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Fort Sam Houston, TX 78234

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14. ABSTRACT

Traumatic brain injury (TBI) is a significant cause of death and disability in military & civilian populations. Cell therapy with mesenchymal stem cells (MSCs) shows promise for TBI. Because not all MSCs are equivalent, further work is needed to define optimal MSC products to minimize risk and maximize benefit. Here we proposed to establish a panel of assays to evaluate safety and potency of cell therapy products prior to administration. Assay results were used to select MSCs with different profiles for preclinical testing in rats. The maximal tolerated dose of MSCs will be determined in both uninjured and injured rats, and the distribution and retention of labeled cells following intravenous administration will be determined. MSC administration will be tested in rats subjected to TBI, and the impact of the cell therapy will be measured using a combination of histological, functional and behavioral testing. Because the long term consequences of TBI can be devastating and negatively affect quality of life in survivors, any treatment that can reduce the amount of brain damage or enhance brain healing will be of tremendous importance

15. SUBJECT TERMS

Traumatic Brain Injury, Cell Therapy, Mesenchymal Stem/Stromal Cells

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Traumatic brain injury (TBI) is a significant cause of death & disability in both military and civilian populations. Cell therapy with mesenchymal stem cells (MSCs) or other progenitors shows promise for TBI treatment and other types of brain injury, but further work is needed to define the optimal MSC products to minimize risk and maximize benefit. MSC products are not all equivalent in safety and potency, and different MSCs are likely to have different efficacy. Here we implement in vitro testing to evaluate both safety & potency of different MSCs, which will ultimately be validated using an animal model for rat TBI. Maximal tolerated dose of MSCs will be determined in both uninjured and severely injured rats, and the distribution and retention of labeled cells *in vivo* following IV administration will be determined. Finally, rats will be subjected to moderate TBI and the effects of MSC administration will be evaluated.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Traumatic brain injury, cell therapy, mesenchymal stem cells, coagulation, macrophage polarization, vascular permeability

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. In vitro safety and potency testing of human MSCs. Tasks proposed include measuring pro-coagulant activity of MSCs using a variety of methods, evaluating cell surface TF expression and generating cells with TF knocked down (reduced) by siRNA/shRNA in order to determine if TF is actually necessary for pro-coagulant activity and/or in vitro potency in MSCs. To support these efforts, we also proposed to develop and implement assays to measure MSC effects on macrophage polarization and on endothelial permeability. (Estimated percentage of completion: 75%. Expected completion date: June 2019)

Aim 2. Animal testing. In this aim we will determine dose response for "good" and "bad" MSCs in uninjured and injured animals (rats). Biodistribution of labeled cells will be determined following administration. (Estimated percentage of completion: 5% (animal studies just beginning in Year 2). Expected completion date: March 2020).

Aim 3. Testing of MSC administration for TBI. This goal is slated for the 3rd year of the proposal, and has not yet begun (0% completed).

What was accomplished under these goals?

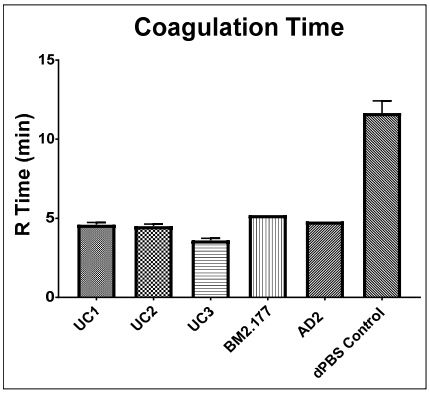
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. In vitro safety and potency testing of human MSCs. Tasks proposed included measuring the pro-coagulant activity of multiple MSCs using a variety of methods, evaluating cell surface TF expression and generating cells with TF knocked down (reduced) by siRNA/shRNA in order to determine if TF is actually necessary for pro-coagulant activity and/or in vitro potency in MSCs. To support these efforts, we also proposed to develop and implement assays to measure MSC effects on macrophage polarization and on endothelial permeability. Tasks & Progress:

A. Measure pro-coagulant activity of multiple MSCs, including MSCs derived from umbilical cord.

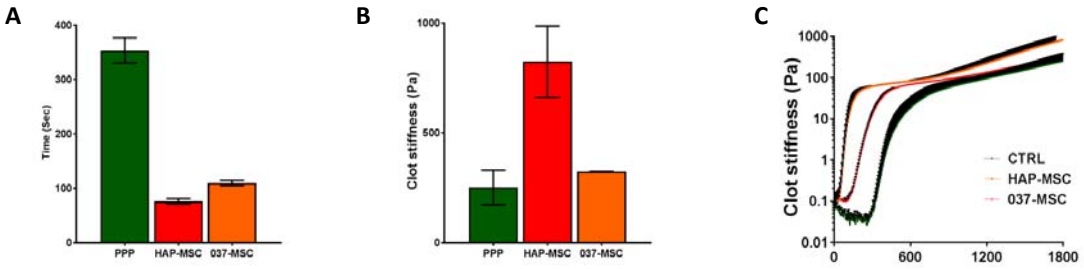
- We investigated >20 different MSCs from different sources, including bone marrow-derived, adipose-derived and umbilical cord-derived tissues. For comparison, primary human fibroblasts were also investigated. Using thromboelastography (TEG),we found that adipose-derived MSCs, umbilical cord-derived MSCs and some but not all fibroblasts have the highest pro-coagulant activity in normal human plasma (as determined by reduction in R-time, time to clot initiation). MSCs derived from bone marrow have lower pro-coagulant activity in this assay and are more variable (a representative TEG experiment is shown in Figure 1). When cells were mixed with whole blood (rather than plasma), we found that the MA value (reflecting clot strength) also increased with the MSCs expressing the most surface TF.

Figure 1. A representative TEG assay to measure pro-coagulant activity of UC-MSCs (umbilical cord-derived MSCs) when added to pooled human plasma. One BM-MSC and one AD-MSC are shown for comparison. Cells were washed extensively in dPBS to remove any traces of culture medium before mixing with platelet-poor plasma at a final concentration of $2x10^4$/ml. dPBS control represents the control with the same volume of dPBS alone (no cells) added to plasma. Reactions were performed in duplicate.



- Rheometry was performed on a subset of MSCs mixed with human plasma (Figure 2). While all human MSCs tested showed pro-coagulant activity, the kinetics and strength of clot formation vary between different human MSCs and point out the need for evaluation prior to human administration.

Figure 2. Evaluation of clotting time, clotting kinetics and clot stiffness by rheometry. One AD-MSC (labeled HAP-MSC) and one BM-MSC (labeled 037-MSC) were mixed with human platelet-poor plasma (PPP) at a concentration of $2x10^4$/ml and analyzed by rheometry. Each sample was analyzed in triplicate. A. R-time (seconds); time to clot initiation. B. Clot Stiffness. C. Kinetics of changes in clot stiffness (average of triplicates).



- 100% of this task is completed, although this testing will still be done on future preparations of cells being prepared for animal administration.

B. Determine cell surface TF expression by flow cytometry. Our previous work showed that there was a good correlation between pro-coagulant activity using TEG and expression of cell surface TF, so surface TF expression was evaluated here using flow cytometry with anti-CD142 antibody.

- We have determined TF expression on >20 different MSCs from different sources. A subset of the data is shown in Table 1 below. Note that both adipose-derived and umbilical cord-derived MSCs show a higher percentage of TF+ (CD142+) cells.

MSC	Tissue of Origin	% CD142+
BM1	Bone Marrow	30.3
BM2	Bone Marrow	28.2
BM3	Bone Marrow	11.4
BM4	Bone Marrow	18.2
BM5	Bone Marrow	11.1
BM6	Bone Marrow	9.5
AD1	Adipose	75.3
AD2	Adipose	83.8
AD3	Adipose	84.3
UC1	Umbilical Cord	72.1
UC2	Umbilical Cord	91.1
UC3	Umbilical Cord	90.4

Table 1. Cell surface expression of TF determined by flow cytometry using anti-CD142 (TF) antibody. BM1-6; bone marrow-derived MSCs from 6 different donors. AD1-3; adipose-derived MSCs from 3 different donors. UC1-3; umbilical cord-derived MSCs from 3 different donors.

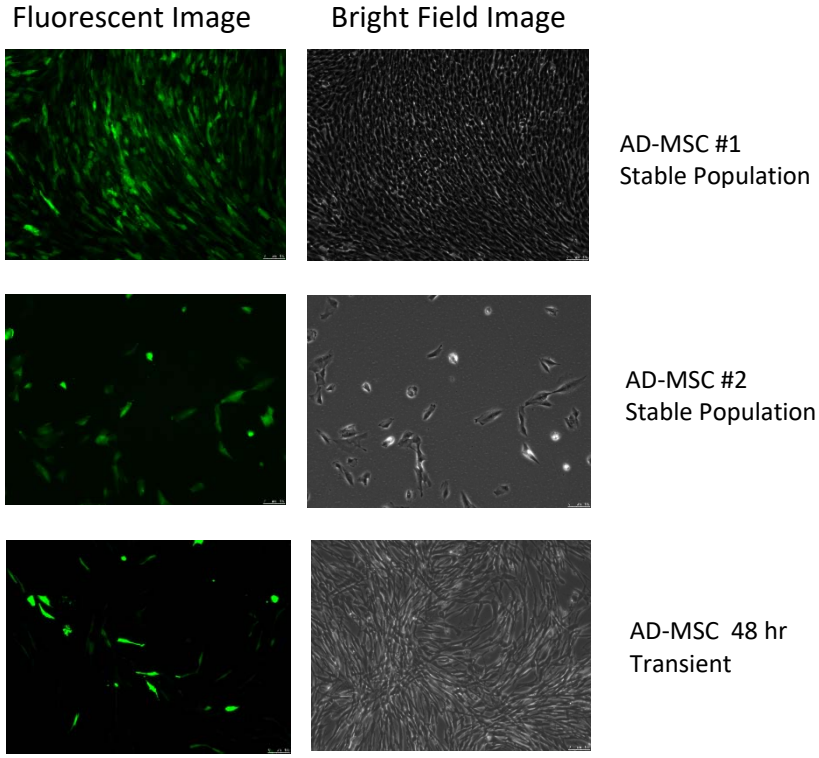
- 100% of this task is done. We have identified MSCs with low TF, medium TF and high TF levels.

C. Generate MSCs with TF "knocked down" (TF-KD cells) using shRNA/siRNA specific for human TF. Determine level of TF in TF-KD cells relative to controls treated similarly with a non-specific siRNA/shRNA using western blotting or flow cytometry.

- Both AD-MSCs and BM-MSCs were generated with TF "knocked down" relative to parental cells without transfection, after transient transfection (harvested at 48 hr). Pro-coagulant activity and TF surface expression were analyzed, and both decreased in the

putative TF-KD cells relative to either untransfected parental populations or cells transfected with a non-specific siRNA. From this we concluded that TF was making a large contribution to pro-coagulant activity. However, we were unable to generate enough TF-KD cells to do functional assays and opted to generate stable TF-KD cells in order to more fully characterize the consequences of reducing TF. The staff member who was carrying out these studies left abruptly, and we (Mr. Delavan in particular) are repeating both the transient TF-KD experiments and the stable TF-KD generation. Although the transient transfections have not yielded levels of transfection high enough to utilize, we have generated stable transfected populations that have taken up and stably incorporated the TFshRNA and control non-specific shRNA plasmids (as judged by resistance to the selection marker puromycin). Green fluorescent protein (GFP) under the control of a constitutive promoter is also contained in the plasmids, and the stably transfected cells express GFP (green fluorescence) (Figure 3). We are currently in the process of testing the levels of TF in the stable cells, as well as doing functional testing.

Figure 3. Transfection of MSCs to generate transient and stable TF-KD cells. MSCs were transfected with pRS shRNA vectors (OriGene) containing shRNA sequences specific for human TF, along with a puromycin resistance gene for stable cell selection and a gene encoding GFP to visualize the transfected cells by fluorescence microscopy. As shown in the top and middle panels, stably selected puromycin-resistant cells express GFP (green cells). As shown in the bottom panel, transiently transfected cells are visible after 48 hr, but the frequency of positive cells does not appear to be enough to reproducibly reduce TF levels (5-10%).



- Although we have had to repeat most of these experiments, an estimated 50% of this task is complete.

D. Test TF-KD cells for pro-coagulant activity using TEG and CAT. Ask if reducing the amount of TF reduces pro-coagulant activity.

- Although this task was previously complete, due to the abrupt departure of the researcher performing this task, we will repeat using new stable TF-KD cells. Currently 0% of this repeat is done, but we anticipate performing this task in the next month.

E. Test TF-KD cells for functional activity using several assays being routinely used in our laboratory. These assays include a modified MLR assay to assess immunomodulation activity, IDO enzyme activity induction under inflammatory conditions, ability to polarize macrophages. Other assays may also be performed with these populations at a later time. Ask if reducing TF reduces functional activity.

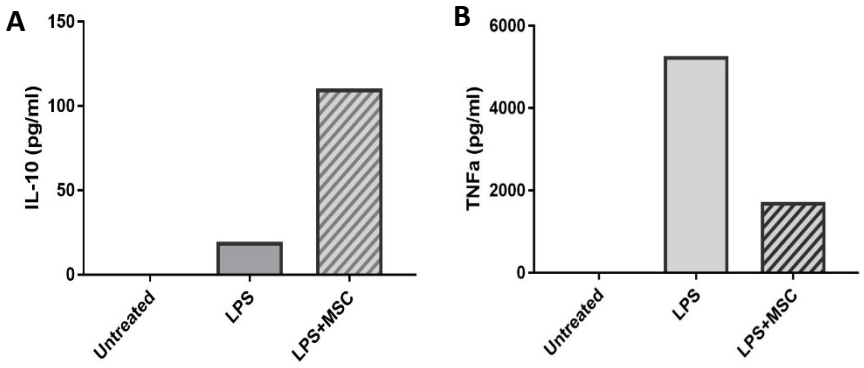
- Due to the abrupt departure of the researcher performing this task, we will repeat using new stable TF-KD cells. Currently 0% of this repeat is done, but we anticipate performing this task in the next 1-2 months.

F. Develop and/or implement assays in our laboratory to evaluate MSC function in macrophage polarization and vascular permeability.

- As described in the progress report for the 1st quarter of this first year, we have implemented an assay designed to assess the influence of MSCs on macrophage polarization. In this assay we use mouse RAW264.7 macrophages to determine the ability of MSC co-culture to increase the ratio of M2 (anti-inflammatory) macrophages to M1 (inflammatory) macrophages. In this assay, the RAW264.7 cells are activated with LPS and plated with serial dilutions of MSCs from various sources. After 24 hr of co-culture,the conditioned medium (CM) is harvested and assayed to determine the levels of secreted IL-10 and TNFa using murine-specific commercial ELISA kits (R&D Systems). An increase in IL-10 and decrease in TNFa secreted by the murine cells indicates an increase in anti-inflammatory M2 macrophages. Using this assay, we found that different human MSCs vary widely in their ability to influence mouse macrophage polarization. In fact, only a few MSCs actually influence polarization in the desired direction in this assay. In general, the BM-MSCs appear to have higher activity in this assay. Since this result seemed a bit surprising and also because there are some biological differences between mouse and human macrophages, we have recently implemented a macrophage polarization assay using human macrophages. First, we have utilized CD14-positive monocytes enriched using magnetic selection from peripheral blood mononuclear cells (PBMCs) obtained from healthy volunteers. CD14+ monocytes were co-cultured with human MSCs and analyzed for pan-macrophage, M1 and M2 surface markers. Although we do see an increase in putative M2 marker-expressing cells, the monocytes do not seem to survive well and only a small number of cells can be analyzed. Therefore, we recently utilized human THP-1 monocytes (ATCC) in a macrophage polarization assay. The results there have been much more encouraging, and the use of immortal monocyte cells is more convenient for actual use as a screening assay. As shown in Figure 4, THP-1 cells were cultured either alone or with MSCs and activated with LPS. Levels of secreted IL-10 and TNFa were evaluated using a commercial ELISA system. A representative experiment is shown in Figure 4; 3 different BM-MSCs have been tested thus far. Co-culture of THP-1 cells with MSCs blunts the TNFa response to LPS but increases IL-10 secretion (indicative of a higher

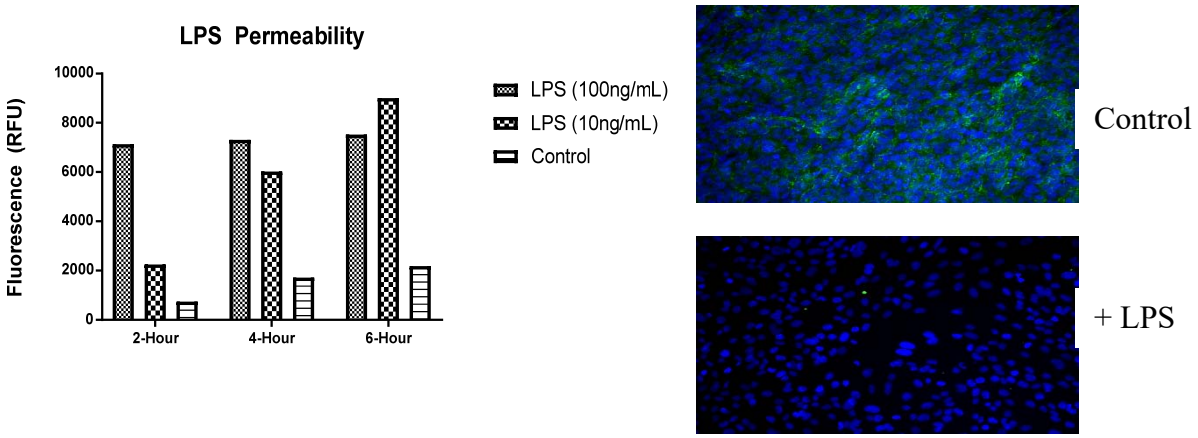
M2/M1 ratio, towards a more anti-inflammatory phenotype). Analysis of the cells by flow cytometry is consistent with more M2-like macrophages when MSCs are present.

Figure 4. BM-MSC co-culture with monocytes influences macrophage polarization. Human THP-1 monocytes were cultured alone or with human MSCs at a 10:1 (monocyte:MSC) ratio overnight, then treated with either vehicle alone or with 1 ug/ml LPS. Conditioned medium (CM) was harvested at 6 hr (for TNFa) and 24 hr (for IL-10). Concentrations of IL-10 (graph A) and TNFa (graph B) in the conditioned media were determined using the ELLA™ automated immunoassay analyzer (Protein Simple, R&D Systems). Values are an average of 3 replicates per sample. An increase in IL-10 and a decrease in TNFa is indicative of a higher M2/M1 ratio in the presence of MSCs. MSCs alone did not produce detectable amounts of either IL-10 or TNFa either with or without LPS (not shown). Untreated; THP-1- CM without LPS treatment. LPS; THP-1-CM harvested after 6 hr (for TNFa) or 24 hr (for IL-10) treatment. LPS+MSC; CM derived from THP-1/BM-MSC co-cultures harvested after 6 hr or 24 hr of treatment. Results shown are for one representative BM-MSC.



- To analyze the effects of MSCs on vascular permeability (important for reduction of endothelial dysfunction and to maintain blood brain barrier integrity), we have been developing an assay to assess endothelial monolayer permeability in transwell culture. Although we have had some technical problems with this assay, we have been able to use both a FITC-Dextran dye assay and immunostaining for tight junction proteins to detect disruption of the endothelial monolayer using human endothelial cells. As shown in Figure 5, we are able to disrupt the monolayer with LPS treatment. Preliminary data (not shown) suggests that co-culture with BM-MSCs has an effect on this disruption, but we have not yet been able to assess recovery.

Figure 5. Endothelial cell permeability assay development. LPS treatment disrupts tight junctions in hMEC/D3 human blood brain barrier cells. Left side (graph); hMEC/D3 cells were plated on fibronectin-coated transwell inserts and allowed to achieve confluence & form tight junctions between cells. In this experiment, no cells were seeded in the bottom chamber. For assay of MSC influence on the permeability, MSCs to be tested will be seeded on the bottom. Cells were treated for 24 hr. with vehicle control or with LPS at either 10ng/ml or 100ng/ml. After 24 hr, FITC-Dextran dye was added to the top chamber and medium from the bottom chamber was sampled at 2, 4 and 6 hr. Fluorescence in the medium in the bottom chamber reflects movement of the dye through the endothelial monolayer. Right side; hMEC/D3 cells were seeded on fibronectin-coated chamber slides and allowed to achieve confluence and form tight junctions. Cells were treated for 24 hr. with vehicle alone (Control) or with 100ng/ml LPS. Slides were fixed and stained with anti-claudin 5 antibody (green) to visualize tight junctions and with DAPI (blue) to visualize nuclei.



- Although we had proposed to have these assays implemented and testing completed by the end of this 1st year, we have had some technical problems and personnel problems that have delayed completion. Nevertheless, we will continue these studies in the first quarter of the second year and expect to have this done by July 2019.

G. At the end of testing, identify "good" and "bad" MSCs for in vivo testing. "Good" MSCs will be defined as MSCs with low TF and pro-coagulant activity and high potency in in vitro functional assays. In vitro potency will need to be validated in vivo (in Aim 2) to determine efficacy in vivo. (Note that we use "potency" to describe in vitro function and "efficacy" to describe function in vivo).

- We have already identified "good" and "bad" MSCs to be used for in vivo testing, based on their TF levels and performance in several functional assays (MLR, IDO). We will continue testing of our collection of MSCs from different sources in the macrophage polarization assay and the vascular permeability assay, in order to further narrow the choices for animal testing.

Aim 2. Animal testing. In this aim we will determine dose response for "good" and "bad" MSCs in uninjured and injured animals (rats).

A. Generate animal protocol for rat TBI. Obtain IACUC approval.

- Dr. Darlington has written an animal protocol and obtained the necessary approvals for submission to the IACUC. The protocol will be reviewed at the April 2019 IACUC meeting.
- An estimated 75% of this task is done. We anticipate obtaining approval in April 2019.

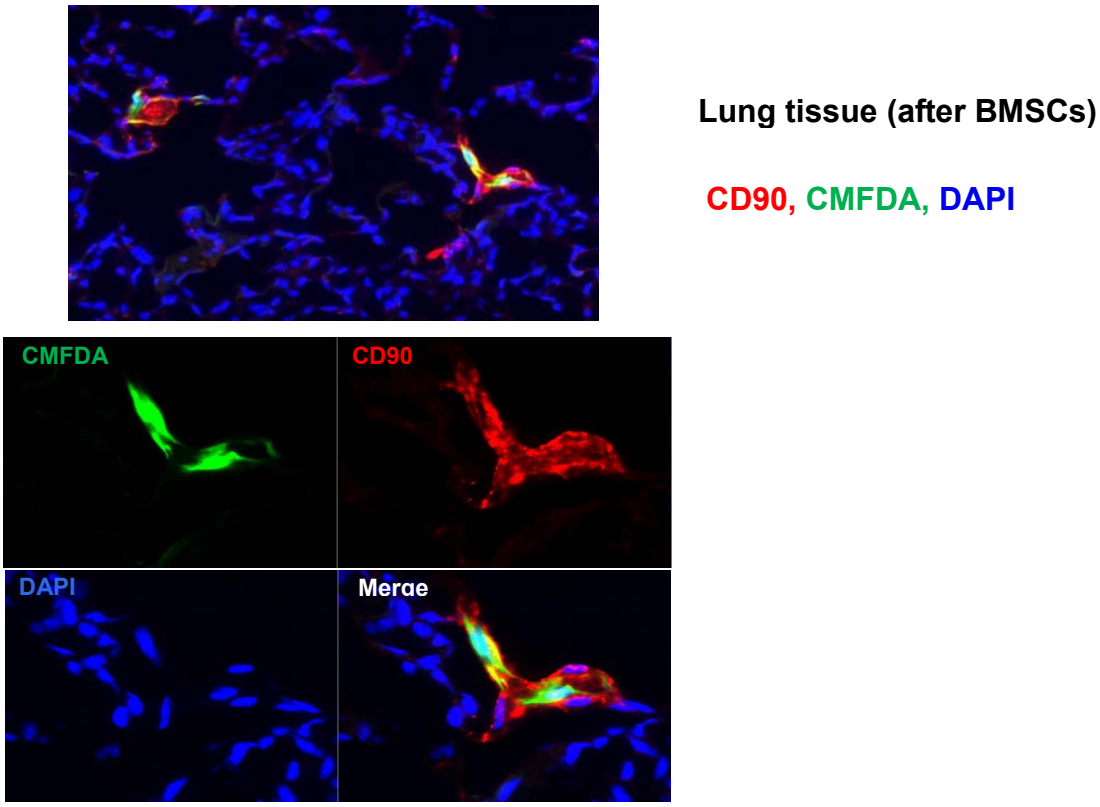
B. Dose response study to determine maximum tolerated dose for MSCs injected intravenously into normal uninjured rats.

- Under a modification to Dr. Wu's existing animal protocol, the maximum tolerated dose for adipose (high TF) and bone marrow (low TF) MSCs has been determined. As predicted, the rats can tolerate more of the bone marrow MSCs with lower TF (up to 20 million/kg body weight for BM-MSCs; 10 million/kg for AD-MSCs).
- An estimated 25% of this task is done. Next we will inject our "good" and "bad" human MSCs identified in Aim 1.

C. Biodistribution studies using labeled MSCs to determine how long they last in the bloodstream and where they go after administration.

- Although we have not yet administered labeled human MSCs into rats, we have injected labeled rat BM-MSCs. As shown in Figure 6, CMFDA-labeled BM-MSCs can be detected in the lungs at early time points (4 hr after administration). Lung tissue was processed for detection of the label (CMFDA, green) and immunostaining with an MSC marker (CD90, red). Nuclei were stained blue using DAPI. At the same time point, labeled cells were no longer detectable in the blood by flow cytometry. Labeled human BM-MSCs will be analyzed similarly.

Figure 6. Detection of CMFDA-labeled rat BM-MSCs in rat lung at 4 hr. after IV administration. BM-MSCs were labeled before injection with CMFDA. Rats were sacrificed 4 hr. following injection of 10^6 cells/kg and processed for immunostaining. A representative fluorescent image is shown. The CMFDA label (green) co-localizes with an MSC marker, CD90 (red). Nuclei are stained with DAPI (blue) to visualize cell location.



D. Implement rat TBI model in our laboratory.

- These rat TBI studies have not yet begun (proposed for the 2nd year). Animal protocol for model development at our facility with our personnel is pending.

E. Dose response study to determine maximum tolerated dose for MSCs injected intravenously into rats subjected to TBI. These studies have not yet begun (proposed for the latter part of the 2nd year).

Aim 3. Administration of MSCs to rats subjected to TBI and analysis.

- Studies not begun, proposed for the (optional) 3rd year. Pending establishment of a reproducible rat TBI model.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Numerous training activities are available to our personnel. Recently, all of us participated in Arbinger Institute Outward Mindset training designed to facilitate team cohesion & productiveness. In addition, our cell therapy team has recently received specialized training in equipment use, including for the Agilent Seahorse and an updated Olympus microscope, camera & imaging software.
In addition, the PI and many of our team attended and presented at several conferences in the past year, including RegenMed SA, AABB Annual Meeting, Military Health System Research Symposium (MHSRS), and the International Society for Cell Therapy Annual meeting.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Several abstracts and posters have been presented at local, regional and national meetings.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue and finish the in vitro studies described in Aim 1; some of these results will be included in a paper currently in preparation and others will be presented elsewhere. Once our pending animal protocol is approved, we will begin TBI model development. Once TBI model development is complete, the dose response in injured and uninjured animals will be compared (it is very possible that the MSC tolerance in injured animals will be altered). Biodistribution studies with human MSCs can begin soon for comparison to the distribution with rat MSCs.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report, since the results have not yet been published. We do anticipate making significant contributions to both the cell therapy and TBI fields.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

A member of our technical staff (Mr. Estlack) left abruptly, making it necessary for us to buy new reagents and repeat some of the work that he had done (especially the TF-knockdown experiments). Although this has set our timeline for finishing these experiments back some, we are making progress and anticipate being finished soon. Because of the redistribution of workload, some of the other experiments (in particular the vascular permeability assays) have also been delayed.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Accepted Manuscript:

1. Christy BA, Herzig MC, Delavan C, Cantu C, Salgado C, Bynum JA & Cap AP. Human Primary Fibroblasts Perform Similarly to MSCs in Assays Used to Evaluate MSC Safety and Potency. In Press, *Transfusion*.

Manuscripts in Preparation for Submission or Resubmission:

2. Christy BA, Herzig MC, Delavan C, Salgado C, Cantu C, Lovelace S, Jensen K, Garcia L, Montgomery RK, Bynum JA & Cap AP. Use of Multiple Potency Assays Exposes Differences Between Human Mesenchymal Stem Cells. In preparation for submission.
3. Herzig MC, Christy BA, Montgomery RK, Delavan CP, Jensen KJ, Lovelace SE, Cantu C, Salgado CL, Bynum JA & Cap AP. Interactions of Human Mesenchymal Stromal Cells with Peripheral Blood Mononuclear Cells in a Mitogenic Proliferation Assay. In preparation for resubmission.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Published Abstracts:

1. Christy B, Herzig MC, Estlack L, Delavan C, Chance T, Montgomery RK, Cantu C, Salgado C, Bynum JA & Cap AP. Activity of Human Fibroblasts in Mesenchymal Stromal Cell Potency Assays. International Society for Cell Therapy 2018.
2. Herzig MC, Christy BA, Montgomery RK, Delavan C, Estlack L, Jensen KJ, Lovelace S, Cantu C, Salgado C, Bynum JA & Cap AP. Analysis of the Interactions of Human Mesenchymal Stem Cells with Peripheral Blood Mononuclear Cells in a Mixed Lymphocyte Reaction (MLR) Assay. International Society for Cell Therapy 2018.
3. Christy BA, Cantu C, Parida BK, Herzig MC, Bynum JA & Cap AP. Human Mesenchymal Stromal Cell Interactions with Innate Lymphoid Cells. AABB Annual Meeting 2018. Transfusion 58(S2):22A.
4. Herzig MC, Christy BA, Cantu C, Bynum JA & Cap AP. Human Mesenchymal Stromal Cells Have a Therapeutic Window for Immunosuppression in the Mixed Lymphocyte Reaction Assay. AABB Annual Meeting 2018. Transfusion 58(S2):61A.
5. Chance T, Rathbone CR, Christy BA, Estlack LE, Delavan CP, Cap AP & Bynum JA. Uniformity and Functionality of Exosomes Isolated from Different Preparations. AABB Annual Meeting 2018. Transfusion 58(S2):68A.
6. Bareis A, Wu X, Darlington DN, Cap AP, Bologna CR, van Nispen JA, Hildreth KE, Barraza D, Williams CE, Dubick MA, Torres Filho IP, Garcia BL, Manos ME, Strain MM, Chavez R, Trapolsi D, Trevino A, Garza T, Crimmins S, Winkler CJ, Gomez BI, McIntyre MK, Chao T, Little JS, Burmeister DM, Chu GC-Y, Heard TC, Fahy JA, Apple DA, Cohen K, Pamplin J, Serio-Melvin M, Veazey SR, Gonyeau KE, Howard EA, Edwards TH, Scott LLF, Parker J, Hall K, Garcia L, Delavan C, Cantu C, Bynum JA, Herzig MC, **Christy B**, del Balzo LA, Ramos ME, Teng B, Keesee JD, Garciamarcano J, Sosanya NM, Tongkhuya S, Shaffer LJ, Kowalczewski CJ, Christy, R. Proceedings of the 6th Annual United States Army Institute of Surgical Research Summer Undergraduate Research Internship Program 2018. *J Transl Med* 2018, 16(Suppl 3):305. DOI 10.1186/s12967-018-1671-8.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Other abstracts in the last year not listed above:

1. Delavan C, Christy B, Becerra S, Herzig M, Bynum J & Cap A. Assay Development to Assess the Influence of Cell Therapy Agents on Blod Brain Barrier Endothelial Cell Function. RegenMed SA meeting, Feb. 2019.
2. Christy BA, Herzig MC, Estlack LE, Delavan CP, Chance T, Montgomery RK, Cantu C, Salgado C, Bynum JA & Cap AP. Activity of Human Fibroblasts in Mesenchymal Stromal Cell Potency Assays. Military Health System Research Symposium 2018.
3. Herzig MC, Christy BA, Montgomery RK, Delavan CP, Estlack LE, Jensen KJ, Lovelace S, Cantu C, Salgado CL, Bynum JA & Cap AP. Analysis of the Interactions of Human Mesenchymal Stem Cells with Peripheral Blood Mononuclear Cells in a Mixed Lymphocyte Reaction (MLR) Assay. Military Health System Research Symposium 2018.
4. Delavan C, Montgomery R, Herzig M, Christy B, Bynum J & Cap AP. Comparison of Direct vs. Transwell MLR Assays For Evaluation of Human Mesenchymal Stromal Cell Immunomodulation Activity
5. Chance T, Rathbone CR, Christy BA, Estlack L, Delavan C, Cap AP & Bynum JA. The Homogeneity and Functionality of Exosomes Isolated from Different Preparations. Military Health System Research Symposium 2018.
6. Estlack LE, Delavan C, Herzig MC, Christy BA, Bynum JA & Cap AP. Cellular Therapy Products Enhance Angiogenic Potential of Human Umbilical Vein Endothelial Cells. Military Health System Research Symposium 2018.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Not applicable.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report yet.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Barbara A. Christy, PhD
Project Role: Principal Investigator
Researcher Identifier: 0000-0001-5318-653X
Nearest person month worked: 6
Contribution to Project: Oversaw entire project, designed & carried out experiments, performed administrative tasks.

Name: Maryanne C. Herzig, PhD
Project Role: Co-Investigator
Nearest person month worked: 4
Contribution to Project: Helped design & carry out all experiments.

Name: James A. Bynum, PhD
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Intellectual contributions & administrative support.

Name: Xiaowu Wu, MD
Project Role: Co-Investigator
Nearest person month worked: 3
Contribution to Project: Designed & performed animal experiments.

Name: Daniel Darlington, PhD
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Design of animal experiments; wrote animal protocol.

Name:	Andrew P. Cap, MD, PhD
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to Project:	Task Area Manager, Provided intellectual contributions and administrative support.

Name:	Christopher Delavan, MS
Project Role:	Research Technician
Nearest person month worked:	6
Contribution to Project:	Carried out much of the work described in this project, including MSC culture, assay development and more.

Name:	Larry Estlack, BS
Project Role:	Research Technician
Nearest person month worked:	4
Contribution to Project:	Provided technical support.

Name:	Christi Salgado, MS
Project Role:	Research Technician
Nearest person month worked:	2
Contribution to Project:	Provided technical support.

Name:	Carolina Cantu, MS
Project Role:	Research Technician
Nearest person month worked:	1
Contribution to Project:	Flow cytometry analysis and interpretation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No changes in senior personnel.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Dr. Lora Watts, University of the Incarnate Word, San Antonio, TX.
Dr. Watts is a collaborator who has provided consultation during this grant period, to advise us in setting up our rat model for TBI.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Mesenchymal Stem Cell Therapy for Traumatic Brain Injury

Award #BA170080

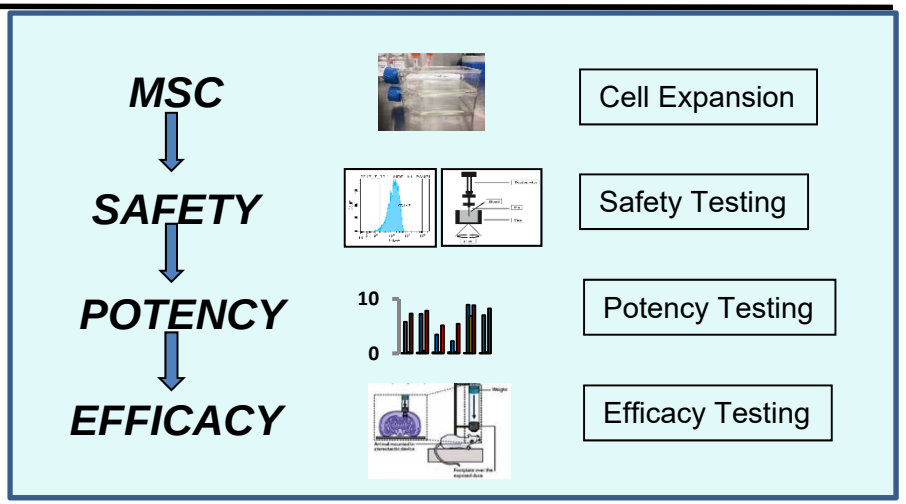
PI: Barbara Christy, PhD **Org:** US Army Institute of Surgical Research **Award Amount:** $1,455,907

Study Aims

- Objective 1: Develop *in vitro* testing protocols to evaluate safety and efficacy of MSCs.
- Objective 2: Preclinical testing for the identification of safe MSC dose in rats.
- Objective 3: Preclinical efficacy testing in a rat model of TBI.

Approach

- Multiple human MSC products will be evaluated for pro-coagulant potential to predict safety for IV delivery.
- Assays to evaluate potency (including immunomodulation, endothelial permeability) will be developed and implemented.
- Maximum tolerated dose will be determined in injured and non-injured rats; distribution & retention of cells will be followed.
- MSCs will be infused into rats subjected to TBI; effects on brain damage & recovery will be monitored.



Characterization of safety & potency underway for multiple MSCs.
Development of macrophage polarization and blood-brain barrier endothelial cell permeability are ongoing (potency testing).

Timeline and Cost

Activities CY	18	19	20	
In Vitro Safety Testing				
In Vitro Potency Testing				
MSC dosing/distribution (rats)				
MSC testing in TBI				
Estimated Budget ($K)	**$454**	**$540**	**$462**	

Updated: April 14, 2019

Goals/Milestones (Example)

CY18 Goals –In vitro testing to identify "good" & "bad" MSCs
- In vitro safety testing
- In vitro potency testing (including new assay development)

CY19 Goal – Preclinical dosing and distribution studies
- Establish TBI protocols in our laboratory
- Determine maximal tolerated dose in both injured and uninjured rats
- Label cells and follow distribution & survival in injured and uninjured rats

CY20 Goal –
- Infuse "good" and "bad" MSCs into rats subjected to TBI
- Evaluate brain injury and recovery using multiple methods

Comments/Challenges/Issues/Concerns
- No major issues at this time

Budget Expenditure to Date

Projected Expenditure: $454K
Actual Expenditure: $454K