperature will be obtained because of the potential for a fever during therapy.
If fistula closure is not achieved after 12 weeks from the second procedure, the patient will receive standard therapy.


Identification of patient with fistula by GI or CT Surgery
RN consult for history review and inclusion/exclusion criteria
Patient meets inclusion criteria and informed consent is obtained
Yes
Endoscopic assessment and picture.
Fistula measuring > 15 mm
Fistula measuring 4-15 mm
Patient excluded. Standard therapy
Lipoaspirationappointment scheduled
SVF processing
Endoscopic administration of final
No
Patient excluded. Standard therapy

3.2 Primary Study Endpoints

SVF harvested without complication and a treatment regimen is established for applying the SVF suspended in fibrin glue in the fistulous tract.

3.3 Secondary Study Endpoints

- Fistulous tract reduction and/or closure within 16 weeks of therapy documented by measurement and photography of the fistula in endoscopic/bronchoscopic evaluation.
- Cardinal symptoms to be reduced, documented by history and physical examination.
- Fistula recurrence to be less, documented by long term follow up.

3.4 Primary Safety Endpoints

Study procedures will be stopped if adverse reactions occur with either the SVF, the fistula itself, or the lipoaspiration wound and the patient will be referred for standard therapy. This would include but not limited to:

a. Signs of intravascular application of TISEEL which can lead to intravascular coagulation, thromboembolic events and acute hypersensitivity reactions.
b. Signs of adverse reactions to the lipoaspiration procedure include an allergy to the Hunstad solution, infection at the puncture wound site or risk of bleeding at the puncture wound site. Risks include bleeding or infection at the site and bruising for approximately one week. No adverse consequences of fluid overload have been associated with low volume lipoaspiration (< 5 liters).
c. Signs of increased fistula size and/or aggravation of symptoms associated with fistulous tract such as paroxysmal cough, dysphagia, wheezing, abdominal distention, chest pain, dyspnea, pneumonia.

For documented contamination or positive culture determined once the product has already been administered, patient will undergo a standard clinical assessment to include vital signs, symptom history, serum electrolytes, liver function tests, CBC and blood cultures x 2. If clinically appropriate, the patient would be admitted to Mayo Clinic Hospital for inpatient overnight observation until documented as medically stable by qualified medical personnel

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Adults ≥ 18 years old
- Adults ≤ 90 years old
- Aerodigestive fistula opening size between 2 mm and 15 mm in diameter (as measured by comparing its size with the open mouth of a biopsy forceps)
- Inpatient or outpatient setting
- Recurrent or de novo fistulas
- A prior diagnosis of ADF in which the standard therapy has failed or is not applicable
- Fistula location may include:
    - Tracheopharyngeal

  - Tracheoesophageal
  - Bronchoesophageal
- Fistula etiology may include:
  - Secondary to previous malignancy with complete remission
  - Secondary to radiotherapy as long as complete remission for 5 years has been achieved and documented
  - Congenital with or without previous treatment
  - Secondary to surgical interventions or endoscopic therapies such as dilation and esophageal manipulation
  - Post prolonged tracheal intubation
  - Secondary to foreign body ingestion
  - Secondary to thoracic trauma/crush injuries
  - Secondary to caustic ingestion
  - Secondary to pneumonectomy or mechanical ventilation
  - Esophagomalacia
- The ability of subjects to give appropriate consent or have an appropriate representative available to do so
- The ability of subjects to return for follow up endoscopic assessment as established.

4.2 Exclusion Criteria

- Exposure to any investigational drug or procedure within 3 months prior to study entry.
- Patients with allergy to fibrin glue (TISSEEL) or anesthetics
- Patients with active/ongoing malignancy such as esophageal, lung, tracheal, thyroid, oropharyngeal or gastric cancer
- Patients on active regimen of chemotherapy
- Patients receiving radiation
- Diabetics with poor glucose metabolic control exhibited by an HbA1c > 9
- If there is evidence, in endoscopy, of dysplastic-appearing mucosa such as Barrett's dysplasia near the fistula, this will be excluded.Patients that require surgical intervention at the fistula area for any reason
- BMI of <16 (may difficult lipoaspiration procedure)
- Women who are pregnant or nursing or women of childbearing potential who are unwilling to maintain contraceptive therapy for the duration of the study
- Clinical signs of respiratory tract or pleuro-pulmonary infections
- Prolonged (> 6 months) use of steroids
- Drug or alcohol dependence
- Active infectious disease positive for HIV, HTLV, HBV, HCV, CMV (IgM > IgG) and/or syphilis
- End of life

4.3 Subject Recruitment, Enrollment and Screening

Recruitment efforts will include the following:

- From the Co-Investigators' clinical practices.

- Referring physicians.
- Word of mouth

Enrollment will include the evaluation and documentation of inclusion/exclusion criteria done with the consent. Family members identified by the patient will be included in the education and informed consent process if the patient prefers so. Subjects of child bearing potential are required to use appropriate contraceptive method(s).
After the patient and family agree, the study RN will examine the patient for the feasibility and appropriateness of the patient to include:

- Review of inclusion and exclusion criteria
- Review of current symptoms.
- Prior fistula treatment and management will be documented.

If patient is eligible, an appointment for endoscopic/bronchoscopic evaluation with the study investigators will be scheduled. During this evaluation:

- A picture of the fistula will be obtained.
- Fistula characteristics such as size, location and appearance will be documented.
- If the fistula measures up to 1.5 cm the patient will continue to be eligible.

Qualified study personnel will be consulted to review the patient for the following:

- Assessment of the ability to harvest adipose tissue. The abdomen will be used as the primary site however if unable to obtain an adequate amount, the hips will be used.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

A subject may be withdrawn from the study prior to that subject completing all of the study related procedures. Some reasons include:

- Signs of intravascular coagulation, thromboembolic events and acute hypersensitivity reactions secondary to TISSEEL.
- Signs of adverse reactions to the lipoaspiration procedure including allergy to the Hunstad solution, infection at the puncture wound site, or bleeding at the puncture wound site.
- Signs of increased fistula size and/or aggravation of symptoms associated with fistulous tract such as paroxysmal cough, dysphagia, wheezing, abdominal distention, chest pain, dyspnea, or pneumonia.
- Failure of subject to adhere to protocol requirements.
- Clinical signs that the primary research intervention is exposing the subject to an unacceptable level of risk.
- Subject decision to withdraw from the study (withdrawal of consent).

Sudden study treatment termination would occur for any noted signs of increasing infection, pulmonary complications or cardiac arrest. Conventional treatment, as appropriate, would continue with any subjects withdrawn from the study group.

Those participants that withdraw or are withdrawn from the study will be referred to their primary care physician for continued follow-up. A summary of the clinical procedures and patient response will be written and sent to the participant's provider of choice.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Safety and feasibility information will be collected for the withdrawn subjects up to the date of withdrawal. Documentation of withdrawal will include whether the withdrawal resulted from a decision by the subject or by the investigator, the reasons for the withdrawal, if known and whether the withdrawal is from all components of the research study or just the primary interventional component. If follow-up information is required, permission will be obtained from the subject.

5 Study Drug

5.1 Preparation and Administration of Study Drug

The investigational agent is autologous, uncultured stromal vascular fraction. The fistulous area will be estimated upon consent. Patients with a fistula measuring up to 1.5 cm in diameter will have 80-100mL of fat aspirated. Lipoaspiration will be performed in the Regenerative Medicine Therapy Suites and processed in the Kiso located on the 3rd floor of the same building.

The product dispensed by the KISO machine (SVF pellet) will be diluted in 2 ml lactated ringers solution. A total of 10 million MNCs (containing MSCs cells) are expected to be contained in this pellet-LR Solution combination. A 0.5ml sample will be reserved for gram stain, culture, endotoxin testing, cell count and viability. The remaining product will be combined with 1 ml of Thrombin Solution of TISSEEL (500 IU Thrombin/ml) to be injected with Sealer Protein Solution (3000 KIU/ml; as per manufacturer guidelines). The solution as injectate would be delivered through a [Wilson Cook; varies] 19 or 22 gauge endosonographic needle circumferentially 3-5 mm from the fistulous tract in 0.1 to 0.3 ml aliquots [either sequentially or suspension followed by Sealer]. During surgical closure of fistula endoscopically, the product/TISSEEL combo will be injected around the fistula, then topically over the fistula.

5.2 Treatment Regimen

Patients will receive approximately $5.0x10^6$ mononuclear cells (MNCs) of SVF per cubic centimeter of fistulous area. The fistula upper limit diameter will be 1.5 cm. Therefore, the total area of the fistula would measure 1.8 cm^2 at the most. Most aerodigestive fistulas are contiguous in nature. We estimate that the fistulous tract will measure 1 cm at the most. Fistula volume will have an upper limit volume of 1.8 cm^3. As recommended by the manufacturer, the volume of TISSEEL to be utilized will be determined by dividing the fistula volume by 4 to yield the final amount of TISSEEL needed to re-suspend the SVF.

Fistulous Area	Required TISSEEL amount
$1.8cm^2$	0.5ml

5.3 Isolation of Stromal Vascular Fraction (SVF) using KISO:

The lipoaspirated tissue KISO system is used in this study for automated SVF cell isolation from human lipoaspirate. The KISO system employs disposable tissue processing bag kit, Collagenase Solution (LRS). The sterile disposable tissue processing bag kit contains all-in-one 3 chamber tissue processing bag; plastic connection tubings; stopcock manifold controlling delivery of fluid reagents; a standard spike designed for connection with standard clinical LRS bag; and standard contamination-free safe environment, consistency between multiple isolations, and compact size of the device. It contains chambers for (1) adipose tissue digestion, and separation of crude SVF from adipocytes; (2) waste collection; (3) further SVF purification by filtration, concentration, standard catheter tip syringe, minimizing potential contamination of the sample. Movement of mixing cell suspension in the bag with external agitator and separating SVF cells by filtration within the waste chamber. After digestion SVF cell suspension is delivered to another chamber with embedded mesh (mesh-in-the-bag). Smaller size mesh retains cell clamps and adipocytes which passed the mesh in tissue digestion chamber. Eventually, filtered SVF cell suspension will be incubated, mixed and deconstructed, using a standard protocol under sterile conditions.

The KISO system employs disposable tissue processing bag kit, SERVA cGMP Collagenase (SERVA, Cat. No. 17454) enzyme, and a standard bag of Lactate Ringers Solution (LRS). The sterile disposable tissue processing bag kit contains all-in-one 3 chamber tissue processing bag; plastic connection tubings; stopcock manifold controlling delivery of fluid reagents; a standard spike designed for connection with standard clinical LRS bag; and standard luer lock 60 mL output syringe. All-in-one 3 chamber tissue processing bag provides contamination-free safe environment, consistency between multiple isolations, and compact size of the device. It contains chambers for (1) adipose tissue digestion, and separation of crude SVF from adipocytes; (2) waste collection; (3) further SVF purification by filtration, concentration, and collection. Adipose tissue is delivered to the processing chamber via input nozzle with a standard catheter tip syringe, minimizing potential contamination of the sample. Movement of fluids between the chambers in such 3 chamber tissue processing bag is mediated by gravity eliminating the need for a pump, and is controlled by single stopcock valve integrated in processing bag. Adipose tissue processing chamber with embedded mesh (mesh-in-the-bag) allows performing multiple steps for SVF separation in one compartment: two consecutive washings of the adipose tissue, enzymatic digestion, phase separation (an aqueous fraction of tissue and a fatty fraction of the digested tissue sample) thus maintaining the same compact dimensions, minimizing contamination risk, and further facilitating fat tissue disruption by mixing cell suspension in the bag with external agitator and separating SVF cells by filtration.

External agitator and heating plate provide the best optimized environment for adipose tissue digestion and SVF cell separation. All the wastes are separated and collected automatically within the waste chamber. After digestion SVF cell suspension is delivered to another chamber with embedded mesh (mesh-in-the-bag). Smaller size mesh retains cell clamps and adipocytes which passed the mesh in tissue digestion chamber. Eventually, filtered SVF cell suspension is drawn into a standard luer lock 60 mL output syringe and centrifuged (600g, 10 min) to remove the fluid and recover SVF cell pellet. The resulting fluid with stromal vascular fraction (SVF) will be centrifuged for 10 minutes. Total preparation time for the SVF is expected to take approximately 1 hour. The isolated SVF cells are then suspended in 1-2 mL Lactated

Ringer's solution. This product is then combined with the recommended quantity of TISSEEL fibrin glue (TISSEEL). The sample of SVF in Lactated Ringers is the final product that will be administered, along with TISSEELvia a minimally invasive endoscopic procedure into and around the patient's fistula.

5.4 Subject Compliance and prior therapy

To continue in the study, the subject must comply with all components of the study including every follow up appointment for assessment of fistula closure.
Patient history related to fistula management will be documented at the initial visit. Fistula therapies outside of the study protocol will not be permitted. We would exclude patients who require further endoscopic or surgical intervention for management of the fistula.

5.5 Packaging

No special packaging will be involved in this study. After cell preparation as outlined above, the TISSEEL applicator containing the cells will be labeled with " CAUTION: NEW DRUG – LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE. FOR AUTOLOGOUS USE ONLY, NOT EVALUATED FOR INFECTIOUS SUBSTANCES and will be placed in a cooler for transportation of the therapy room located in the same building.

Mayo Clinic Cell Therapy Laboratory
4500 San Pablo Road S, Jacksonville, FL 32224
(904) 956-1867

Stromal Vascular Fraction **IRB #**_____
Lot Number: XXXXXXX
Expiration Date: XXXXXX **Subject #**_____
Cell Dose: XXXXXX

Subject Name_____
Released for Clinical Use: *DATE*
CAUTION: NEW DRUG – LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE.
FOR AUTOLOGOUS USE ONLY, NOT EVALUATED FOR INFECTIOUS SUBSTANCES

5.6 Blinding of Study

This is an open-label pilot study in which every participant will receive the treatment of interest and will be aware of it.

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

Lipoaspirate will be received at the Regenerative Medicine Therapy Suites following protocol for "Receipt of Biospecimens for Clinical Trials".

Study supplies (KISO and TISSEEL kits) will be received at the Regenerative Medicine Therapy Suites per protocol "Receiving Reagents & Supplies into the Cell Therapy Laboratory". Supplies will be reviewed and checked for expiration prior to use in this study.

5.7.2 Storage

Excess SVF product will be disposed of according to appropriate handling of bio-hazardous waste.

5.7.3 Dispensing of Study Drug

Regular study SVF product reconciliation will be performed to document cells assigned, cells dispensed, cells returned, and cells remaining. This reconciliation will be logged on the SVF product reconciliation form, which will be signed and dated by the study team.

5.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of SVF product dispensed and remaining product. This reconciliation will be logged on the SVF product reconciliation form, which will be signed and dated. Any discrepancies noted will be documented and investigated, prior to destruction of unused study cells. Cells destroyed will be documented in the study files.

6 Study Procedures

6.1 Visit 1

1. Screening to identify interested and qualified subjects.
2. When an interested, qualified subject is identified, a study RN consult will be initiated.
3. The study RN will assess patient eligibility.
4. If inclusion criteria are met, participation in this pilot clinical trial will be offered.
5. A subsequent visit will be scheduled to obtain radiographic studies for baseline assessment of the fistula if not available already.
6. If the patient already has radiographical evidence and assessment of the fistula meeting the study criteria and the patient agrees to participation, a delegated study team member will obtain written consent.
7. The endoscopist/cardiothoracic surgeon will assess the radiographical fistula characteristics, and thoroughly document them on the medical records. Undermining and/or tunneling will be assessed in centimeters, when present.
8. Initial assessment information will be obtained and documented on the case report form (CRF).
9. Visit 2 will be scheduled for the lipoaspiration, endoscopy procedure, and SVF application.

6.2 Visit 2

1. Qualified study personnel will prepare the patient for the lipoaspiration procedure to obtain the adipose tissue.
2. 80-100 cc's of adipose tissue will be removed by lipoaspiration and inserted into a sterile specimen container. The specimen will be labeled with the patient's name and Subject ID #.A sticker indicating "Research Patient" will also be applied.

3. The adipose tissue will be processed and stromal cells isolated.
4. The study RN will be paged to deliver the product and return to the endoscopist/CT surgeon for application per the study procedure. Patient will be transported to the endoscopy suites.
5. Post-procedural measures will include an immediate contrast study if no contraindications. Other radiographic studies for bronchopleural fistula can be performed within the next 24 hours if clinically indicated.
6. If a stent is placed over the repaired fistula, the patient will be scheduled for stent removal in 1 month.

6.3 Visit 3

1. The patient will be evaluated by the physician who performed the procedure.
2. If a stent was placed over the repaired fistula after the SVF administration, the stent will be removed during this visit.
3. If clinically indicated, a radiographic study such as but not limited to barium swallow will be obtained in order to assess fistula evolution. Fistula measurements will include length, width, and depth in centimeters. Undermining and/or tunneling will be assessed in centimeters, when present.
4. Assessment of the lipoaspiration site will be completed.
5. All assessment findings and the treatment provided will be documented in the EMR and in the CRF by the study RN and/or physician who performed the procedure.
6. Every image will be available and/or uploaded to QREADS in accordance with the Mayo Clinic Photography policy.
7. Assessment for presence of adverse events will be completed and documented in the CRF. Any question of potential adverse events will be referred to the consultant.
8. Patient will receive a trimestral return visit until study endpoints are reached.

6.4 Follow-up Visits

Follow-up visits will be scheduled at month 4, 7, 10 and yearly for 5 years, to obtain information about fistula healing. Assessment for presence of adverse events will be completed and documented in the CRF. Any question of potential adverse events will be referred to the consultant. Each visit will include clinical assessments, exams, education, and imaging as clinically indicated.

6.5 Study Visit Table

Study Activity	Visit 1 Screening (two weeks prior to Day 1)	Visit 2 Day 0	Visit 3 1 month	Visit 4 4 month	Visit 5 7 month	Visit 6 10 month	Visit 7 Year 1	Year 2-5 follow-up
Inclusion/Exclusion criteria review	X							
Review med list	X							
Informed consent	X							
Medical History	X	X						
Physical exam (BMI, Ht, Wt, BP)	X	X						X
Temperature	X	X	X	X	X	X	X	X
Clinical Assessment	X	X	X	X	X	X	X	X
Fistula assessment with imaging	X	X	X	X	X	X	X	X
Lipoaspiration		X						
Application of SVF		X						
Adverse event evaluation		X	X	X	X	X	X	X
Patient education	X	X	X	X	X	X	X	X

7 Statistical Plan

7.1 Sample Size Determination

The size of the pilot study was based on factors of patient recruitment, time, and available resources. Patient recruitment determination was based on a review of the number of patients seen annually by Gastroenterology, Cardiothoracic Surgery and Otolaryngology with a diagnosis of aerodigestive or bronchopleural fistulae. We estimate that each of the latter departments treat around 5 patients with potential inclusion criteria for our study every year (15 patients per year in total).

7.2 Statistical Methods

Analysis will proceed according to the specific objectives following intention-to-treat principles. Sample sizes are too small for any formal comparisons; therefore all data will be summarized numerically and displayed graphically as appropriate.

The primary objective relates to the safety and feasibility of implementing the harvesting and application of SVF on aerodigestive fistulae and no statistical analysis will be conducted related to this objective except to assist in summarizing the proportions of patients for whom complications occur that may impact the feasibility of conducting a future trial.
The secondary objective relates to the efficacy of SVF regarding time to fistula closure. For this aim, we will graphically display the size of each patient's fistula until closure is achieved. Separately we will also summarize the number and percent of patients with the following secondary endpoints: (1) greater than 20% fistula healing within 4 weeks after start of treatment; (2) greater than 60% fistula healing within 4 months after start of treatment and (3) in-vitro cell characterization, differentiation and quantification.

All study results will be taken into consideration when determining whether the use of SVF for treating aerodigestive fistulae warrants further study in a larger randomized control trial.

8 Safety and Adverse Events

8.1 Definitions

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as the first application of the SVFs to the fistula until the end of the first year follow-up visit to assess the fistula status. Adverse events may fall into three areas:

1. A positive culture of the study product.
2. Fistula complication e.g. infection, further progression, stent migration with fistula recanalization, TISSEEL intravascular injection with DIC, perforation, TISSEEL allergy.
2. Lipoaspiration complications e.g. infection at site of incision.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition.
The NIH Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be used to grade the adverse event.
At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator will instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

8.2 Recording of Adverse Events

At each contact with the subject, the study team will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results will be recorded in the source document.

All adverse events occurring during the study period will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period will be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet

and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.
Should any of the tested samples yield a positive culture, the Regenerative Medicine Therapy Suites Medical Director, Dr. Shapiro, and the PI, Dr. Woodward will be notified. An immediate assessment of results will be performed to determine if the source is from the product (True positive) or due to a lab contaminant. Colony count, identity of the organism, if immediately available, and specimen handling and process will all be evaluated when determining the source of the microbial contamination.

If during follow-up visits, the fistulous tract increases in size or if there are clinical or radiological signs of a worsening condition, perforation or infection, the PI will be notified, the patient will be informed and offered standard of care.
No study subject will be further treated until root-cause analysis is performed and appropriate actions to address the findings are completed.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures within 24 hours.
Information will be collected on the adverse event worksheet (*and entered in the REDCap database)*:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB. Non-UPIRTSOs will be reported to the IRB at the continuing review.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious adverse reactions where there is evidence to suggest a causal relationship between the study drug and the adverse event and any clinically important increase in the rate of serious adverse reactions will be reported as a serious adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

8.4 Stopping Rules

Study enrollment and treatment procedures will be suspended in the event that two successive patients experience a Serious Adverse Event during the procedure or within the first 2 weeks after treatment. The study may also be suspended or terminated at the direction of the DSMB based on the review criteria outlined in the Data Safety Monitoring Plan (DSMP). The study would only be resumed after a thorough review of the incidents and any corrective and preventive actions have been put in place along with consultation between the study team and the IRB.

8.5 Medical (Clinical) Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Internal Data and Safety Monitoring Board

The internal DSMB will review the study data as outlined in section 3.4 related to the primary safety outcomes. This internal board will be comprised of (Albert G. Hakaim, M.D., Brian Lacy, M.D., Ph.D, and Frank Lukens, M.D.). They will evaluate the study according to the DSMP.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.
Hard copy data such as consent forms will be stored in locked file cabinets; electronic data will be stored in secure web-based database (REDCap) that will be designed with the help of the statistician.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinic and office charts, laboratory notes, memoranda, evaluation checklist, recorded data from automated instruments, photographic images, subject files, and records kept at the laboratories involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded in the REDCap database. A printed REDCap source document will be used to capture the subject's clinical data. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not answered, it will be documented as "N/D". If the item is not applicable to the individual case, it will be documented as "N/A". Errors will be identified by a strike-through and corrected data.

Data Management

Study data will be managed in a study specific REDCap system.

Data Security and Confidentiality

The REDCap system has built in system for control of access, data integrity and audit trails. Access and confidentiality are controlled in a manner similar to other institutional systems.

Data Quality Assurance

The principal investigator will be accountable to verify the accuracy, completeness, and validity of the data recorded.

Data Clarification Process

A report will be run on the REDCap program to identify missing elements in the data capture. A designated study team member will make corrections in the data and the principal investigator will verify.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject's source documents and case report form, REDCap database and regulatory documents.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717 whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. Regenerative Medicine Therapy Suites, etc.), and has adequate space to conduct the monitoring visit.

This study will be monitored on a routine basis during the conduct of the pilot. The Mayo Clinic Office of Research Regulatory Support can provide assistance with clinical monitoring for the pilot support the sponsor-investigator. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration regulations.

Study participants will have follow-up visits at 4 months, 7 months, 10 months, and 1 year as well as yearly for 5 years after treatment to assure they continue to heal and there are no questions or complications.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. Cell Therapy Laboratory, etc.).
Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to all regulations and Mayo Clinic research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This pilot study will be financed either by external grants or internal grants from Mayo Clinic.

12.2 Conflict of Interest

None

13 Publication Plan

The Principal Investigator will be responsible for manuscript preparation and submission to a suitable journal for publication of results.
Registration on ClinicalTrials.gov will be completed as appropriate.

14 References

1. Cherubino M, Rubin JP, Miljkovic N, Kelmendi-Doko A, Marra KG. Adipose-derived stem cells for wound healing applications. *Ann Plast Surg.* Feb 2011;66(2):210-215.
2. G. Vantrappen JH. *Diseases of the esophagus*2012.
3. Hyman S. Abrams MT. Esophagorespiratory Fistulae. *JAMA Archives of Otolaryngology.* 1954;60(3):371-374.
4. Murthy S, Gonzalez-Stawinski GV, Rozas MS, Gildea TR, Dumot JA. Palliation of malignant aerodigestive fistulae with self-expanding metallic stents. *Dis Esophagus.* 2007;20(5):386-389.
5. Balazs A, Kupcsulik PK, Galambos Z. Esophagorespiratory fistulas of tumorous origin. Non-operative management of 264 cases in a 20-year period. *Eur J Cardiothorac Surg.* Nov 2008;34(5):1103-1107.
6. Richter GT, Ryckman F, Brown RL, Rutter MJ. Endoscopic management of recurrent tracheoesophageal fistula. *J Pediatr Surg.* Jan 2008;43(1):238-245.
7. Park KJ, Ryoo SB, Kim JS, et al. Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohn's disease: a pilot clinical trial. *Colorectal Dis.* May 2016;18(5):468-476.
8. Lee WY, Park KJ, Cho YB, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells.* Nov 2013;31(11):2575-2581.
9. Sanz-Baro R, Garcia-Arranz M, Guadalajara H, de la Quintana P, Herreros MD, Garcia-Olmo D. First-in-Human Case Study: Pregnancy in Women With Crohn's Perianal Fistula Treated With Adipose-Derived Stem Cells: A Safety Study. *Stem Cells Transl Med.* Jun 2015;4(6):598-602.
10. Mizushima T, Takahashi H, Takeyama H, et al. A clinical trial of autologous adipose-derived regenerative cell transplantation for a postoperative enterocutaneous fistula. *Surgery Today.* 2016;46(7):835-842.
11. Aho JM, Dietz AB, Radel DJ, et al. Closure of a Recurrent Bronchopleural Fistula Using a Matrix Seeded With Patient-Derived Mesenchymal Stem Cells. *Stem Cells Transl Med.* Oct 2016;5(10):1375-1379.
12. Diaz-Agero Alvarez PJ, Bellido-Reyes YA, Sanchez-Giron JG, Garcia-Olmo D, Garcia-Arranz M. Novel bronchoscopic treatment for bronchopleural fistula using adipose-derived stromal cells. *Cytotherapy.* Jan 2016;18(1):36-40.

13. Alvarez PD, Garcia-Arranz M, Georgiev-Hristov T, Garcia-Olmo D. A new bronchoscopic treatment of tracheomediastinal fistula using autologous adipose-derived stem cells. *Thorax.* Apr 2008;63(4):374-376.
14. Casteilla L. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. *World J Stem Cells.* 2011;3(4):25.
15. Manoj M. Lalu LM, Christina Pugliese, Dean Fergusson, Brent W. Winston, John C. Marshall, John Granton, Duncan J. Stewart, for the Canadian Critical Care Trials Group. Safety of Cell Therapy with Mesenchymal Stromal Cells (SafeCell): A Systematic Review and Meta-Analysis of Clinical Trials. *PLoS One.* 2012;7(10).