Award Number: W81XWH-13-2-0024

TITLE: Stem Cell Therapy to Improve Burn Wound Healing

PRINCIPAL INVESTIGATOR: Carl Schulman, MD, PhD, MSPH, FACS

CONTRACTING ORGANIZATION: University Of Miami
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TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Stem Cell Therapy to Improve Burn Wound Healing

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5b. GRANT NUMBER

W81XWH-13-2-0024

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Miami M
Miller School of Medicine Ryder Trauma Center
1800 NW 10th Avenue, Suite 221
Miami, FL 33136

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Thermal injuries are a significant source of morbidity in times of war, constituting 5% to 20% of all injuries and 4% of all deaths. Although these are usually not life threatening, they cause significant morbidity to the patient and disruption of a deployed military unit. Hypertrophic scaring occurs frequently in operative (grafted) and non-operative burn wounds and can lead to the formation of scar contractures. Contractures represent a great source of morbidity to burn patients. Scar contracture rates have not evolved with improvements of burn care despite the use of treatments designed to mitigate the effects of hypertrophic scarring including scar massage, topical treatments, steroid injections, and compression garments. Mesenchymal stem cells (MSC's) have been used in a variety of clinical applications to repair and regenerate damaged tissue. Previous work by our group has demonstrated the safety and efficacy of delivering bone marrow cells including MSC's to chronic wounds with significant improvement in healing and scarring. Application of mesenchymal stem cell (MSC) therapy to severe burn wounds represents the opportunity for improved outcomes where alternate therapies are limited and often ineffective.

15. SUBJECT TERMS

wound healing, stem cell therapy, mesenchymal stem cells, MSC

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

Section I - A brief introduction covering the purpose and scope of the research effort.

The purpose of the research effort is to evaluate the safety and efficacy of mesenchymal stem cells in the treatment of burn wounds. The scope of the research is a Phase I and II clinical trial.

BODY

OVERALL PROGRESS

Section II - A brief description of overall progress to date plus a separate description for each task or other logical segment of work on which effort was expended during the report period. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. If this award includes the recruitment of human subjects for research or a clinical trial, report progress on subject recruitment (i.e., number of subjects enrolled versus total number proposed).

A. Personnel:

- Jeffrey McBride added as a key member of the study team.
- Carol Kittles, her name was removed from the study team list.

B. Approvals:

UM IRB approvals:

1. Deviations' report after UM RCQA Audit:

Deviations report 01-11-16

Deviations report 09-04-15

Deviations report 12-30-15

Deviations reported to UM IRB on 30 Dec, 2015 and resubmitted on 14 Jan, 2016
IRB Approval 31 Oct 2016
IRB Approval letter sent on 13 Dec 2016

2. Continuing Review 2016 CR00004284 sent to UM IRB on 4 Apr, 2016

Documents submitted with the report:

- IRB#20120925 Badiavas Sponsor DSMB review 09Feb2016
- DSMB Report March 2016 Drs. Elgart and Pust.
- HRP-2XX-FORM- Supplemental Information for Continuing Review-Dr. Schulman
- Continuing review 2016 Grant Progress report
- 2016 Deviation report Continuing review
- 2016 CR Deviation Report 12APR2016
- Appendix #1 Deviations' report after UM RCQA Audit

IRB Approval 25 Apr 2016
IRB Approval letter sent on 26 Apr 2016

Continuing Review 2016 CR00004284 approved by UM IRB, this report was sent to the Department of Defense on 11 May 2016.
Continuing Review Acceptance Memorandum from the Department of Defense was received on 14 Jun 2016.

3. Modification # 18 sent on 19 Jul, 2016

- Jeffrey McBride added as a key member of the study team.
- Carol Kittles removed from the study team list.

IRB Approval 03 Aug 2016
IRB Approval letter sent on 03 Aug 2016

C. <u>Donors:</u>

No changes.

- **Screened: 2**
- **Screen failed: 1**
- **Enrolled: 1**

D. <u>Patients-Recipients:</u>

Screened: 18
Screen failure: 6
Withdraw consent: 2
Enrolled: 10

- **Recipient #R001:**
  - **Patient completed his participation in the study. No adverse events reported.**
- **Recipient#R002:**
  - **Patient was a screen failure. No adverse events reported.**
- **Recipient #R003:**
  - **Screening visit (visit 1) completed on 15 Jan 2015.**
  - **First MSCs application (visit 2) on 20 Jan 2015.**
  - **This subject was not eligible for a second MSCs application because the wound was completely healed.**
  - **A serious adverse event was reported by the subject on 07 Apr, 2015. The patient has complained about gastrointestinal symptoms; this event is unlikely related to the study. Patient received a referral to GI.**
  - **Antral ulceration biopsy reported Gastric Cancer. Patient was successfully treated for Gastric Cancer. Final Adverse event report completed by Dr. Schulman.**
  - **Final visit completed on 12 Jul 2016. No other adverse events reported.**
- **Recipient #R004:**
  - **This patient did not show up on 08 Apr 2015 (Visit 3). We have been unable to contact this patient by phone. We sent two certified letters. We will continue trying to contact the subject by phone.**
  - **No adverse events reported.**
  - **Patient lost to follow up.**

- **Recipient #R005:**
  - **Patient was a screen failure. No adverse events reported.**

- **Recipient #R006:**
  - Patient completed his participation in the study on 17 Jan 2017. No adverse events reported.

- **Recipient #R007:**

  - **Informed consent forms signed on 17 Nov, 2015**
  - **Patient did not return to clinic.**

- **Recipient #R008:**

  - **Patient was a screen failure because. No adverse events reported.**

- **Recipient #R009:**

  - **Patient withdrew from study. No adverse events reported.**

- **Recipient #R010:**

  - **Final visit scheduled on 15 Feb 2017.**
  - **Adverse events reported:**
    - **Left upper extremity weakness: Severity Mild. Unrelated to study treatment. No actions taken with study treatment.**
    - **Persistent Hypertension: Severity Mild. Unrelated to study treatment. No actions taken with study treatment. Resolved on 01 Jan 2017**

- **Recipient #R011:**

  - **Patient completed his participation in the study on 01 Feb 2017. No adverse events reported.**

- **Recipient #R012:**

  - **Informed consent forms signed on 05 Apr 2016**
  - **Visit 1 screening on 05 Apr 2016**
  - **Visit 2 completed on 08 Apr 2016, first stem cells application.**
  - **Visit 4 completed on 20 Apr 2016, second stem cells application.**
  - **Patient is coming to the clinic for follow up visits.**

- **Recipient #R013:**

  - **Informed consent forms signed on 06 Sep, 2016**
  - **Visit 1 screening on 06 Sep, 2016**
  - **Visit 2 completed on 09 Sep 2016, first stem cells application.**
  - **This subject was not eligible for a second MSCs application.**
  - **Patient is coming to the clinic for follow up visits.**

- **Recipient #R014:**

  - **Informed consent forms signed on 24 Oct, 2016**
  - **Visit 1 screening completed on 27 Oct, 2016**

- **Patient was a screen failure due to abnormal labs.**
- **No adverse events reported.**

- **Recipient #R015:**

  - **Informed consent forms signed on 13 Jan 2017**
  - **Visit 1 screening completed on 17 Jan, 2017**
  - **Visit 2 completed on 19 Jan 2017, first stem cells application.**
  - **This subject was not eligible for a second MSCs application.**
  - **Patient is coming to the clinic for follow up visits**

- **Recipient #R016:**

  - **Informed consent forms signed on 18 Jan, 2017**
  - **Visit 1 screening completed on 19 Jan, 2017**
  - **Visit 2 completed on 20 Jan 2017, first stem cells application.**
  - **This subject was not eligible for a second MSCs application.**
  - **Patient is coming to the clinic for follow up visits.**

- **Recipient #R017:**

  - **Informed consent forms signed on 24 Jan 2017**
  - **Visit 1 screening completed on 26 Jan 2017**
  - **Patient was a screen failure due to abnormal labs.**
  - **No adverse events reported.**

- **Recipient #R018:**

  - **Informed consent forms signed on 27 Jan 2017**
  - **Visit 1 screening completed on 27 Jan 2017**
  - **Patient was a screen failure due to abnormal labs.**
  - **No adverse events reported.**

E. **Research Monitor**: Clinical Research Operations & Regulatory Support (CRORS), University of Miami, has been providing support with the following areas:

- Good clinical practice
- Good documentation practice
- Informed consents
- Responsibilities for IND/IDE holders
- Investigational product
- Regulatory binder/trial master file
- Protocol compliance
- Assistance with the Audit process

Monitor visits:

- **18 and 21 Mar 2016**
- **01 Apr 2016**
- **18 May 2016**
- **01 Jun 2016**
- **16 and 17 Nov 2016**

F. Sponsor's Scientific Summary :

All cytokine analysis of mixed MSCs and Pre and Post treatment PBMCs have been completed in all patients treated thus far. While no significant related adverse events have been noted thus far, elevations specifically in IFN-γ and TNF-α could indicate a subclinical response to the administered allogeneic MSCs. No significant cytokine elevations have been noted to date in any treated patient (Figures A, B & C). These findings support the immune privileged/immune suppressive effects of administered allogeneic MSCs with the lack of host immune response to these cells in burn patients. In an effort to gather further information on burn patients, we continue to monitor baseline cytokine levels in all screened or treated subjects in to better define differences in 2nd degree burn injury between individuals. We have found that there is a variability in the pretreatment PBMC production levels of IL10, IFN-γ and TNF-α with the greatest differences noted for IL10. These levels have not been previously examined in second degree burn patients and tend to support disparity in the immune response to burn injury between patients. A better understanding of the differences in baseline (post burn, pretreatment) PBMC cytokine production may be helpful in better evaluating a host's overall response to second degree burn injury and predicting their response to MSC therapy. We are continuing to monitor these changes and correlate them to clinical outcome.

The swiftness of response to allogeneic MSCs has been among the important observations we have made in the past year. Rapid Epithelialization and re pigmentation in particular are among these findings. There are likely complex materials released by MSCs that are responsible for these changes as engraftment of delivered cells into the wound bed would likely require more time. In examining materials released by delivered cells, we have made a significant observation in finding the presence of extracellular vesicles in the saline fluid (containing MSCs) administered to burn patients (Figure D). Extracellular vesicles are membrane bound particles released by cells that carry numerous regulatory elements including proteins, peptides, nucleic acids and transcription factors. Extracellular vesicles are involved in complex intracellular signaling and appear to be responsible for delivering many of the effects attributed to several cell types including MSCs. In preparing MSCs for administration, they are placed in saline once thawed and delivered to patients usually within two hours. We have found within that time MSCs release billions of extracellular vesicles into the saline. This finding demonstrates that the MSCs being delivered are metabolically active and responsive. The observation of finding extracellular vesicles in the fluid we are delivering could help to explain the rapid clinical response we are seeing. We are continuing to examine the release of these vesicles by our donor MSCs and better characterized them. A more thorough analysis of extracellular vesicles released by donor MSCs could lead to improved methods for treating burn patients with stem cells and stem cell derived materials.

Figure A

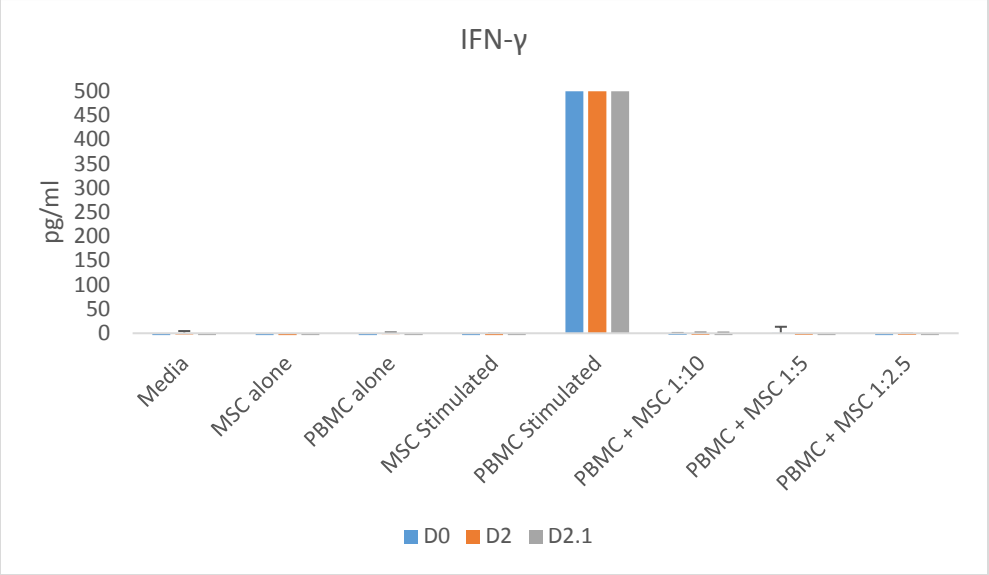

Figure A: **IFN- γ ELISA Assay.** All mixture ratios of PBMC and donor MSCs are non-reactive. PBMC stimulated is the positive control. Media, MSC alone, MSC stimulated and PBMC alone did not produce appreciable levels of IFN- γ. **(**D0 = Blood sample taken at screening, prior to administration of donor MSCs; D2 = Blood sample taken one hour after the second application of donor MSCs; D2.1 = Blood sample taken one week after second applicatioin of donor MSCs.)

Figure B

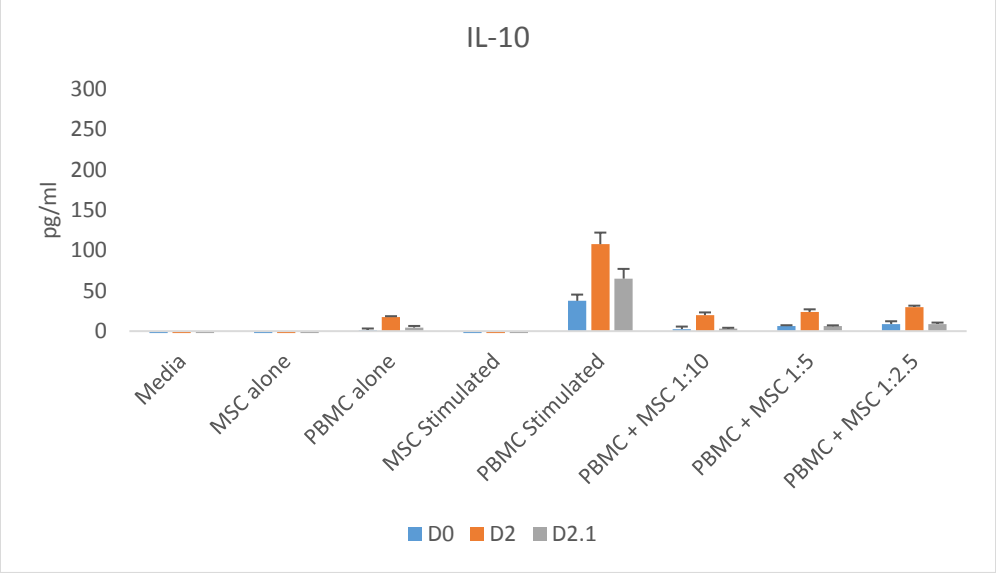

Figure B: **IL-10 ELISA Assay.** Small, non significant increase in mixture ratios of PBMC and donor MSCs PBMC stimulated is the positive control. This patient did demonstrate elevated levels of IL-10 in stimulated PBMC but this was variable among patients. Media, MSC alone and stimulated MSC did not produce appreciable levels of IL-10. **(**D0 = Blood sample taken at screening, prior to administration of donor MSCs; D2 = Blood sample taken one hour after the second application of donor MSCs; D2.1 = Blood sample taken one week after second applicatioin of donor MSCs.)

Figure C

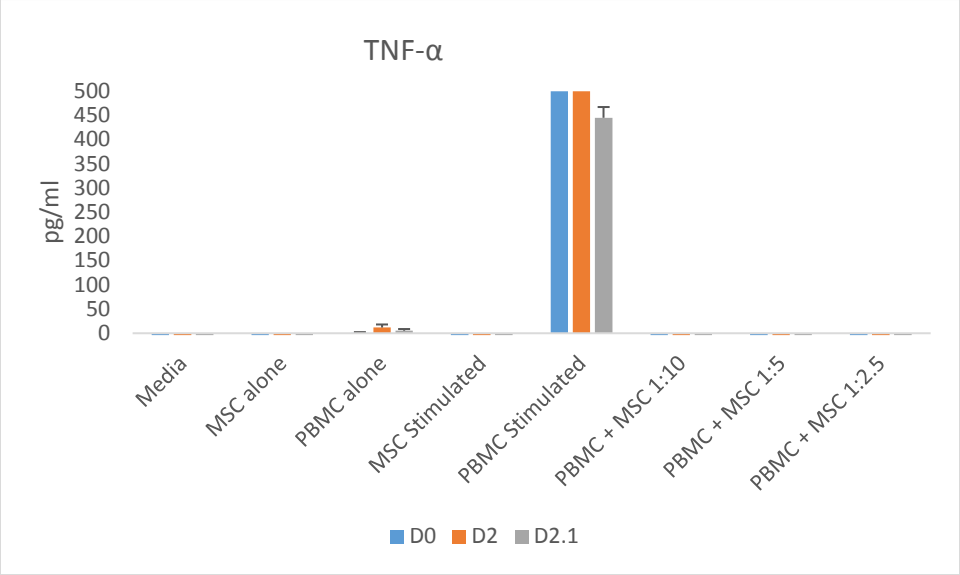

Figure C: TNF- α ELISA Assay. All mixture ratios of PBMC and donor MSCs are non-reactive. PBMC stimulated is the positive control. Media, MSC alone, MSC stimulated and PBMC alone did not produce appreciable levels of TNF- α. **(**D0 = Blood sample taken at screening, prior to administration of donor MSCs; D2 = Blood sample taken one hour after the second application of donor MSCs; D2.1 = Blood sample taken one week after second applicatioin of donor MSCs.)

Figure D

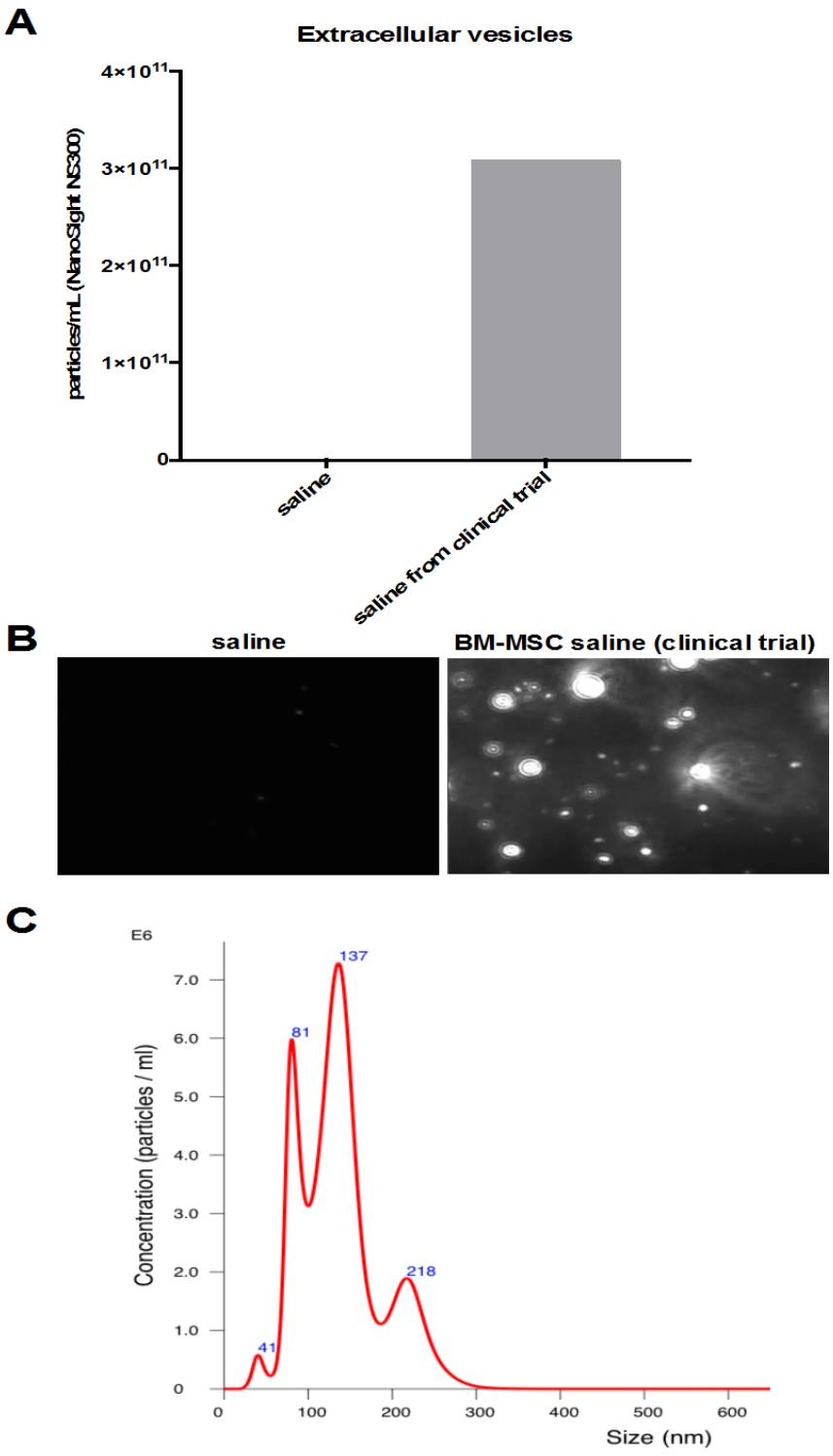

Figure D. Extracellular vesicles are found within the saline buffer from passage 3 BM-MSC given topically to burn patients. (A) quantification of vesicle particle number concentration using NanoSight. **(B)** NanoSight video-derived images showing extracellular vesicles (diluted 1:500 in saline). **(C)** Particle size distribution and concentration (diluted 1:500 in saline)

PROBLEM AREAS

Section III: Problem Areas.

(a) A description of current problems that may impede performance along with proposed corrective action.

- **The enrollment for the study is slow; we are working in a partnership with ISR as a second clinical site for the clinical trial.**

(b) A description of anticipated problems that have a potential to impede progress and what corrective action is planned should the problem materialize.

N/A

CONCLUSION

UPCOMING PERFORMANCE PERIOD

Section IV - A description of work to be performed during the next reporting period.

A. Continue donors recruitment, screening and Bone Marrow Aspiration as needed.
B. Complete all 20 recipients screening visits and MSCs application.
C. Data collection.

ADMINISTRATIVE COMMENTS

Section V - Administrative comments (Optional) - Description of proposed site visits and participation in technical meetings, journal manuscripts in preparation, coordination with other organizations conducting related work, etc.

Quad Chart updated

Stem Cell Therapy To Improve Burn Wound Healing

Insert ERMS/Log Number and Task Title Here

W81XWH-13-2-0024

PI: Carl Schulman **Org:** University of Miami **Award Amount:** $3,060,000

Study/Product Aim(s)

- Perform Phase 1 Trial of Allogeneic MSCs in Burns
- Perform Phase 2 Trial of Allogeneic MSCs in Burns
- Collect Tissue Repository for Biomarker Evaluation

Approach

Free text.

Insert a picture or graphic here, with a caption, that represents the proposed work

Accomplishment: Place a description of the latest scientific accomplishment here. Limit the comments to three lines or less to make them fit; be succinct. These comments are valuable since they show progress.

Timeline and Cost

Activities CY	14	15	16	17
Obtain IRB approval and start donor recruitment,; Hire essential personnel				
Obtain donor MSCs and apply to patients				
Enroll 80 patients for randomized trial, follow for 12 months				
Collect samples for repository				
Estimated Budget ($K)	**$230**	**$1200**	**$1300**	**$330**

Updated: 02/14/2017

Goals/Milestones

CY13 Goal – Phase 1 Trial

☑ Obtain UM and DOD IRB approval, hire essential personnel

CY14 Goals – Phase 1 and Phase 2 Trial

☑ Obtain donor MSCs and apply MSCs to patients. Maintain cells ready for application

☑ Start screen and Recruit 20 patients for safety/dose studies

CY15 Goal – Continue Phase 1 and, Start Tissue Repository

☑ Continue donors recruitment, screening and Bone Marrow Aspiration as needed.

☑ Continue patients screening and enrollment.

☑ Collect blood and tissue samples to perform functional studies

CY16 Goal – Complete phase 1 Trial and Collect Tissue Repository

☑ Continue donors recruitment, screening and Bone Marrow Aspiration as needed.

☑ Continue patients screening and enrollment.

☑ Collect blood and tissue samples to perform functional studies

CY17 Goal – Complete phase 1 Trial, Start phase 2 trial and Collect Tissue Repository

☐ Continue donors recruitment, screening and Bone Marrow Aspiration as needed.

☐ Complete the screening and enrollment of 20 patients for safety/dose studies

☐ Start the screening and enrollment of 80 patients in randomized trial

☐ Collect blood and tissue samples to perform functional studies

☐ Follow patients for at least 6 months after the last MSCs application

Comments/Challenges/Issues/Concerns

- Patients-Recipients: Screened: 18; Screen failure: 6; Withdraw consent: 2; Enrolled: 10

Budget Expenditure to Date

Projected Expenditure: $2,633,566

Actual Expenditure: $2,266,493