CLINICAL STUDY PROTOCOL

Phase I study to examine the safety and efficacy of allogeneic MSCs in suppressing inflammation in patients with small abdominal aortic aneurysm (AAA)

IRB #1510579216

IND 16579

Indiana University School of Medicine
1801 N. Senate Blvd.
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July 15, 2021

ETHICS AND REGULATORY COMPLIANCE STATEMENT

The procedures set forth in this protocol are designed to ensure that the sponsor(s) and principal investigator(s) abide by the International Conference on Harmonization (ICH) current Good Clinical Practice (cGCP) guidelines, current Good Laboratory Practice (cGLP) guidelines, the Declaration of Helsinki, and applicable local regulatory requirements and laws in the conduct, evaluation, and documentation of this study.

Protocol Name	**Phase I study to examine the safety and efficacy of allogeneic MSCs in suppressing inflammation in patients with small abdominal aortic aneurysm (AAA) .**
Investigational Product Name	Allogeneic mesenchymal stem cells
Date: (dd-mmm-yyyy)	June 2017
Author	Michael P. Murphy, MD

PROTOCOL HISTORY

Date (dd-mmm-yyyy)	**Description**
7-Oct- 2015	Response to "Clinical Hold"
24-Mar-2016	Remove IUH as clinical performance site
12-May-2016	Add 5 year long term f/u, add serum analysis
00-July-2016	CRC for infusion
00-Nov-2016	Change AAA size to 3.5-5.0 (inclusion criteria)
22-Nov-2016	Add Day 14 f/u +/- 5 days to schedule
06-Dec-2016	Corrections to the f/u schedule
29-Dec-2016	Add HLA typing, remove temp log, PET CT: add month 12, change Mo 1 to a range of 2-6 weeks, add agent orange exposure to data capture.
03-JAN-2017	Removed Obstructive Sleep Apnea from exclusion, Removed month 18 & 14 labs and ECG's Removed infectious disease labs (except HIV and Hep. C) from baseline testing. Decreased the number and amount of blood specimens obtained.
April-2017	$30 stipend added for travel reimbursement
May-2017	AAA size from 3.5 to 3.0. Remove Pet CT's. CT at baseline and 12-month f/u.
July-2017	Remove f/u HbA1C's, remove all direct bilirubin tests, add: Karen Lynn & Ashley Gutwein. Increase peripheral blood draws for ancillary studies to 34 ml.
Sept.-2017	Remove open label statements, replace with double blind. Remove overnight stay. Complete ECG and labs on day 3. Add Day 1 phone call. Remove use of home oxygen from exclusion. Add Month 12 QoL questionnaires to the follow-up schedule. Remove ICS contact info.
Dec-2017	Increase the age of subjects from 80 to 85 in the inclusion criteria. Remove the physical exams to be completed by the PI from Months 6, 12, 18 & 24.
Jan-2018	Adding Janet Klein and Dr. Dalsing. Adding Methodist Hospital and the CRC as performance sites.
February 2018	Change MSC cell manufacturing source to Case Western Reserve University in Cleveland, Ohio, and/or Longeveron, LLC in Miami, Florida.
05 JUN 2018	Remove the Day 3 ECG. Page 15: changed the age to 85 (this was changed 1.4.18).
27 JUN 2018	Remove Day 3 from the Follow-Up schedule.
18 JUL 2018	Clarification of (6.1.3 page 17) MSC handling, storage and accountability. Remove Longeveron, LLC, add Tiffany Liang, remove Keisin Wang and Linden Green. Re-enter omitted ECG, CTA, and U/S

	tests into schedule of events from previous AMD clerical error. This is not new testing.
20 SEP 2018	Remove baseline CT and replace with (previous) PET CT and again at Day 14. SOC CTA at month 12 to remain. Replace the Month 6 QoL Questionnaire on the Follow up schedule (protocol & ICS).
22 APR 2019	Inpatient stay after study product infusion changed from 4 hours to 2 hours. All subjects (VA & IUH) will receive a $30 gift card for the first 5 study visits, no mileage requirement. Patients will be reexamined changed from day 3 & 7 to days 7 & 14.
15 JUL 2021	Add unblinding of subjects at Month 24 follow-up visit. Add off-site storage of VA specimens to SSF (VA HIPAA Auth). Remove Maryanne Bowyer-Cherry.

CONTACT INFORMATION

Sponsor: Indiana University School of Medicine	**Primary Study Contact, Principal Investigator:** Michael P. Murphy, MD
	Protocol Author: Michael P. Murphy, MD
Coordinating Investigator and Principal Investigators An updated list of Principal Investigators (PI), investigation sites, and institutions will be maintained separately. The definitive list will be provided in the clinical study report.	

PROTOCOL SIGNATURE PAGE

Phase I study to examine the safety and efficacy of allogeneic MSCs in suppressing inflammation in patients with small abdominal aortic aneurysm (AAA)

As an Investigator for this Study, I have read the Clinical Trial Protocol. I agree to make available to the Sponsor, Indiana University School of Medicine (or its designee), original source documents and all regulatory documents pertaining to this Study. I agree to cooperate fully with the Sponsor with the conduct of study-related audits.

By my signature below, I agree to conduct this Study in accordance with the Clinical Trial Protocol, current Good Clinical Practice (cGCP) and Good Laboratory Practice (cGLP) guidelines, obligations as set forth in Title 21 CFR Parts 812, 54, 5,6 and 11 (as applicable), and any applicable regulatory laws. I will make no changes to protocol-defined procedures without written permission from the Sponsor.

I understand that Investigational Use Products may be used **only** for the purposes explicitly described in this protocol.

I further agree to treat the results of this Study as confidential information and will not submit the results of the Study for publication without prior written authorization from the Sponsor.

Michael P. Murphy, MD	Michael P. Murphy	7/15/2021
PRINTED NAME	SIGNATURE	DATE

SYNOPSIS

Title of Study	Phase I study to examine the safety and efficacy of allogeneic MSCs in suppressing inflammation in patients with small abdominal aortic aneurysm (AAA)
Objectives	Assess the safety and efficacy of systemic (IV) administration of escalating doses of allogeneic MSCs in modulating immune cell phenotypes and suppressing aortic inflammation in patients with small AAA.
Planned Number of Subjects and Duration of Involvement	36 patients
Patient Population	Abdominal aortic aneurysms (AAA) patients
Investigational Product Name	Allogeneic mesenchymal stem cells
Methodology Overview	In a double blind, Phase I trial, 36 patients with AAA measuring 30-50mm in maximal transverse diameter (MTD) will be randomized in a 1:1:1 fashion to receive either 1.0 $x10^6$ MSCs/kg (n=12), 3.0 $x10^6$ MSCs/kg. (n=12), or placebo (Plasmalyte A) (n=12) delivered intravenously. The primary safety endpoints will be incidence of treatment related adverse events at 24 months post MSC-administration. Exploratory primary efficacy endpoints will be changes in circulating inflammatory cell phenotypes and aortic inflammation as measured by CT. The treatment period will involve long-term follow-up through 5 years. The incidence of surgical intervention, aneurysm related death, quality of life, and major adverse cardiac events will be recorded.

Table 1 Abbreviations

AAA	Abdominal Aortic Aneurysm
AE	Adverse Event
AMI	Acute Myocardial Infarction
ASC	Adipose derived MSC
bMSC	Bone marrow derived MSC
CTSI	Clinical and Translational Sciences Institute
DSMB	Data Safety Monitoring Board
eGFR	Effective Glomerular Filtration Rate
GVHD	Graft Versus Host Disease
IFN-γ	Interferon-γ,
IL	Interleukin
IND	Investigational New Drug Application
IV	Intravenous delivery
MACE	Major Adverse Cardiac Event
LC-MS	Liquid chromatography-mass spectrometry
MCP-1	Monocyte Chemotactic Protein-1
MLR	Mixed Lymphocyte Reaction
MMP	Matrix Metalloproteinases
MNC	Mononuclear cell
MSC	Mesenchymal Stromal Cell
pMSC	Placenta derived MSC
18-FDG PET/CT	18-florodeoxyglucose positron emission tomography/computed tomography
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
SAE	Serious Adverse Event
TIMP	Tissue Inhibitors of Metalloproteinases
TNF-α	Tumor Necrosis Factor-α,
Treg	T regulatory cell
TSG-6	TNF- secreted protein 6
VAMC	Veterans Administration Medical Center
WBC	White Blood Cell Count

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2. INTRODUCTION.

Each year 200,000 people are diagnosed with AAA in the U.S. and 15,000 will inevitably die from rupture.[1] Approximately 39,000 endovascular or open surgeries are performed annually to prevent complications of AAA with two-year costs of $105,745 and $108,344 (2012 U.S. dollars), respectively.[2] The risk of rupture accelerates with AAA diameter thus an effective therapy that could mitigate AAA expansion would have a significant impact on patient survival, quality of life, and health care costs. In a recent randomized, placebo-controlled trial; doxycycline, an inhibitor of a key protease implicated in AAA formation, matrix metalloproteinase-9 (MMP), failed to reduce AAA expansion.[3] The pathogenesis of AAA begins with early T-cell and macrophage activation with subsequent recruitment of neutrophils and activation of MMPs.[1] The result is smooth muscle cell apoptosis, elastin degradation, and loss of structural integrity of the aortic wall. Extinguishing this inflammatory cascade in its initiation may prove more effective than targeting later phase proteases.

Mesenchymal stromal cells (MSC) have pleiotropic immunomodulatory properties relevant to the pathogenesis of AAA.[4,5] Our laboratory has shown that MSCs induce formation of immunosuppressive T-regulatory cells, decrease cytotoxic CD28-T cells, promote polarization of macrophages to the anti-inflammatory M2 phenotype, and inhibit neutrophil infiltration. Furthermore, human MSCs delivered intra-venously significantly decreased AAA inflammation and expansion in murine models, evidence that MSCs warrant further evaluation for this purpose in the context of a clinical trial.[6]

The objective of this study is to conduct a single center, double blind, Phase I trial to test the safety of systemic (intravenous, IV) administration of allogeneic bone marrow derived MSCs and compare two doses (1 million and 3 million MSCs/kg. in modulating inflammatory responses in patients with small AAA. The rationale for this investigation is: (1), there are no effective treatment options that suppress AAA inflammation and expansion; (2), there is an established safety profile of MSCs in previous clinical trials[7-11]; and (3), compelling preliminary data supporting this concept.

3. DESCRIPTION OF THE INVESTIGATIONAL PRODUCT

3.1 Overview

Human bone marrow derived MSCs increase T- regulatory (Treg) cells and decrease CD4+ and CD8+ cytotoxic T-cells in co-culture with peripheral blood mononuclear cells collected from AAA patients. Furthermore, we have demonstrated that human MSCs increase Tregs in blood and aortic tissue in mouse models of AAA while significantly decreasing aneurysm expansion. Treg induction presents a novel approach that may clinically suppress the inflammatory processes pathogenic in AAA formation.

3.2 Preclinical Data using adult human allogeneic bone marrow derived MSCs to support this IND.

3.2.1 The allogeneic MSCs used for these experiments are provided by Case Western Reserve University in Cleveland, Ohio. MSCs were obtained from 50 mL of bone marrow aspirated from a young, healthy donor. Mononuclear cells were cultured in 175 cm^2 flasks. Adherent cells were harvested and passaged (P1) into new flasks and at 70-80% confluence, they were further expanded to a second passage only (P2). MSCs used in these preclinical studies were manufactured using the exact processes that will be used for the MSCs to be used in the clinical trial detailed in this IND, thus we expect that MSC potency will be equivalent.

3.2.2 Circulating $CD4^+FoxP3^+$ T-regulatory cells are decreased in patients with AAA and significantly increased after co-culture with Human MSCs. PBMC specimens were isolated from patients undergoing endovascular AAA repair (n=10) and healthy subjects (n=5) and were stained with fluorochrome labeled

antibodies for CD3, CD4, CD25, and after permeabilization, for FoxP3, as previously decribed.[9] We found that there were significantly fewer circulating Treg cells in AAA patients (0.46 ± 0.07%) as compared to healthy controls (0.67+0.16%, *P*=0.01), (**Fig.1A).** PBMCs from AAA patients were then co-cultured with MSCs or saline for 7 Days and Treg were then quantified with FACS. MSCs significantly increased Treg at 7 days compared to control (2.56±0.06% vs. 1.07±0.04%, *P*< 0.01) (**Fig. 1B, C).**

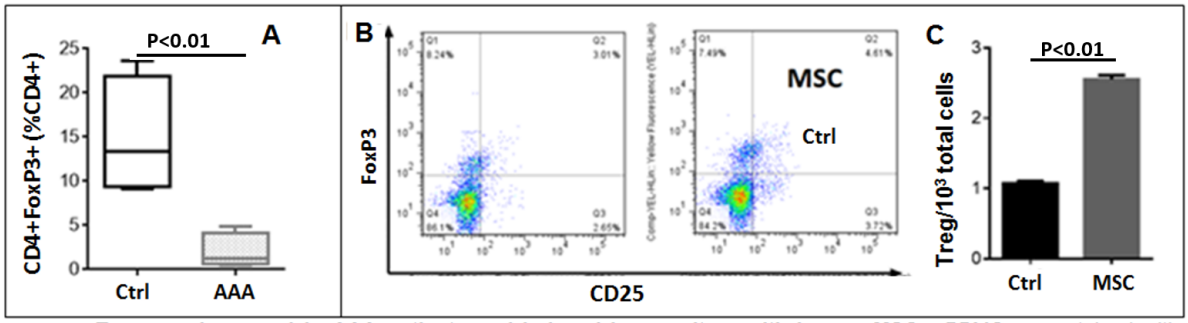


Figure 1. Treg are decreased in AAA patients and induced in co-culture with human MSCs. PBMC were stained with fluorochrome labeled antibodies for CD3, CD4, CD25, FoxP3 (after permeabilization). There were significantly fewer circulating $CD4^+FoxP3^+$ Treg in AAA patients (0.46±0.07% vs. Healthy controls (0.67±0.16%, *P*=0.01) (**A**).PBMCs from AAA patients were co-cultured with human MSCs or 0.9% NaCl (control) for 7 Days. $CD4^+CD25^+Foxp3^+$ cells (Treg) were quantified with flow cytometry (**B**). PBMC cultured with MSC were significantly enriched for Treg at 7 Days (2.56±0.06%) vs. control (1.07+0.04%, *P*<0.01) (**C**).

3.2.3 Human MSCs increase T-regulatory cells in aortic aneurysm tissue in the mouse elastase AAA model. C57Bl/6 mice were given hMSCs ($1x10^6$) or PBS 24 hours after aortic elastase perfusion. Mice were sacrificed at Days 1, 4, and 7 and aortas harvested. Immunohistochemical (IHC) staining of aneurysm sections (**Fig. 2A**) demonstrated that hMSCs significantly increased murine Treg as compared to controls at each time point (**Fig. 2B**).

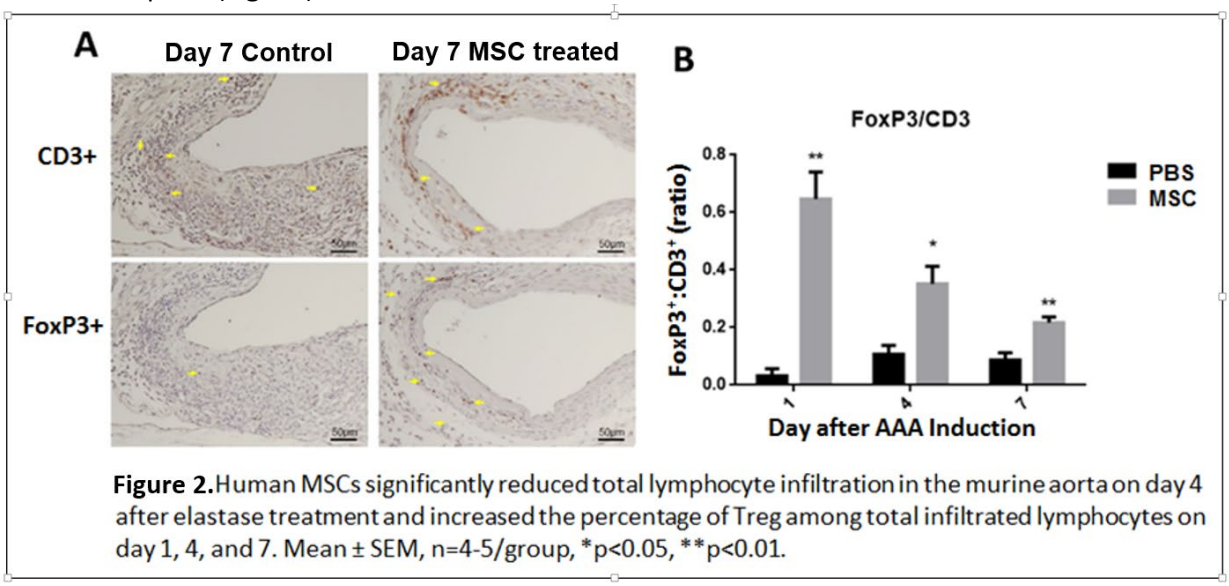


Figure 2. Human MSCs significantly reduced total lymphocyte infiltration in the murine aorta on day 4 after elastase treatment and increased the percentage of Treg among total infiltrated lymphocytes on day 1, 4, and 7. Mean ± SEM, n=4-5/group, *p<0.05, **p<0.01.

3.2.4 Human MSCs increase immune suppressor function in the mouse elastase AAA model. Using the murine elastase AAA model, $1x10^6$ human MSCs or phosphate buffered saline (control) were injected IV (n=5/group) at the time of elastase application. At 72 hours the mice were sacrificed and $CD4^+CD25^+$ cells were isolated from PBMC using magnetic beads followed by cell sorting to isolate Tregs

($CD4^+CD25^+CD127^{lo}$) and $CD4^+CD25^+$ T-cells (responder cells) . The cells were cultured in varying ratios: Treg: Tresponder of 0.1:1.0; 0.5:1.0; and 1:1, in 96 well culture plates coated with 100 µL of 5.0 µg/mL anti-CD3 and anti-CD28. Controls were the $CD4^+CD25^-$ responder cells plated alone. Cell proliferation was measured using a modified 3-[4,5-dimethyl-2-thiazol-yl]-2,5-diphenyltetrazolium bromide assay with a Premix WST-1 Cell Proliferation Assay System as described. Percentage suppression was determined at each ratio of Treg: Tresp by comparison with $CD4^+CD25^-$ cells (Tresp) plated alone. We found that immunosuppressive activity was significantly increased in Tregs from MSC treated compared to control treated mice (**Fig. 3**).

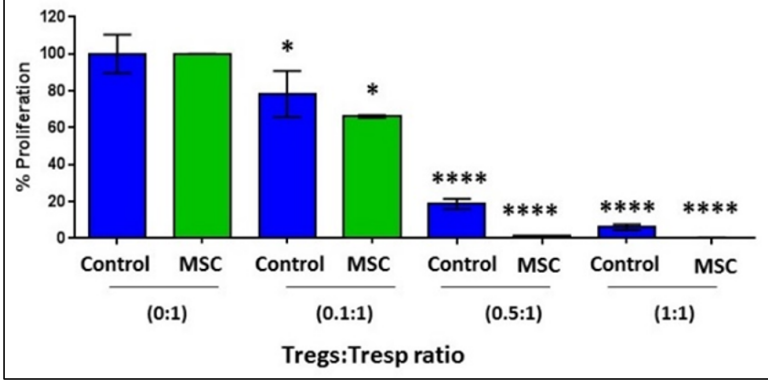


Figure 3. Intravenous administration of human MSCs promote murine Treg suppressor function in the mouse AAA model. There was a significant reduction in proliferation with Tregs isolated from MSC treated mice compared to controls (Tresp) at 0.5:1 and 1:1 ratios (Treg:Tresp).Statistically significant values relative to proliferative arm with no regulatory T cell (0:1) are indicated (one-way ANOVA with Dunnett's multiple comparison test).* P-value ≤0.05 **** P-value ≤0.0001

3.2.5 Circulating $CD4^+CD28^-$ and $CD8^+CD28^-$ T-cells are increased in patients with AAA and significantly decreased after human MSC infusion in the murine elastase AAA model. The mononuclear cell fraction of peripheral blood (PBMC) was isolated from patients with AAA and age matched healthy subjects and analyzed with FACS. Percentages of $CD4^+CD28^-$ and $CD8^+CD28^-$ T-cells were significantly greater in AAA patients compared with healthy controls. Adjusting for normal accumulation of $CD28^-$ T cells in healthy controls, 60.4% of the AAA patients had elevated levels of $CD4^+CD28^-$ T cells and 19.8% showed increased levels of $CD8^+CD28^-$ T cells in the peripheral blood (**Fig.4 A)**. MSCs or phosphate buffered saline (PBS) were infused in C57Bl6 mice at the time of AAA induction. $CD4^+CD28^-$ and $CD8^+CD28^-$ T-cells and Neutrophils ($Ly6G/C^+$) from aortic aneurysm tissue specimens were quantified using flow cytometry at Day 7 after MSC or PBS infusion revealing a significant decrease in all of these pathogenic inflammatory cell phenotypes (**Fig.4B**).

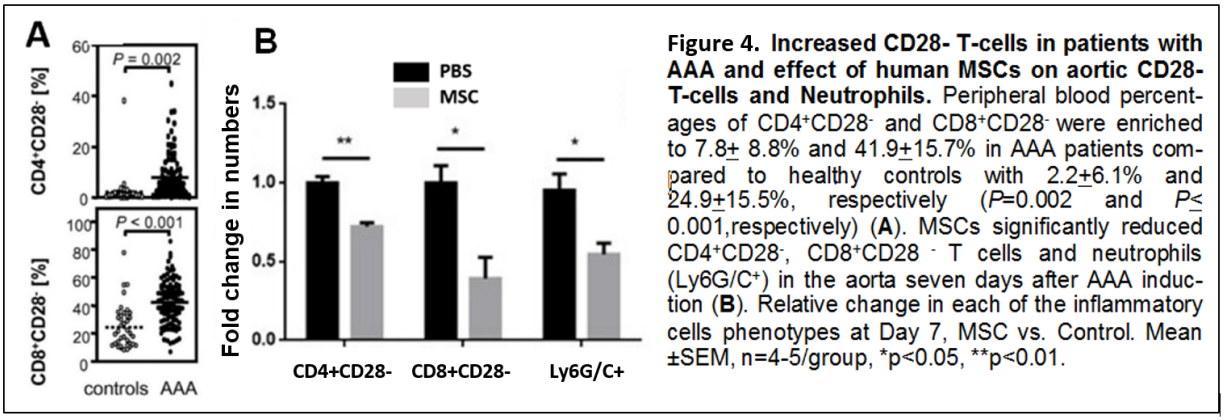


Figure 4. Increased CD28- T-cells in patients with AAA and effect of human MSCs on aortic CD28- T-cells and Neutrophils. Peripheral blood percentages of $CD4^+CD28^-$ and $CD8^+CD28^-$ were enriched to 7.8± 8.8% and 41.9±15.7% in AAA patients compared to healthy controls with 2.2±6.1% and 24.9±15.5%, respectively (P=0.002 and $P\leq$ 0.001,respectively) (**A**). MSCs significantly reduced $CD4^+CD28^-$, $CD8^+CD28^-$ T cells and neutrophils ($Ly6G/C^+$) in the aorta seven days after AAA induction (**B**). Relative change in each of the inflammatory cells phenotypes at Day 7, MSC vs. Control. Mean ±SEM, n=4-5/group, *p<0.05, **p<0.01.

3.2.6 Human MSCs preserve elastin and collagen and inhibit matrix metalloproteinase-9 activity in the murine elastase AAA model. Using the topical elastase AAA model, we performed histological comparisons of the aneurysmal tissue to assess the effects of MSCs on elastin and collagen fiber content in the extra-cellular matrix of the aortic wall. We found that compared to control mice, MSC treated mice retained elastin lamellae and collagen fibers (**Fig. 5A-C**). Elastin is the primary architectural protein of the aorta and is destroyed by production of MMP-9 by activated inflammatory cells leading to loss of tensile strength and aneurysm formation. MMP-9 serum levels are elevated in about 50-70% of patients with AAA. MSCs have been shown to secrete tissue inhibitors of metalloproteinsase-1 and -2 that inhibit MMP-2 and -9 activity *in vitro. We* found that compared to control treated AAA mice, MSCs significantly decrease serum MMP-9 activity (**Fig. 5D**).

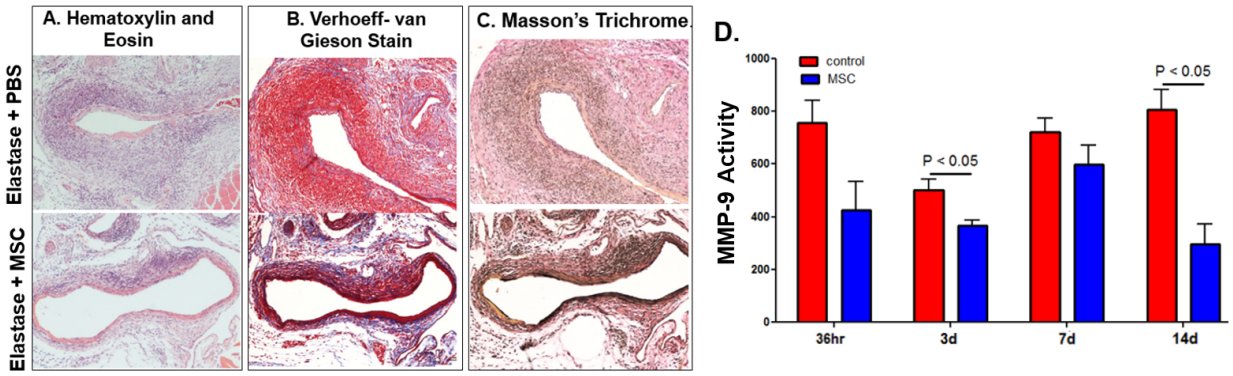


Figure 5. **Representative images after staining fixed sections of the murine aorta 14 days after aneurysm induction (40x magnification).** Hematoxylin and Eosin staining reveals preservation of the tunica media in MSC treated compared to phosphate buffered saline treated mice (**A**) , MSC treated mice showed reduced aortic elastin fragmentation (**B**), and collagen degradation (**C**). Using the Anaspec Sensolyte MMP-9 assay kit MMP activity was measured in relative fluorescence units at Ex/Em 490/520, as previously described. MMP-9 activity was significantly decreased compared to PBS treated mice at Day 3 (365.54 ± 21.76 RFU SEM vs 501.66 ± 40.51 RFU SEM, $p < 0.05$) and at Day 14 (295.13 ± 77.89 RFU SEM vs 805.56 ± 76.53 RFU SEM, $p < 0.05$), with a trend towards decreased MMP-9 activity at Day 7 ($p = 0.8$) (**D**).

3.2.7 Systemic infusion of human MSCs suppresses IL-17, -23, and IFN-γ and AAA expansion in the mouse elastase model. We delivered hMSC IV on day 1 after perfusion of the aorta with elastase. We then measured aortic diameter by video micrometry at Day 14. Compared to baseline, aortic diameter was significantly decreased in hMSC-treated compared with saline treated mice (**Fig. 6A**).

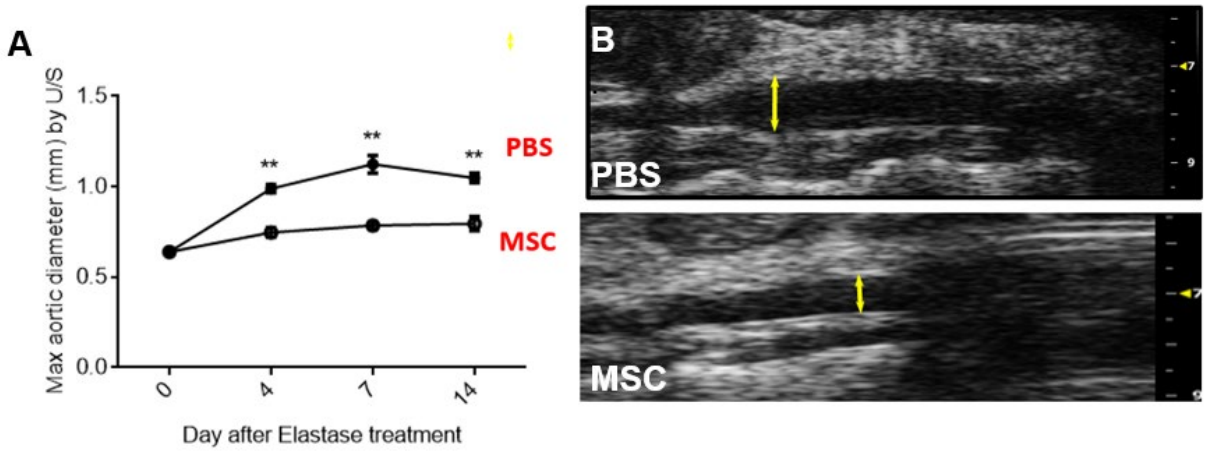


Figure 6. Human bone marrow MSCs attenuate AAA expansion in the murine elastase model. MSCs significantly suppressed murine elastase induced aortic expansion by day 4 compared to vehicle (**A**). Mouse aorta ultrasound images of at baseline and days 4, 7 and 14 post elastase treatment or 1 x 106 human MSCs (i.v.) (**B**). N=4-10/group, **p<0.01.

3.2.8 IL-10 responses are deficient in AAA patients and robustly enhanced after MSC administration *in vivo* in the murine elastase AAA model. Serum levels of the anti-inflammatory cytokine IL-10 are significantly decreased in patients with AAA as compared to risk factor matched subjects without AAA , whereas as serum levels of TSG-6 and PGE_2 are increased in AAA patients. *This suggests that an impaired host IL-10 response to tissue injury and inflammation may contribute to AAA formation* (**Fig. 7**). Clinical grade MSCs exposed to TNF-α in culture robustly secreted 12x the levels of IL-10, 7.6x levels of TSG-6, 3.6x levels PGE2, and 1.6x levels of TGF-β from unstimulated conditions (**Fig. 8A**). Using the same MSC stock we then administered $1x10^6$ cells to our mouse AAA model. Using species specific antibodies for human and mouse IL-10 we discovered that mouse serum levels of *human* IL-10 peaked at Day 3 and then were undetectable by Day 7. Serum levels of *mouse* IL-10 were significantly increased at Day 7 and continued to increase to Day 21 (when the experiment was terminated) in MSC treated treated AAA mice (**Fig. 8B**). Thus it appears that MSCs induce a delayed endogenous host IL-10 response that may sustain the anti-inflammatory effect after human MSCs are cleared. Tr1 regulatory cells are induced by exogenous IL-10 and exert immunoregulatory control via secretion of IL-10. Thus we plan to enumerate Tr1 in addition to $FoxP3^+$ Tregulatory cells in Aim 1 of this proposal.

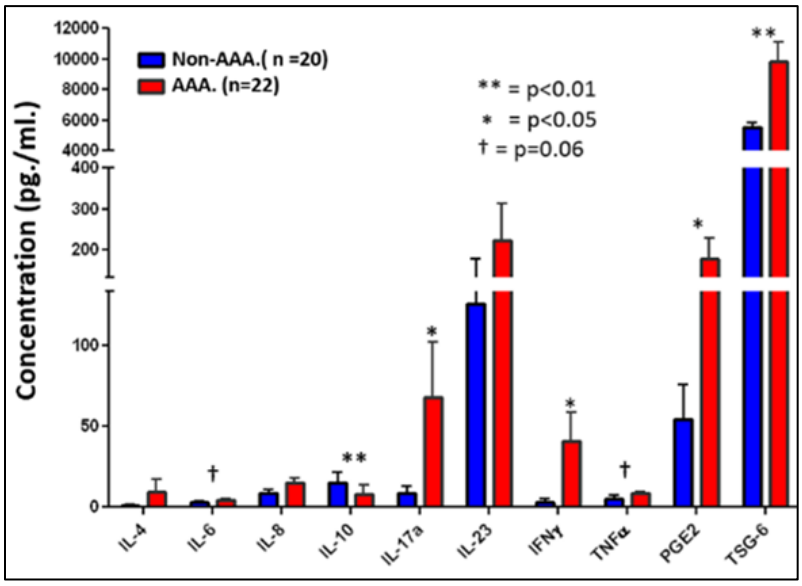


Figure 7. IL-10 is deficient in patients with AAA. Blood samples were collected from patients being screened AAA. Using the Bio-plex multi-analyte system we found significant differences in, IL-10 (14.36 ± 0.51 vs. 8.03 ± 1.01), IL-17a (9.00 ± 4.16 vs. 67.82 +/32.93), IFN-γ (3.22 ± 1.69 vs. 40.63 ±17.66), TNF-α (5.14± 1.84 vs. 8.32 ± 0.99), PGE_2 (54.3 ± 21.02 vs. 176.69 ± 43.1), and TSG-6 (545.8 ± 358 vs. 9826.53 ±1280), pg./ml; non-AAA vs. AAA, respectively, P< 0.01

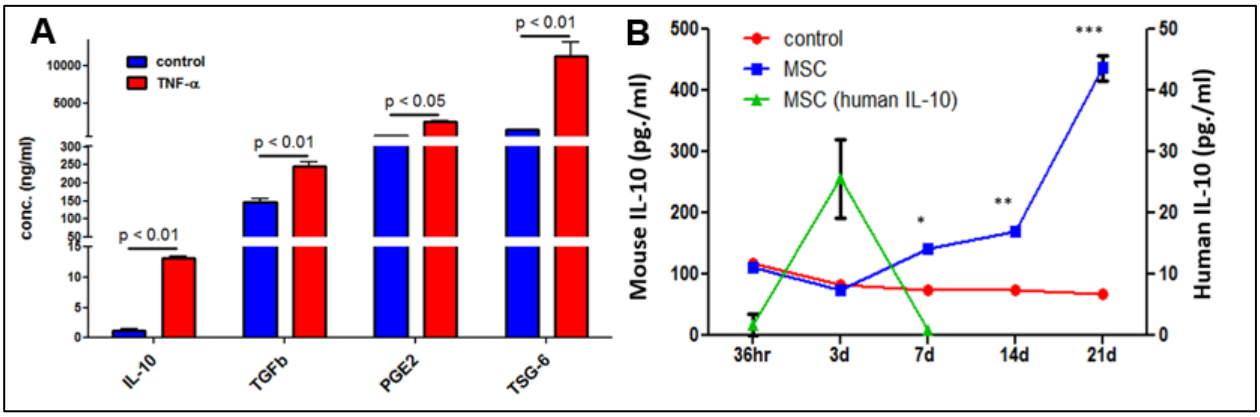


Figure 8. Mesenchymal Stromal Cells robustly secrete IL-10 *in vitro* and induce an endogenous IL-10 response *in vivo*. MSCs were cultured to 90% confluency and 10ng/ml of TNF-α was added. After 24 hours the supernatant was collected and analyzed by ELISA. IL-10 increased from 1.12 ± 0.31 to 13.23 ± 0.25 pg/ml, TGF-β ↑ 148.08±11.29 to 245.12±19.74 pg/ml., PGE2 ↑from 704.12±21.2 to 2586.52±142.69 ng. /ml., and TSG-6 ↑from 1474.82 ±11.41 to 11276.06 ±1802.06 ng. /ml.(±SEM) (**A**). Using our murine elastase model we administered 1 x106 human MSCs after AAA induction and peripheral blood (n=5/per time point) was collected. Using ELISA with species specific antibodies we found that there was an initial surge of human IL-10 peaking at Day 3 (25.59 ± 11.11 pg./ml) and undetectable at Day 7. Murine IL-10 significantly increased at Day 7 (148.63 ± 23.62 SEM pg./ml) in MSC treated mice vs. control (73.71± 12.02 pg./ml.) and peaked at Day 21 (426.28 ± 21.52 pg./ml) vs. control (69.21±19 pg./ml), when the experiment was terminated. (±SEM) * P< 0.05, **P<0.01, and ***P< 0.001 compared to control (**B**).

3.3 Proposed Intended Use Statement

Allogeneic MSCs are a novel therapeutic option to suppress growth of small AAA. Human MSCs are intended for investigational use only by selected investigators familiar with their use and experienced in conducting clinical studies. Human MSCs may only be administered to human subjects participating in clinical studies sponsored/approved by Indiana University School of Medicine, and who have provided formal written consent.

3.4 Study Objectives.

The principal objective of this study is as follows:

Assess the safety and efficacy of administration of allogeneic MSCs in patients with small AAA.

This study will test the hypothesis that administration of MSCs in doses of 1x 10^6 MSCs/kg. and 3x 10^6 MSCs/kg demonstrate safety in patients with small AAA. This trial will also test the hypothesis that MSCs, in a dose dependent fashion (1 x10^6 MSC/kg. vs. 3.0 x 10^6 MSC/kg.), promote the frequency and immune suppressor function of Treg cells and decrease AAA inflammation as measured by computed tomography. In an double blinded, Phase I trial, 36 patients with AAA measuring 35-50 mm in maximal transverse diameter (MTD) will be randomized in a 1:1:1 fashion to receive intra-venously either a single dose of 1 x10^6 MSCs/kg., 3 x10^6 MSCs/kg. , or placebo (Plasmalyte A) (n =12/group). The primary safety endpoints will be incidence of treatment related adverse events accrued over 24 months. Efficacy measures are changes in frequency and immune suppressor function of Tregs, number and cytotoxic activity of CD4+/CD8+ CD28- T-cells, activated monocytes, and changes in aortic inflammation as measured by PET CT compared to baseline. Secondary endpoints measured out to year 5 will include changes in IL-10 and Tr1 levels. Duplex ultrasound measurements of the AAA, which is considered standard of care, will be recorded as change in aortic inflammation during long-term follow-up. Incidence of surgical intervention, aneurysm related death, quality of life, and major adverse cardiac events will be recorded through year five (5).

Completion of this study will determine if MSCs are safe in patients with AAA and provide critical insights that will direct a placebo-controlled, Phase II randomized trial.

4.0 STUDY OVERVIEW

4.2 Study Approach

This protocol is designed to describe a Phase I single center, double blind study that will enroll 36 patients having a diagnosis of degenerative infrarenal abdominal aortic aneurysms measuring 35-50 mm. in diameter by Computed Tomographic imaging. The PI, Michael P. Murphy MD will be unblinded. Co-Investigators, nurse coordinators and study subjects will be blinded. At Month 24 post-study product infusion, subjects will have the option to be unblinded to their investigational product.

4.3 Study Duration

It is expected that the study will be completed within 8 years. Each patient will be followed for 24 months for assessment of safety with long-term follow-up through year 5 after enrollment.

5. STUDY POPULATION

5.1 Sample Size and Target Study Population

5.1.1 Sample size

The total number of individual subjects is expected to reach up to 36.

5.1.2 Study population

The study population will be representative of adults at least 40 years of age and at most 85 years of age with small (30-50mm) AAA. Children will not be eligible for this study because AAA is a degenerative and inflammatory disease of the aged. It is anticipated that at minimum, enrollment will reach approximately 1-16 qualifying adults per month for 1–2 years.

5.1.3 Alignment with intended study population

The study population includes patients likely to benefit from allogeneic MSC transplantation. It will also include other patients who will be controls.

5.2 Recruitment Methods

5.2.1 Recruitment for Study

Eligible patients will be invited to participate in the study on a first-come basis, subject to Indiana University School of Medicine weekly recruitment goals. Patients will be recruited for this study from the Richard Roudebush VA Medical Center and IU Health Methodist Hospital. Patients will be informed of the possible risks of the procedure and will be required to give informed consent before study-specific procedures can proceed. A minimal financial inducement will not be offered. Each subject will be informed that no personally relevant clinical information will be derived from the collected data, and that the only possible benefit to the subject is a decrease in AAA expansion rates. Medications will be documented and certain medications may be held. Each subject's involvement in the study will be limited to the period between signing of the informed consent form (ICF) and the completion of study specific procedures.

5.2.2 Duration of study activities

Enrollment is anticipated to continue for approximately 1–2 years. In the event that additional studies are going to be conducted, new protocols will be developed specifically for those studies.

5.3 Patient Selection. The eligibility criteria for prospective enrollment of subjects are shown in **Table 1**.

Table 1. Inclusion/Exclusion Criteria for Enrollment of Subjects

Inclusion criteria	1. Be ≥ 40 and ≤85 years of age. 2. Have diagnosis of non-inflammatory degenerative infrarenal abdominal aortic aneurysms measuring 30-50mm. in diameter by Computed Tomography (CT) scan. 3. Females of childbearing potential must be willing to use one form of birth control for the duration of the study. Female participants must undergo a blood or urine pregnancy test at screening.
Exclusion criteria	1. Inflammatory AAA defined by a thickened aortic wall and retroperitoneal fibrosis and adhesions of peritoneal organs, and elevated erythrocyte sedimentation rate or in the opinion of investigator. 2. Mycotic AAA defined as saccular morphology, a positive blood culture, fever, or in the opinion of the investigator. 3. Symptomatic, Saccular, or any AAA associated with thoracic aorta dilatation > 5.0 cm. 4. Infra-renal AAA associated with Marfan's or Ehlers-Danlos Syndrome or other connective tissue disorders 5. Common or external iliac artery aneurysm > 30 mm. in maximal transverse diameter. 6. AAA due to dissection. 7. Allergy to iodine contrast. 8. History of untreated or recurrent cancer within the last 5 years, except basal cell skin carcinoma.

	9. eGFR< 30mL/min. 10. Any condition requiring immunosuppressant medications (e.g., for treatment of organ transplants, psoriasis, Crohn's disease, alopecia areata, rheumatoid arthritis, scleroderma, lupus). 11. Acute coronary syndrome in the last 30 days prior to enrollment. * 12. CHF hospitalization within the last 30 days prior to enrollment. * 13. HIV or HCV positive. 14. Contraindication to Computed Tomography or known allergy to contrast media. 15. Any bleeding diathesis defined as an INR ≥ 2.0 (off anticoagulation therapy) or history of platelet count less than 70,000 or hemophilia. 16. Pregnant or breast feeding women. 17. Significant hepatic dysfunction (ALT or AST greater than 2 times normal). 18. Life expectancy less than two years. 19. Inability to provide written informed consent due to cognitive or language barriers (interpreter permitted). 20. Presence of any clinical condition that in the opinion of the PI or the sponsor makes the patient not suitable to participate in the trial. *As defined by the standard definitions of CHF and ACS by the American Heart Association.
Participant Withdrawal Criteria	At the discretion of the investigator or at the request of the participant. The reason for withdrawal will be documented, specifically if the reason is due to an adverse event.

6. STUDY MATERIALS

6.1 Investigational Product

6.1.1 Identity of the investigational product

The clinical grade allogeneic MSCs required for this Phase I study will be provided by the Case Western Reserve University in Cleveland, Ohio. MSCs will be cultured from mononuclear cells obtained from a single healthy donor and expanded to passage 2.

6.1.2 Safety issues

Enrolled patients will be randomized to receive 1 or 3 million MSC/kg or placebo IV. This dose has been extrapolated from our preclinical model[6] and spans the range of MSC doses used in previous clinical trials.[7-11] Hare, et al, tested the safety of allogeneic MSCs in patients with acute myocardial infarction randomized to doses of 0.5, 1.6, and 5 million MSCs/kg in a similar dose escalation study without treatment related adverse events.

6.1.3 Handling, storage, accountability

MSCs will be transported using a liquid nitrogen dry shipper validated for 4 days to maintain temperature for long-term storage. The temperature is logged upon arrival and becomes part of the official shipping documentation. The integrity and temperature of the cell containers are checked on arrival and results documented. The study product should not be at room temperature for more than four hours.

6.1.4 Required training and MSC administration

MSCs will be manufactured at the Case Cancer Comprehensive Center Cellular Therapy Lab at Case Western Reserve University in Cleveland, Ohio with supervised and trained personnel. The MSCs, in the

appropriate dose, will be shipped to the Cell Therapy Laboratory at University Hospital where the MSCs will be thawed and diluted 1:1 with Plasmalyte A and administered within 4 hours of the thawing process. All personnel in the Stem Cell Laboratory are qualified in the processing on MSCs for patient administration. The thawed MSCs will then be administered to the patients in a monitored setting with telemetry and pulse oximetry.

MSC Administration. This is a randomized, double blinded trial and after enrollment, patients will be randomized in a 1:1:1 fashion to receive placebo (Plasmalyte A), $1x10^6$ MSC/kg., or 3×10^6 MSC/kg. The rationale for selection of these doses was presented in the Research Strategy. IV MSC infusions will occur in a monitored inpatient room on the Surgical Intensive Care Unit at the Roudebush VA Hospital or the Clinical Research Center at IU Health University Hospital. Patients will be pre-medicated with hydrocortisone (25-50 mg. IV) and diphenhydramine (Benadryl, 25-50 mg. IV). All subjects will be monitored throughout the MSC infusion procedure with vital signs and pulse oximetry beginning 15 minutes prior to infusion and ending 2 hours post procedure. They will also be evaluated for clinical signs of pulmonary distress including progressive or sustained hypoxia, dyspnea, and tachycardia. If enrolled patients develop any of the following conditions within 24 hours of planned infusion, MSC delivery will be halted:

a. Fever (Temperature increase to ≥100.4°F)
b. Upper respiratory tract infection diagnosed by independent health provider.
c. Significant shortness of breath
d. Unanticipated change in consciousness or neurological change
e. Major adverse cardiac event

All patients will be admitted for continued observation to the SICU at the Roudebush VA Hospital or the Clinical Research Center at IU Health University Hospital. The patient will be observed for two hours after the study product infusion and then discharged home. The patient will be re-examined on Days 7 and 14 for treatment related adverse events.

6.1.5 External studies

In the event that plans are made to conduct additional studies, new protocols describing the relevant handling, storage, accountability, and training procedures will be prepared specifically for those studies.

6.2 Other Study Materials

6.2.1 Materials to be provided by study site

- Study notebooks to maintain study documents, including signed ICFs, all applicable information, and additional forms to be collected and retained by the study institution during the course of the study.
- Example spreadsheets for tracking patient information.

6.2.2 Materials to be provided by external testing facilities

There are no external testing facilities associated with this study.

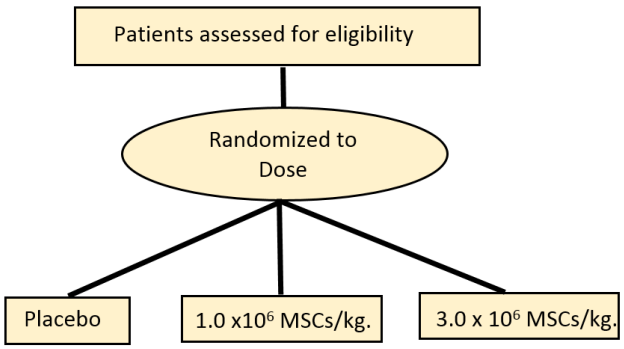
7. STUDY PROCEDURES

7.1 Workflow

MSC preparation. Donor infectious disease testing will be performed by CLIA certified Laboratories. Allogeneic donor Infectious Diseases testing will include anti-HIV-1/2 and anti-HCV. Testing will be repeated if it exceeds 30 days. Potential donors whose testing is incomplete or not available within 30 days will not be eligible to donate bone marrow and testing will be repeated to confirm ID markers. Potential donors testing positive for any of these infectious diseases will NOT be eligible.

Randomization Scheme and MSC Dosing. The PI, Michael P. Murphy MD., will determine inclusion in the study. After that decision has been rendered, the patient will be screened for enrollment. After enrollment, 36 patients will be randomized to receive MSCs or placebo IV through an antecubital vein (**Figure 1**).

Figure 1. Randomization Scheme.



Serum samples will be collected pre-MSC administration, 1 and 6 months' post MSC-treatment and analyzed in the Cellular Therapy Laboratory of University Hospital to determine if patients develop antibodies against allogeneic MSCs.

Baseline Screening and Follow-Up Schedule. The following evaluations will be carried out at baseline to determine if patient is eligible for study: (1) Baseline blood tests (complete blood count, serum chemistries and liver panel); (2) infectious disease panel and HLA typing; (3) medical history and physical exam; (4) concomitant medications; (5) pregnancy test for women of childbearing age ;(6)12 lead ECG; (7) 18-fluorodeoxyglocose (FDG) uptake with positron emission tomography/computed tomography (PET/CT) will be performed at baseline and Day 14. Computed tomographic angiography of the abdominal aorta and iliac arteries (as part of "Standard of Care") will be performed at month 12. Duplex ultrasound of the aorta will be performed at month 12 while the patients are enrolled in the trial to monitor AAA size. Follow up studies are detailed in **Table 2**, below. Subjects will return for long-term follow-up visits at six month intervals from year 1 (+/- 4 weeks) through year 5 (+/- 4 weeks). Window ranges for follow-up visits are guidelines for scheduling and will not be considered a protocol deviation if visits fall outside of these ranges. See **Table 3**, below, for details on the long-term follow-up visits.

- Annual CT abdominal exams beginning at year 1 are considered Standard of Care.

- MSCs products will be thawed and diluted 1:1 with Plasmalyte. Samples for cell viability (i.e. Trypan Blue and/or 7AAD) and 14-day bacterial sterility testing will be obtained. Viability >70% will need to be entered in the Certificate of Analysis before the product is released for administration.

- In the event of a positive sterility test result after products infusion, the physician and patient will be informed, and the results reported to the IRB and FDA. The positive result will be reported in an information amendment submitted to the IND in a timely manner, preferably within 30 calendar days after initial receipt of the positive culture test result (21 CFR 312.31).

    - Should cultures for detection of bacteria and fungi become positive, the Principal Investigator of the study will be notified. The PI will then notify the patient and the appropriate antibiotics will be started based on sensitivities of the identified organism.

    - A physical examination of will be conducted within 24 hours at which time a complete blood count will be obtained. Should there be an elevate white blood cell count (> 11.5K), the patient will be admitted to the hospital for intravenous antibiotics or antifungal medications. Should there be no clinical evidence of infection the patient will be treated for 7 days with oral antibiotics or anti-fungal medication

    - A systematic review of potential sources of contamination will be conducted with emphasis on skin preparation and draping and the components of the cell transport and preparation system. An examination of the sterile packaging for disposable components and the process of sterilization of non-disposable components will be performed as part of this review.

    - An infection as a result of contamination will be reported as an adverse event within 15 calendar

Table 2. Follow-Up Schedule

Procedures	**Baseline**	**Day 0 MSC (4-6 hr. visit)[9]**	**Day 7 F/U (+/- 2 days)**	**Day 14 F/U (+/- 5 days)**	**Mo 1 F/U (+/- 1wk)**	**Mo 6 F/U (+/- 2wk)**	**Mo 12 F/U (+/-2 wk)**	**Mo 18 F/U (+/-2 wk)**	**Mo 24 F/U (+/-4 wk)**
Informed Consent	**X**								
Medical History	**X**								
Physical Exam	**X**	**X**	**X**	**X**	**X**	**X**			
Vital Signs	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Con. Medications	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
AE/SAE Evaluations	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
QoL Questionnaires	**X**					**X**	**X**	**X**	**X**
Infectious Disease Labs[1]	**X**								
Laboratory Evaluations	**X[2]**				**X[3]**				
12 Lead ECG	**X[8]**				**X**				
Duplex Ultrasound	**X**					**X**	**X**	**X**	**X**
Randomization	**X**								
PET CT	**X**			**X**					
CT Angiogram[9]							**X**		**X**
Blood Sample[4]		**X[5]**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Infusion Reaction Assessment[6,7]		**X**		**X**					

1. Infectious disease testing including basic HIV, Hepatitis C testing. HLA Typing will be performed at baseline, but is not exclusionary to enrollment, nor a protocol deviation if HLA results after study product infusion.
2. Baseline labs ONLY = CBC with differential, Glucose, Sodium, Potassium, Chloride, CO2, BUN, Creatinine, eGFR, hs-CRP, CPK, HbA1C, Total Bilirubin, Total Protein, Albumin, Alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), platelet count, PT/INR, Pregnancy (childbearing Females-baseline only).
3. Follow-up labs = CBC with differential, Glucose, Sodium, Potassium, Chloride, CO2, BUN, Creatinine, eGFR, hs-CRP, CPK, Albumin, platelet count.
4. Peripheral blood draw for ancillary studies is up to 34 ml.
5. Day of MSC administration blood sample to be collected prior to study product infusion (can be done at physical exam if within 2 hours of infusion).

6. Infusion reaction assessment will include: clinical observation for signs of tachycardia and cutaneous flushing and vital signs (blood pressure, heart rate, respiratory rate, and temperature) at 15 min intervals throughout infusion starting 15 min prior to infusion. Post infusion vital signs will be monitored at 15 min, 30 min, 1 and 2 hours.
7. Oxygen saturation will be continuously monitored throughout infusion by pulse oximetry starting 15 min prior to infusion through 2 hours post infusion.
8. Baseline ECG may be obtained any time prior to study product infusion.
9. CT Angiograms beginning at year 1 and performed annually throughout subjects' participation are considered Standard of Care.

Table 3. Long-Term Follow-Up Schedule

	Mo 30 (+/-4wks)	**Mo 36 (+/-4wks)**	**Mo 42 (+/-4wks)**	**Mo 48 (+/-4wks)**	**Mo 54 (+/-4wks)**	**Mo 60 (+/-4wks)**
Duplex Ultrasound[1]	**X**	**X**	**X**	**X**	**X**	**X**
CT Angiogram[3]		**X**		**X**		**X**
QOL Questionnaire	**X**	**X**	**X**	**X**	**X**	**X**
AE/SAE Evaluations	**X**	**X**	**X**	**X**	**X**	**X**
Blood Sample[2]	**X**	**X**	**X**	**X**	**X**	**X**

1. Duplex ultrasound measured every six months is considered standard of care.
2. Peripheral blood draw for ancillary studies is 34 ml.
3. CT Angiograms performed annually are considered Standard of Care.

7.2 Study Data

Eligible subjects will be invited to participate in the study on a first-come basis. Potential donors must provide informed consent to participate. To be eligible for participation, subjects must be between 40 and 80 years of age with an AAA 30-50 in MTD.

7.2.1 Collection of data

At the discretion of the Investigator and subject to Indiana University School of Medicine requirements, data (**Table 1**) will be collected from each eligible subject providing signed informed consent.

7.3 Procedures for Study Closure

7.3.1 Routine study close-out

The study will end when Indiana University School of Medicine has obtained all data necessary to complete its studies of the test product. Study close-out will follow Indiana University School of Medicine standard procedures and may include, but is not limited to, review of regulatory documents, collection of completed case report forms, reconciliation of study records, removal or destruction of ancillary study supplies, and informing the Investigator of remaining obligations (e.g., record retention, final report submission to the IRB, financial disclosure updates, etc.).

7.3.2 Suspension or premature termination of the study

This study may prematurely terminate at any time because of a regulatory authority decision, a change in opinion of the IRB, or at the discretion of the Investigator or Sponsor. If this trial is temporarily suspended or prematurely discontinued, Indiana University School of Medicine will promptly notify the Investigator(s) and provide instructions. If the study is temporarily suspended, Indiana University School

of Medicine will provide guidance on timing and procedures for resuming the study. If the study is prematurely discontinued, all study materials must be collected and all study forms completed to the extent possible. All such materials must be returned to Indiana University School of Medicine upon request.

8. DATA QUALITY ASSURANCE

The study site will be responsible for the accuracy of data. Indiana University School of Medicine or its agent may periodically conduct monitoring visits to ensure the quality of data collection.

9. STATISTICAL METHODS

The safety of systemic administration of allogeneic MSCs will be measured by treatment-related adverse events. Treatment-related adverse events will be categorized by overlapping systems and severities. The three categories of systems are cardiovascular, respiratory, or infectious. Two categories of severity will be serious adverse (SAE) and major adverse cardiac events (MACE). For completeness, instances of adverse events may appear in more than one category. Within each of these categories adverse events will be listed in descending order of frequency for the treatment-group. In addition, for each category, the sum and difference between the two routes of delivery of the proportions will be reported as percent incidence. Confidence Intervals at the 95% confidence level and *P*-values for these four groups will be calculated. Since four previous trials have not reported adverse events with MSC treatment, confidence intervals will be generated by the method of the Wilson Score Interval because they are robust, with good coverage probability even for small number of trials and are not degenerate near zero. These intervals will be used to summarize the data rather than as any formal inferential statement. No adjustment for multiplicity will be made.

Primary attention in Aim 1 will focus on the detection of cellular immunosuppression activity coincident with MSC administration in both proliferation (six decreasing and one increasing) and function (three decreasing functional outcomes). Ten mixed model repeated measures with a fixed term for MSC dose and their appropriate baseline covariate will be fit. The denominator degrees of freedom will be estimated by the method of Kenward and Rodger.[12] A compound symmetry covariance structure will be fit if the model with the unstructured covariance does not converge. For these data the directionality of superiority or inferiority will be assessed. Specifically, the alternative hypotheses are that (1) three T-cell counts ($CD4^+CD28^-$, $CD8^+CD28^-$ T-cells and Th-17^+ T-cell) will be decreased; (2) $CD28^-$ T-cell function will be inhibited (decreased; as measured by IFN-γ–producing cells, Perforin-producing cells, and CD57 expression); (3) T-regulatory cells ($CD4^+Fox3P^+$ T-regs) will be increased; and (4) monocytes will be decreased (fewer $CD45^+CD14^{++}CD16^-$, $CD45^+CD14^+CD16^+$, $CD45^+CD14dim/^-CD16^{++}$). To assess the individual hypotheses, the model-derived, confidence interval for the intercept will be reported. Confidence intervals at the 95% level from the same models will be constructed to estimate the difference, or contrast, between routes of administration

9.1 Bias Minimization

All subjects meeting the specified eligibility criteria will be enrolled on a first-come basis.

9.2 Planned Analyses

The clinical study report will contain only summary data analyses reflecting safety profiles, and summary data analyses of efficacy. All patient data will remain coded during analyses to maintain patient confidentiality.

10. ADVERSE EVENT REPORTING

10.1 Adverse Events and Stopping Rules

Adverse events occurring during the enrollment period should be documented by the Investigator in progress notes, but will not be collected or analyzed by Indiana University School of Medicine unless considered serious by the Investigator. Adverse events will be reported according to federal guidelines for safety reporting for Investigational New Drug Application (IND).

Serious adverse events (SAEs) encountered during study enrollment will be documented by the Investigator and reported to Indiana University School of Medicine immediately upon discovery. SAEs are defined under current Good Clinical Practice (cGCP) guidelines as events that result in one or more of the following:

- life-threatening illness or injury;
- permanent impairment of a body structure or a body function;
- medically necessary in-patient hospitalization;
- medical or surgical intervention necessary to prevent permanent impairment to body structure or function; or
- fetal distress, fetal death, or congenital abnormality.

Serious events that affect the rights, safety, or welfare of subjects must be documented on a form and be reported immediately to Indiana University School of Medicine and to the Investigator's IRB according to that IRB's policies.

Major Adverse Cardiac Events (MACE). MACE endpoints will include: death, nonfatal myocardial infarction, including, Q-wave, non Q-wave, and CK/CKMB elevation, stroke, transient ischemic attack (TIA), hospitalization for heart failure, hospitalization for hypo or hypertension, life-threatening arrhythmia, and aneurysm rupture, which will be collected throughout the 6-month time period.

Severity Assessment. We will use the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, for detailed descriptions of Severity Grades. The CTCAE schema is classified by body system and event using the MedDRA hierarchy and provides descriptions of events that qualify under each severity rating**.**

The following table contains general descriptions of Adverse Event Severity Grades.

Severity Grade	Description
1	Mild. Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
2	Moderate. Minimal, local, or non-invasive intervention indicated or limiting activities of daily living (i.e. preparing meals, shopping for groceries/clothes, managing money, using telephone, etc.)
3	Severe or medically significant but not immediately life-threatening. Hospitalization or prolongation of hospitalization indicated OR disabling OR limiting self-care (e.g. bathing, dressing, feeding self, using toilet, taking medications, etc.)
4	Life-threatening consequences; urgent intervention indicated.

5	Death. Death related to adverse event.

3. Stopping Rules

With any of the events listed below, the trial will be stopped, no additional patients will be enrolled and the FDA, the NIH, DMSB, and IRB will be notified. A comprehensive review of the cases will be conducted by the principal investigator, study coordinator, DMSB, and selected specialists to determine the etiology of each event. A decision to permanently halt or resume the study will be rendered by the aforementioned boards independent of the principal investigator.

- Expansion of AAA by > 7 mm. MTD in five or more patients in the MSC treatment group in a 6-month period.
- Two or more aortic aneurysm ruptures in the MSC treatment group within a 12-month period.
- One non-pulmonary embolism within a 12-month period indicative of acute respiratory distress syndrome (ARDS).
- One death related to MSC infusion.

10.2 Sponsor Contact for Serious Adverse Event Reporting

Michael P. Murphy, MD
1801 N. Senate Blvd.
MPC2, #3500
Indianapolis, IN 46202

11. RISK ANALYSIS

11.1 Potential Risks of the Investigational Product and Clinical Investigation

Study sites will not be provided with an investigational device.

The types of risk associated with injection of allogeneic MSCs align with those associated with injection of autologous bone marrow mononuclear cells (ABMNCs), most notably rash, mild fever, and myalgia. These risks are mild and rare. These risks are all stated in the consent form**.**

11.2 Potential Benefits of the Investigational Product and Clinical Investigation

Subjects are not expected to benefit in any way from their participation in the study.
The studies made possible by the data collected in this study are expected to lead to effective therapeutic strategies using MSCs or their secreted products to suppress expansion of small AAA negating the need for surgery and preventing rupture.

11.3 Minimization of Risks

Although the risk to subjects participating in the study is anticipated to be minimal, the clinician, at his/her discretion, will not collect data from those individuals for whom collection is judged to pose an unusually high risk of physical or mental harm or discomfort. Participation in this study poses no risk to study personnel other than that normally encountered during standard practice. These risks will be minimized by adherence to the following guidelines:

- Personnel should wear appropriate personal protective equipment to avoid contact of the eyes or skin with hazardous materials or products derived from biological sources.

12. INVESTIGATOR RESPONSIBILITIES

12.1 Site Qualification and Study Oversight

The PI is responsible for general administration of the study.
Before the study, the PI must:

- Obtain approval to conduct the study from the study site's IRB;
- Sign the Signature Page him/herself and have all sub-investigators sign the Signature Page and return it to Indiana University School of Medicine;
- Provide financial disclosures to Indiana University School of Medicine for themselves and all sub-investigators participating in study conduct, per Title 21CFR 54 (see **Section 12.4** below).

During the study, the PI must ensure that:

- The study is conducted ethically;
- Case report forms (CRFs), including Subject ICFs, are provided with each transfer of data requiring informed consent; and
- All other study forms are completed as instructed by Indiana University School of Medicine.

In the case of completion or termination of the study or an Investigator's role in the study, or at Indiana University School of Medicine request, all study materials must be returned to Indiana University School of Medicine.

12.2 Case Report Forms/Electronic Data Records

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method(s) used.
Original CRFs are the sole property of Indiana University School of Medicine and should not be made available in any form to third parties, except for authorized representatives of Indiana University School of Medicine or appropriate regulatory authorities, without written permission from Indiana University School of Medicine.

It is the PI's responsibility to ensure completion, review, and approval of all CRFs. CRFs must be signed by the PI or by an authorized staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the PI has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs.

12.3 Access to Source Documents

Indiana University School of Medicine or its agents and appropriate regulatory authorities shall be granted direct access to all study-related documents to perform verification that the protocol and all applicable current Good Laboratory Practices (cGLPs), Good Clinical Practices (GCPs), and regulations are being followed and to confirm that study documents are complete and accurate. It is important that Investigator(s) and their relevant personnel be made available during monitoring visits and any audits or inspections, and that sufficient time is allotted for the process.

12.4 Financial Disclosure

Investigators must provide Indiana University School of Medicine with sufficient, accurate financial information in accordance with local regulations to allow Indiana University School of Medicine to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information to Indiana University School of Medicine concerning their relevant financial interests during the course of the study and for 1 year after completion of the study. Conflicts of interest should be disclosed as required by law.

Financial support for this project is provided by Indiana Clinical and Translational Sciences Institute (CTSI).

12.5 Deviations from the Study Protocol

An Investigator may not deviate from the study protocol without prior approval by Indiana University School of Medicine unless the deviations are necessary under emergency circumstances to protect the rights, safety, or well-being of human subjects or the scientific integrity of the clinical investigation. These deviations must be documented and promptly reported to Indiana University School of Medicine and, if applicable, to the IRB providing oversight of the study. Protocol deviations may result in corrective and preventive actions and/or disqualification of the Investigator.

12.6 Record Retention

To enable evaluations and/or audits from regulatory authorities or Indiana University School of Medicine, the PI and all sub-investigators agrees to retain all study records, including copies of all CRFs, UADE forms, and source documents, for 3 years following completion of the project dependent upon the study data. The Investigator must obtain the Indiana University School of Medicine written permission before disposing of any records, even if retention requirements have been met.

If an Investigator relocates, retires, or for any other reason withdraws from the trial, Indiana University School of Medicine must be notified in advance, and study records must be transferred to a designee acceptable to Indiana University School of Medicine. This designee might be another Investigator, another institution, or Indiana University School of Medicine itself.

12.7 Publication Policy

The results of this study will be submitted for publication to a medical journal. The PI agrees that any publication of data from this study will comply with Indiana University School of Medicine publication policy, the instructions to authors outlined by the editor of the journal or conference proceedings where the data is to be published, and the spirit of recommendations made in the good publication practice guidelines (GPP2) of the International Society of Medical Publication Professionals. Indiana University School of Medicine has the right to review any manuscripts, presentations, or abstracts that originate from this study or that utilize these data before they are submitted for publication or other means of communication.

13. ETHICS AND COMPLIANCE

13.1 Informed Consent and De-Identification

13.1.1 Prospectively collected data

All subjects will be given a copy of the IRB-approved ICF to review before their study participation begins. The Investigator will explain all aspects of the study in lay language and answer all of the potential participant's questions regarding the study. If the participant decides to participate in the study, s/he will

be asked to sign and date the ICF. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

13.2 IRB Review

The PI is required to obtain IRB oversight of the research study. The IRB must be provided with the Indiana University School of Medicine-approved study protocol. Performance of the study may not begin until written evidence of IRB approval has been provided to Indiana University School of Medicine.
The conduct and performance of this study will be in accordance with applicable Sponsor and Investigator responsibilities as described in Title 21 CFR 812 and other Good Clinical Practice guidance.
IRB/Ethics Committee oversight will be required as human subjects or data from humans are being used. This protocol and the associated informed consent document(s) (if applicable) must be submitted to the IRB for review and approval. Performance of the study at a given site may not begin until written evidence of IRB oversight has been provided to an Indiana University School of Medicine study manager. IRB Review and approval must comply with Title 21 CFR 812 Subpart D.

13.3 Confidentiality of Data and Patient Records

The study institution shall keep all records associated with this study for at least 3 years, as specified in. Investigators will keep all records associated with this study for at least 3 years.

13.3.1 Provisions to Protect the Privacy Interests of Participants

The PI and/or study institution shall provide sufficient information to allow the IRB to evaluate the researcher's provisions to maintain the confidentiality of data.

Privacy data will be maintained in accordance with HIPAA and other applicable policies and local law.

All records will be kept in a locked file in the PI's office. All patient records and labels will be deidentified with regards to the patient's name, medical record number, address, phone number, social security number, and date of birth.

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