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TITLE: Regenerative Rehabilitation: Towards the Optimization of Rehabilitation Strategies to Improve the Efficacy of Regenerative Therapies for Treatment of VML Injury

PRINCIPAL INVESTIGATOR: Christopher L. Dearth, PhD

RECIPIENT: The Henry M. Jackson Foundation for the Advancement of Military Medicine in collaboration with the Uniformed Services University of Health Sciences

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14. ABSTRACT

The proposed Regenerative Rehabilitation based study seeks to optimize a rehabilitation protocol such that the ability of a regenerative therapy to create more functional skeletal muscle tissue is increased. We hypothesize that rehabilitative exercises will promote improvements in the overall strength of the injured muscle. As such, this research represents a significant advancement in the understanding of this underrepresented component of care for severe extremity trauma. If/when successful, the knowledge developed is primed to transition into clinical practice and may prove to be pivotal to achievement of clinically meaningful regenerative and functional outcomes for Wounded Warriors.

15. SUBJECT TERMS

Volumetric muscle loss, Regenerative medicine, Regenerative Rehabilitation, Orthopeadic Trauma

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1. INTRODUCTION:

Traumatic extremity injuries are the hallmark of recent military conflict. The battle mortality rate for US forces has decreased from 30% in WWII to less than 10% in the OIF/OEF conflicts. However, the decreased mortality rate has been accompanied by an increase in the percentage and absolute number of seriously injured SM. These SM survive but are left with extraordinary, life affecting injuries; especially complex and severe extremity injuries. Indeed, extremity wounds make up the most common survivable injuries of modern military conflict and comprise two-thirds of initial hospital costs to the DoD.

***The volumetric loss of skeletal muscle is a limb-associated morbidity that persists despite what may be considered a surgical success for either the amputated or reconstructed limb*.** VML injury was operationally defined by US Military surgeons as a traumatic or surgical loss of a large volume of skeletal muscle that results in chronic functional impairment. Since that time, it has been established that VML accounts for 63% of total disability among military limb reconstruction patients with a disability rating. VML injuries that cause disability present heterogeneously across the appendicular musculoskeletal system. That is, in VML patients from a general population of combat casualties both upper and lower extremities can suffer VML injury that leads to disability. Among subjects with reconstructed limbs following open tibia fractures, below the knee VML, specifically, is a frequent and debilitating problem. Additionally, patients with late amputation following an attempted reconstruction are at risk of continuing disability associated with their VML even after the late amputation. Lastly, *VML does not present as a static disabling condition, but rather an insidious degenerative process that worsens with time[7] and may eventually contribute to the development of deleterious secondary health conditions (i.e., low back pain & osteoarthritis)*.

***Current regenerative paradigms for VML injury have yielded promising pre-clinical results, but require augmentation to achieve a clinically important difference*.** The only regenerative therapy tested to date for clinical VML is a biological decellularized extracellular matrix (ECM). Biological scaffolds are designed to chemoattract endogenous cells that are capable of orchestrating a myogenic response. The clinical data demonstrate modest initial functional improvements, without a robust regenerative response that would support an even great functional improvement. As a muscle stem cell-based therapy, autologous minced muscle grafts have been quantitatively shown to promote regeneration of approximately two-thirds of the fibers and strength lost in a rat tibialis anterior (TA) muscle model. The regenerated fibers are derived from donor muscle progenitor cells and become innervated within 8 weeks of implantation. However, in a porcine VML model the regenerative outcomes dissipate, and much like ECM present fairly extensive fibrosis.

***Regenerative and Rehabilitative Medicine: A Necessary Synergy for Functional Recovery from VML Injury*.** Looking forward to the requirements for true clinical success in re-establishing function to traumatized limbs with VML injuries, the need to establish comprehensive regenerative and rehabilitative therapies (i.e., Regenerative Rehabilitation) is imminent. The benefits of Regenerative Rehabilitation is likely to enhance independent treatment efficacy. However, the science to elucidate the biologic underpinnings of Regenerative Rehabilitation based approaches are still in its infancy and countless questions exist that are in need of comprehensive evaluation.

2. KEYWORDS:

Volumetric muscle loss, Tissue engineering, Regenerative medicine, Regenerative Rehabilitation, orthopeadic trauma

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Primary Goal & Overarching Hypothesis:

An overarching objective of our research program is to develop individualized, patient-specific (i.e., personalized medicine) Regenerative Rehabilitation based therapies capable of providing the highest level of functional performance and optimal QoL for SMs and veterans with traumatic extremity injuries. However, before this can be achieved, numerous aspects of those injuries, including VML injuries, require further investigation. Thus, pre-clinical Regenerative Rehabilitation themed studies which seek to elucidate these key questions, particularly those focused on VML injuries, are crucial to understanding the fundamental mechanisms related to the interplay between mechanobiology and tissue regeneration. Thus, the ***primary goal*** of this study is to develop a synergistic Regenerative Rehabilitation treatment program which facilitates optimal functional outcomes following VML injury. The proposed work will test the ***overarching hypothesis*** that optimization of timing of a gold standard rehabilitation therapy will improve the efficacy of a comprehensive regenerative medicine treatment strategy and thus facilitate improved skeletal muscle form and function compared to either the rehabilitation or regenerative interventions in isolation. The overarching hypothesis will be tested in the following Specific Aims:

	Timeline	**USUHS**	**Status**
Specific Aim I: Evaluate the Effects of Dosing and Scheduling of ITA for Reduction of Fibrotic Tissue Deposition in a Rat Hindlimb Model of Volumetric Muscle Loss.			
Major Task 1: Optimize the optimal dosage of administration of ITA within a VML injury to facilitate reduced fibrosis.	**Months**	**POC**	--
Subtask 1: Obtain IACUC & ACURO Approvals	1-2	Dearth	**Complete**
Subtask 2: Perform Rodent Surgeries	2-6	Dearth / Goldman	In Progress
Subtask 3: Perform Cellular, Molecular, & Histological Analyses	3-7	Dearth / Goldman	In Progress
Subtask 4: Data Reduction & Dissemination	6-8	Dearth / Goldman	In Progress
Major Task 2: Optimize the optimal duration of administration of ITA within a VML injury to facilitate reduced fibrosis.	**Months**	**POC**	--
Subtask 1: Obtain IACUC & ACURO Approvals	1-2	Dearth	**Complete**
Subtask 2: Perform Rodent Surgeries	2-6	Dearth / Goldman	In Progress
Subtask 3: Perform Cellular, Molecular, & Histological Analyses	3-7	Dearth / Goldman	In Progress
Subtask 4: Data Reduction & Dissemination	6-8	Dearth / Goldman	In Progress
Milestone(s) Achieved: Determination of an optimized dose & duration of administration of ITA within a VML injury to facilitate reduced fibrosis.			

	Timeline	USUHS	Status
Specific Aim II: Evaluate Regenerative Outcomes associated with Administration of ITA in Concert with Regenerative Medicine Therapies in a Rat Hindlimb Model of Volumetric Muscle Loss			
Major Task 1: Evaluate the combined use of MMG + ITA at improving regenerative outcomes following VML.	**Months**	**POC**	--
Subtask 1: Obtain IACUC & ACURO Approvals	6-7	Dearth	**Complete**
Subtask 2: Perform Rodent Surgeries	7-11	Dearth / Goldman	Pending
Subtask 3: Perform Cellular, Molecular, & Histological Analyses	8-12	Dearth / Goldman	Pending
Subtask 4: Data Reduction & Dissemination	9-13	Dearth / Goldman	Pending
Major Task 2: Evaluate the combined use of ECM + ITA at improving regenerative outcomes following VML.	**Months**	**POC**	--
Subtask 1: Obtain IACUC & ACURO Approvals	6-7	Dearth	**Complete**
Subtask 2: Perform Rodent Surgeries	7-11	Dearth / Goldman	Pending
Subtask 3: Perform Cellular, Molecular, & Histological Analyses	8-12	Dearth / Goldman	Pending
Subtask 4: Data Reduction & Dissemination	9-13	Dearth / Goldman	Pending
Milestone(s) Achieved: Determination of an optimized combination (i.e., antifibrotic & regenerative) therapy which facilitates the highest level of reconstruction of tissue form & function.			

	Timeline	USUHS	Status
Specific Aim III: Optimization of a Regenerative Rehabilitation Program to Improve Functional Outcomes in a Rat Hindlimb Model of Volumetric Muscle Loss.			
Major Task 1: Evaluate the ability of physical rehabilitation (i.e., wheel running) to provide a synergist beneficial impact on tissue regeneration, force production, and ambulation.	**Months**	**POC**	--
Subtask 1: Obtain IACUC & ACURO Approvals	11-12	Dearth	**Complete**
Subtask 2: Perform Porcine Surgeries	12-16	Dearth / Goldman	Pending
Subtask 4: Perform Cellular, Molecular, & Histological Analyses	13-17	Dearth / Goldman	Pending
Subtask 5: Data Reduction & Dissemination	14-18	Dearth / Goldman	Pending
Milestone(s) Achieved: Determination of the efficacy of an optimal Regenerative Rehabilitation therapy for treatment of VML injuries.			

What was accomplished under these goals?

Administrative Activities:

- Study kickoff meeting completed
- Project initiation administrative activities completed
    - Award account set up at HJF
    - BPO set up at USU
    - Scientific contract support staff (post-doctoral fellows) identified, hired & on-boarded (Drs. Dolan & Franco)
        - These positions are supported via leveraged funds from the Extremity Trauma & Amputation Center of Excellence (EACE) and thus are at no cost to the current project
- IACUC protocol submitted & approved
    - Approval achieved on 6DEC2019

Scientific Activities / Preliminary Results:

1) Decrease in body weight is often an indicator of drug toxicity. Each animal's weight was measured at the beginning and end of the treatment regimen (**Figure 1**). No difference in body weight between vehicle and ITA-treated animals were found.

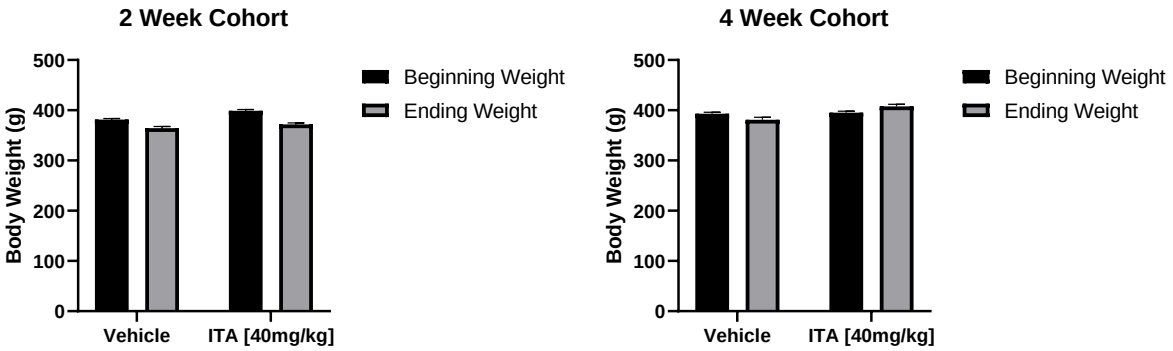

Figure 1: Change in animal body weight at 2 (left) and 4 (right) weeks following subcutaneous injection of vehicle or ITA [40mg/kg]. Bar graph shows mean body weight + SEM.

2) To assess whether ITA (40mg/kg) had an effect on the gross tissue weight of the injured tibialis anterior muscle, weights of the muscle were measured at the study end point for each cohort (**Figure 2**). No difference between vehicle and ITA-treated animals were observed.

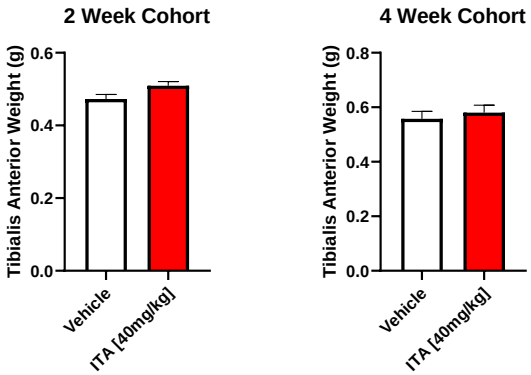

Figure 2: Gross tissue weight of injured tibialis anterior muscles at 2 (left) and 4 (right) weeks following subcutaneous injection of vehicle or ITA [40mg/kg]. Bar graph shows mean body weight + SEM.

3) Analysis of mRNA expression at 2 weeks revealed a significant downregulation for Col1a1, Col3a1, and α-SMA in the ITA-treated animals compared to vehicles controls (**Figure 3**). However, no changes were observed for TGF-β expression.

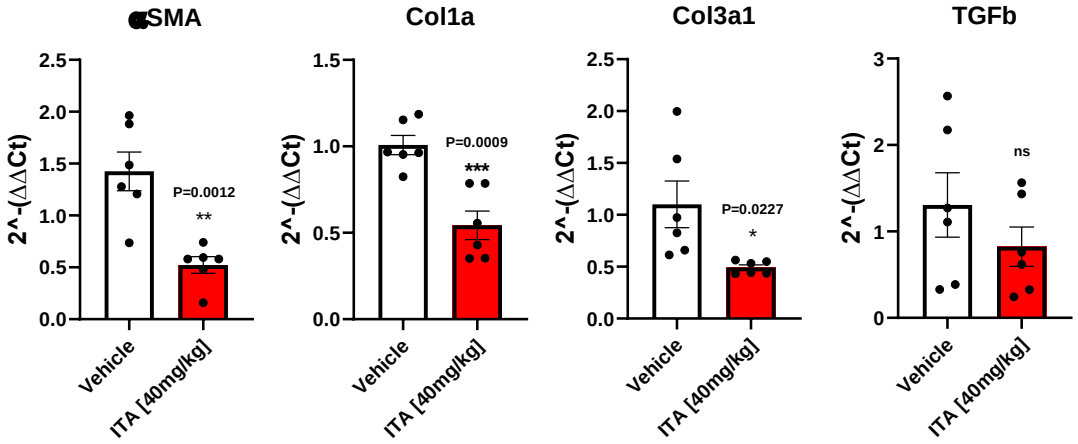

Figure 3: Gene expression analysis for select fibrotic genes at 2 weeks. Graphs show fold change (2^-(ΔΔCt)) relative to vehicle control. The average dCq value of the vehicle controls was used as a calibrator to calculate the ddCq value and fold change was calculated for each animal (Vehicle n=6; ITA n=6). ns=non-significant

4) Both vehicle and ITA-treated animals showed reduced isometric torque compared to uninjured controls (**Figure 4**). However, no significant changes in isometric torque were found between vehicle and ITA groups at 4 weeks.

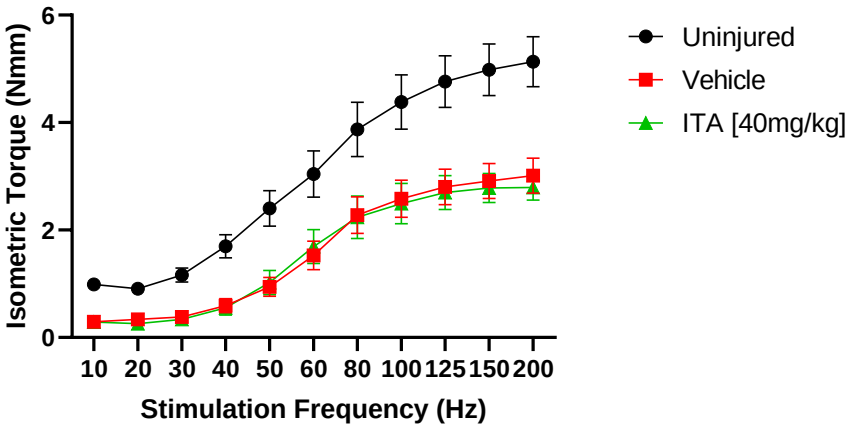

Figure 4: In vivo TA muscle neuromuscular strength was assessed at 4 weeks post injury for uninjured, vehicle, and ITA [40mg/kg] groups

Impact of COVID-19:

Unfortunately, due to the COVID-19 pandemic and the associated closure of USUHS (and subsequent restriction of research related activities), progress on this project was significantly inhibited during the current report period (Yr1) in regards to primary data generation (i.e, in lab work).

However, the investigative team devoted significant effort towards efforts that did not require in lab activities, such as conducing preliminary data analyses, planning of future experiments, and initial knowledge translation efforts.

What opportunities for training and professional development has the project provided?

While the intent of this study is not to provide training opportunities per se, activities have been made available to three of the EACE-USUHS postdoctoral trainees (Drs. Dolan, Franco, & Motherwell). Specifically, Drs. Dolan, Franco, & Motherwell have received training on surgical procedures for an animal model of extremity trauma, post-operative care, neuromuscular force production assays, sample preparation and analyses.

How were the results disseminated to communities of interest?

The investigative team is proud to report that an abstract for this project which was submitted to MHSRS (Abstract # MHSRS-20-01492; "*Effect of Itraconazole on Fibrotic Wound Healing after Volumetric Muscle Loss Injury*") was ***accepted for a podium presentation*** within the "Caring for Traumatic Soft Tissue & Orthopedic Injury within Austere Environments" section. However, of course, subsequently we received notification that MHSRS was cancelled due to COVID-19; but nonetheless, the acceptance demonstrates an acknowledgement by our peers of the quality, relevance to high priority military requirements, and potential impact to the care of injured Service members of our work. In sum, this is a noteworthy accomplishment for a project that is so early on in its period of performance, and ultimately speaks to the positive trajectory it is on.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we hope to fully resume research activities for this project. Specifically, we anticipate finalizing activities within Specific Aim 1, as well as initiating and subsequently finalizing activities within Specific Aim 2.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

As stated above, this research project has incurred significant, previously unanticipated, delays due to the COVID-19 pandemic. More specifically, due to the COVID-19 pandemic, all in lab research activities at USUHS were mandated to be stopped. Thus, at current, the project has experienced ~6 month delay in the originally projected timeline. Everything that can be done to mitigate the deleterious impact of this delay to the study, is being done (e.g., preliminary data analyses, etc.). However, based on the extend of delay incurred already, along with the unknown impact of COVID-19 in the future, it is effectively guaranteed that this study will not be able to meet its originally projected timeline, and thus will need a No Cost Extension (NCE).

Changes that had a significant impact on expenditures

Aside from the timing delay described above, and the resultant under execution of the project related expenditures (relative to the originally projected timeline within the SoW), no other financial impacts have been sustained – i.e., the project is in good fiscal health relative to the current status.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers, and presentations.

Nothing to Report

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

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Christopher L. Dearth, PhD
Project Role:	Principle Investigator
Nearest person month worked: 1	
Contribution to project:	Dr. Dearth coordinated all aspects of the project related activities, including: group / collaborator teleconferences, budgetary management, equipment / supply purchases, and study preparatory activities.
Name:	Stephen Goldman, PhD
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to project:	Dr. Goldman assisted Dr. Dearth with project related activities, including: equipment / supply purchases, and study preparatory activities.

***Note: Drs. Dearth and Goldman are GS DoD employees (part of the EACE), thus their efforts are at no cost to the award.

Name:	Connor Dolan, PhD
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to project:	Dr. Dolan assisted with project related activities, including: experimental procedures; data collection and analysis

Name:	Sarah Franco, PhD
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to project:	Dr. Franco assisted with project related activities, including: experimental procedures; data collection and analysis
Name:	Jessica Motherwell, PhD
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to project:	Dr. Motherwell assisted with project related activities, including: experimental procedures; data collection and analysis

***Note: Drs. Dolan, Franco, & Motherwell are supported via leveraged funds from the EACE, thus their efforts are at no cost to the award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

As noted above, the DoD Extremity Trauma & Amputation Center of Excellence (EACE) is a key partner in this project by providing the infrastructure – e.g., personnel (as described above) – necessary to accomplish the goals of this project.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating 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.

Regenerative Rehabilitation: Towards the Optimization of Rehabilitation Strategies to Improve the Efficacy of Regenerative Therapies for Treatment of VML Injury

W81XWH-19-2-0039
BA180115

PI: Christopher L. Dearth, PhD **Org:** EACE / USU / HJF **Award Amount:** $297,730

The ***primary goal*** of this study is to develop a synergistic Regenerative Rehabilitation treatment program which facilitates optimal functional outcomes following VML injury. The proposed work will test the ***overarching hypothesis*** that optimization of timing of a gold standard rehabilitation therapy will improve the efficacy of a comprehensive regenerative medicine treatment strategy and thus facilitate improved skeletal muscle form and function compared to either the rehabilitation or regenerative interventions in isolation. The overarching hypothesis will be tested in the following Specific Aims:

Specific Aim I: Evaluate the Effects of Dosing and Scheduling of ITA for Reduction of Fibrotic Tissue Deposition in a Rat Hindlimb Model of Volumetric Muscle Loss.

Specific Aim II: Evaluate Regenerative Outcomes associated with Administration of ITA in Concert with Regenerative Medicine Therapies in a Rat Hindlimb Model of Volumetric Muscle Loss

Specific Aim III: Optimization of a Regenerative Rehabilitation Program to Improve Functional Outcomes in a Rat Hindlimb Model of Volumetric Muscle Loss

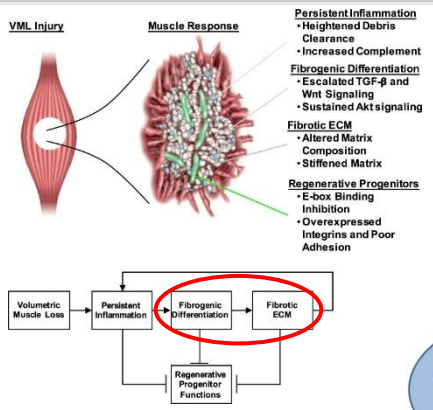

Figure 1: Pathobiology of VML schematic

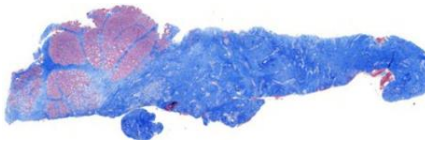

Figure 3: VML clinical tissue biopsy which demonstrates extensive fibrosis

Figure 2: Wounded SM with a VML injury to the lower extremity.

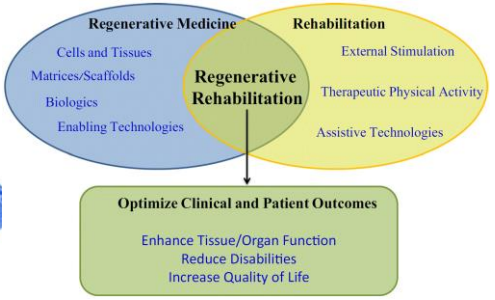

Figure 4: Regenerative Rehabilitation Schematic.

Timeline & Cost

Activities CY	19	20	21	22 (NCE)
Study Initiation				
Specific Aim I				
Specific Aim II				
Specific Aim III				
Estimated Budget ($K)	**$55**	**$75**	**$100**	**$68**

Budget Expenditure to Date
Projected Expenditure: ~$205k
Actual Expenditure: ~$130k

Updated: 29SEPT2020

Goals / Milestones

CY19 Goal – Study Initiation
- ☑ Study kickoff
- ☑ Complete study administrative activities
- ☑ IACUC generation, submission, & approval
- ☑ Initiate specific aim I

CY20 Goals – Study Execution
- ☐ Complete specific aim I
- ☐ Initiate specific aim II

CY21 Goal – Study Completion
- ☐ Complete specific aim II
- ☐ Initiate specific aim III
- ☐ Complete specific aim III
- ☐ Finalize data analyses
- ☐ Complete knowledge dissemination

Effect of Itraconazole on Fibrotic Wound Healing after Volumetric Muscle Loss Injury

Sarah Franco PhD[1,2], Connor Dolan PhD[1,2] , Naveena Basa Janakiram PhD[1,2], Michael S. Valerio, PhD[1,2], Stephen M. Goldman, PhD[1,2], and Christopher L. Dearth, PhD[1,2]
[1]DoD-VA Extremity Trauma and Amputation Center of Excellence, Fort Sam Houston, TX
[2]Department of Surgery, Uniformed Services University of the Health Sciences and Walter Reed National Military Medical Center, Bethesda, MD

Background:
Extremity injuries account for nearly half (47%) of combat and noncombat-related injuries sustained by Service members during conflicts in Iraq and Afghanistan between 2003 and 2014. Volumetric muscle loss (VML) injury—a common component of extremity injuries—was operationally defined by US military surgeons as the traumatic or surgical loss of a large volume of skeletal muscle, which results in a chronic functional impairment. Since that time, it has been established that VML accounts for ~63% of total disability among Service members who sustain traumatic extremity injuries with a disability rating. Importantly, VML injuries can result in life-long disabilities, and there is a critical need to develop regenerative medicine-based therapeutics that can attenuate both the acute and chronic pathobiology of VML.

The pathobiology of VML includes extensive fibrotic tissue deposition which leads to a self-sustaining environment that prevents endogenous or therapeutically induced skeletal muscle regeneration. Critical to the pathogenesis of VML-induced fibrosis is the activation and transdifferentiation of resident fibroblasts and/or circulating marrow-derived cells into myofibroblasts (α- smooth muscle actin+) which mediate the deposition of fibrous ECM components. As such, this transdifferentiation process has been identified as a target for the development of novel anti-fibrotic drugs with broad effects across a number of disease states. A recent screen of FDA approved drugs for their capacity to inhibit myofibroblast transdifferentiation identified the drug itraconazole (ITA) as a potential anti-fibrotic with a mechanism that is reported to be independent of direct TGF-β signaling inhibition.

The goal of this study was to assess ITA for its capacity to mitigate fibrotic wound healing in the context of VML. It was hypothesized that ITA treatment following VML injury would decrease fibrosis compared to vehicle controls.

Methods:
Lewis rats (male; 389.9g ± 1.755) were subjected to a well-established tibialis anterior (TA) muscle-based VML injury model. Animals were randomized to either no repair (NR; n=5), vehicle control (5% DMSO + PEG300; n=24), or ITA (40mg/kg in 5% DMSO+PEG300; n=12). Animals receiving vehicle or ITA were administered subcutaneous injections on the day of surgery, followed by injections every other day and were sacrificed at either 2 or 4 weeks. At the end of the study period, the common peroneal nerve was stimulated using percutaneous needle electrodes and isometric tetanic torque was recorded. At the terminal time points, animals were euthanized, and the TA muscle was collected for either molecular or histological analysis. Gene expression for collagen type I alpha 1 chain (*Col1a1*), collagen type III alpha 1 chain (*Col3a1*), alpha-smooth muscle actin (*αSMA*), and transforming growth factor-beta (*TGFβ*) were analyzed via qRT-PCR for the 2-week time point. Picrosirius red stain will be used to visualize collagen complementary to hematoxylin and eosin staining.

Results:
Analysis of mRNA expression at 2 weeks revealed a downregulation for *Col1a1, Col3a1*, and *αSMA* in the ITA-treated animals compared to vehicle controls (p=0.0009, 0.0227, 0.0012, respectively). However, no changes were observed for TGF-β expression (p=0.2964). When comparing NR vs. vehicle, the NR group had higher mean isometric torque at 50, 80, 100, 125, and 150 Hz (p=0.0283, 0.0374, 0.0235, 0.0493, 0.0457, respectively). A higher mean isometric torque was also observed for the NR group when comparing NR vs. ITA at 100, 125, and 150 Hz (p=0.0274, 0.0478, 0.0392, respectively). However, no differences were observed at other stimulation frequencies compared to the NR group, nor was there a difference between vehicle vs. ITA at any stimulation frequency.

Additionally, there was no difference in peak isometric force among the groups (p=0.1574). Histological analyses are currently underway.

Conclusions:
The results obtained in this study have demonstrated the potential anti-fibrotic effects of ITA in the context of VML injuries. Specifically, ITA inhibited the expression of important pro-fibrotic genes (i.e., *Cola1a1, Col3a1, αSMA*, and *TGFβ*) that are known to be up-regulated following VML-injury. While there were differences in the mean isometric torque at some frequencies, there were no observed differences in the peak isometric force among the groups. Future work will further characterize ITA inhibition on the expression of additional fibrotic genes and proteins, and histological analyses will be used to assess whether these changes result in architectural differences. Furthermore, long-term (8 weeks) effects of ITA administration on both fibrosis and muscle function will also be investigated. If ITA can provide persistent anti-fibrotic effects, it may aid in creating an environment that favors skeletal muscle regeneration and ultimately serve as a therapeutic approach to reducing VML in extremity injuries.

Acknowledgements: Funding was provided by the Department of Defense, specifically the Clinical and Rehabilitative Medicine Research Program (Award# W81XWH-19-2-0039).

Learning Objectives:

1. Understand the critical need to develop therapeutic approaches to facilitate the restoration of tissue form and function following volumetric muscle loss in Service members.
2. Describe the role of the fibrotic wound healing response in the pathobiology of volumetric muscle loss.
3. Discuss the potential applicability of itraconazole in reducing fibrosis and improving the capacity for skeletal muscle regeneration.