Interdisciplinary Research (IDR) Origination Awards

Cover Page

Project Title

Title: Galectin-1 as a Novel Treatment of Secondary Damage Following Volumetric Muscle Loss Injury

Principal Investigator(s) (full-time faculty)

Name (PI listed first)	Department	College
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Track

Track one

Abstract

Volumetric muscle loss (VML) is a severe and debilitating type of musculoskeletal injury that results from trauma or invasive surgeries, characterized by the blunt removal of muscle tissue and progressive secondary damage to the remaining tissue. The severity of VML is sufficient to overwhelm the body's natural regenerative capabilities, resulting in inflammation, scar tissue formation, and long-term loss of muscle function. Herein, we propose the use of Galectin-1 (Gal-1) as a therapeutic intervention to address secondary damage caused by VML. Gal-1 is a carbohydrate-binding protein naturally expressed in muscle to improve the healing of acute injuries, and when delivered exogenously, can also improve muscle health and pathology in models of muscular dystrophy. Using a rat model, we aim to evaluate the therapeutic potential of Gal-1 alone and in combination with muscle transplants, to mitigate secondary damage and restore form and function to VML injured muscle. This study aims to assess the effects of Gal-1 on key pathological markers such as inflammation and fibrosis, mitochondrial function, innervation, and myofiber morphology, as well as its ability to support transplant integration and functional recovery. Our findings will advance the development of comprehensive treatment strategies for VML, addressing both primary VML injury and secondary damage to improve muscle regeneration and functional outcomes.

Summary of Plans for External Funding

Our plan is to use the IDR Award to generate foundational data on Gal-1's efficacy in mitigating secondary damage—such as fibrosis, inflammation, and mitochondrial dysfunction—in a rat model of VML, while also demonstrating its synergistic potential when combined with regenerative therapies like muscle transplants. These proof-of-concept results will form the basis for our external grant proposal targeting the Department of Defense (DoD) through funding mechanisms such as the Defense Medical Research and Development Program (DMRDP) or the Peer-Reviewed Orthopedic Research Program (PRORP). We anticipate preparing and submitting our DoD proposal within 12 to 18 months from now, following the successful completion of the initial data collection phase (AIM 1). This timeline ensures that our proposal is grounded in evidence that directly validates the use and efficacy of using Gal-1 to address the challenges associated with traumatic muscle injuries. By emphasizing the translational potential of our approach, our proposal will highlight how this innovative therapy could significantly improve outcomes for injured service members. Ultimately, our goal is to combine different treatment strategies that focus on the vast complexity of VML injuries and bridge the gap between foundational research and clinical application.

PROJECT NARRATIVE

Volumetric muscle loss (VML) occurs when a significant portion of muscle tissue, including cells, nerves, blood vessels, and extracellular matrix, is lost due to trauma or invasive surgery. This devastating loss of muscle fails to regenerate, replaced instead by aggressive inflammation and dense scar tissue. As a result of the primary VML injury, secondary damage develops in the remaining muscle, characterized by mitochondrial dysfunction, reduced satellite cell activation (muscle stem cells), and progressive denervation. Together, the primary injury and secondary damage result in severe muscle atrophy, long-term muscle dysfunction, and, in extreme cases, amputation **(Fig. 1)**.

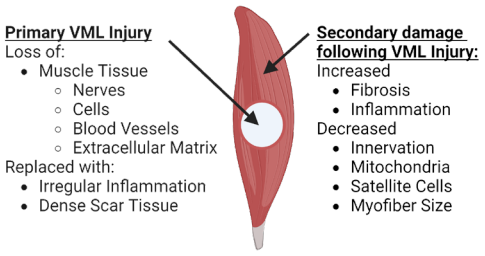

Fig. 1 – VML Pathology: Illustration that highlights the known pathology of VML injuries and the subsequent secondary damage to the remaining muscle tissue. The combination of which leads to severe muscle atrophy, long-term muscle dysfunction, and possible amputation.

Currently, there is no standard of care for VML injuries. Existing therapies—such as muscle transplants, stem cell injections, and biomimetic scaffold implants—have had mild success at restoring form and function to the injured muscle. Likely due to the strong focus on replacing the lost tissue while neglecting the health of the surrounding muscle, where secondary damage further impairs recovery. Novel therapies that directly target secondary damage are urgently needed and, when combined with other supportive treatments, have the potential to synergistically enhance healing and restoration of muscle functional.

We propose investigating the use of Galectin-1 (Gal-1) as a novel therapy to address secondary damage following VML. Gal-1 is a carbohydrate-binding protein naturally produced in skeletal muscle and motor neurons to support regeneration following acute muscle injury. Delivery of human recombinant Gal-1 has also been shown to reduce myofiber damage and improve myofiber size and function in mouse models of muscular dystrophy. Based on known therapeutic effects of Gal-1, ***we hypothesize that Gal-1 treatment will mitigate secondary damage in VML-injured muscle and improve functional and regenerative outcomes, especially when combined with a regenerative therapy (muscle transplant)***. This proposal will systematically evaluate the effects of Gal-1 on critical aspects of VML pathology using a rat model, which we will test through the following specific aims:

Aim 1: Evaluate the therapeutic potential of Galectin-1 alone to mitigate secondary damage in VML-injured muscle. Secondary damage in VML-injured muscle includes mitochondrial dysfunction, reduced satellite cell activation, progressive denervation, chronic inflammation, and fibrosis. Dr. Van Ry's lab has demonstrated the ability of Gal-1 to mitigate many of these complications in a mouse model of muscular dystrophy. To determine whether Gal-1 has a similar therapeutic impact in VML, we will create a VML injury in the tibialis anterior muscle of rats and administer weekly Gal-1 or placebo treatments. Key pathological markers will be assessed at critical time points (7-, 21-, and 42-days post injury) to evaluate whether Gal-1 restores the health and function of the remaining muscle tissue.

Aim 2: Assess the synergistic effects of combining Gal-1 with a muscle transplant to treat primary and secondary VML injuries. Dr. Sorensen has previously shown that muscle transplants stimulate new tissue growth and partial recovery of muscle function following VML, but their efficacy is often limited by poor integration with the remaining tissue. We hypothesize that Gal-1 treatment will enhance these outcomes by addressing secondary damage and supporting integration with the remaining tissue. To test this, we will deliver muscle transplants at the time of VML injury and administer weekly Gal-1 treatments. Six weeks post-injury, we will evaluate muscle function, myofiber size and content, mitochondrial function, and innervation to assess the benefits of this combined approach.

BACKGROUND AND SIGNIFICANCE

Volumetric muscle loss is a critical and persistent challenge in both military and civilian healthcare, where traumatic events—such as combat injuries, high-energy accidents, and invasive surgeries—result in severe and irrecoverable muscle damage. Despite progress in emergency care and survival rates, advancements in treating the long-term consequences of VML remain limited as conventional therapies fall short of restoring both the structural integrity and functional capacity of the injured muscle. The lack of effective treatments not only results in profound physical disabilities, but also imposes substantial economic burdens, with VML-related injuries in the military alone estimated to contribute over $42.4 billion in initial care costs and more than $100 billion in lifetime disability expenses (1-3). Civilian cases of VML are similarly impactful, frequently resulting from high-energy trauma such as motor vehicle accidents (6 million annually in the U.S.), industrial and farm equipment accidents, and invasive surgeries (removal of cancerous tumors). Each year, approximately 150,000 open fractures, 30,000 gunshot wounds, 12,000 surgeries to remove soft tissue sarcomas, and numerous other injuries contribute to the civilian incidence of VML, leading to extensive long-term disability, costly rehabilitation, and chronic comorbidities like metabolic syndrome and cardiovascular disease (4). These injuries collectively contribute to the $400 billion annual economic toll associated with trauma and injury care in the U.S., highlighting the need for more effective therapeutic options (5, 6).

Currently, most VML treatments focus solely on replacing lost tissue. However, these approaches have had limited success due to insufficient integration with the remaining muscle, which, when coupled with ongoing secondary damage, often results in progressive atrophy and loss of muscle function (7-12). The ability to restore innervation at the neuromuscular junction and mitochondrial function in the endogenous tissue is especially critical for improving the functional recovery of the affected muscle, yet these aspects are often neglected in existing treatments (9, 13).

Our proposed study directly addresses this unmet need by evaluating the therapeutic potential of Gal-1 as a treatment for secondary damage following VML. Gal-1 protein therapy has already demonstrated improvements in muscle health and regeneration in models of muscular dystrophy by modulating inflammation, reducing myofiber injury, increasing fiber size, and stabilizing muscle cell membranes (14-16). These effects collectively support a healthier regenerative environment, which, if replicated in a VML context, could shift the therapeutic focus toward a more comprehensive treatment approach.

In this proposal, we aim to assess the impact of Gal-1 on secondary VML injury, including its effects on innervation, mitochondria, and satellite cell activity using a rat model of VML. This model enables a detailed evaluation of Gal-1's capacity to promote a favorable environment for muscle regeneration and to counteract the degenerative challenges that often follow traumatic muscle injuries. Additionally, we will investigate whether combining Gal-1 with a muscle transplant can further enhance muscle regeneration and function, potentially overcoming the limitations of current grafting techniques. By focusing on these secondary aspects of VML, this research seeks to address the full spectrum of complications associated with VML, providing a foundation for therapies that could prevent progressive atrophy and promote functional recovery. Ultimately, our long-term goal is to provide a comprehensive solution for individuals with VML, to reduce their reliance on long-term medical interventions, decrease healthcare costs, and, most importantly, improve their quality of life.

RESEARCH DESIGN AND APPROACH

This study will evaluate the therapeutic potential of Gal-1 as a treatment for secondary damage following VML and its synergistic effects when combined with a muscle transplant. Our approach employs a rat model of VML and integrates a collection of methodologies, including histological, biochemical, and functional assessments, to comprehensively address the proposed aims.

Aim 1: Evaluate the therapeutic potential of Gal-1 alone to mitigate secondary damage in VML-injured muscle **(Fig. 2)**.

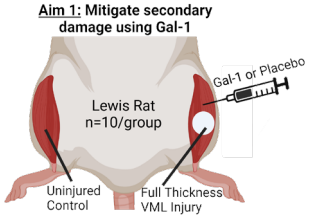

Fig. 2 - Aim 1 Study Design: A full thickness VML injury will be surgically created in the left tibialis anterior (TA) muscle and Gal-1 or a placebo administered weekly. Markers of secondary muscle damage will be measured at 7-, 21-, and 42-days post VML injury.

We've collected preliminary data using skeletal muscle myoblast from VML injured muscle that suggest Gal-1 can mitigate the effects of secondary damage in VML-injured muscle **(Fig. 3)**. To further test this hypothesis in a rat model, we will create a full thickness VML injury in the left tibialis anterior (TA) muscle of adult Lewis rats using a 6mm biopsy punch, the contralateral (right) leg will serve as an uninjured, intra-animal control. The treatment groups will consist of rats receiving either weekly intramuscular injections of Gal-1 or saline as a placebo, the first injection will be given at the time of surgery. Notably, we will be using Human Recombinant Galectin-1, produced and purified in the lab of Dr. Van Ry as described in Vallecillo-Zuniga et al (14).

Critical markers related to VML pathology and secondary damage will be evaluated at 7-, 21-, and 42-days post injury. The selected time points are intended to capture critical points of the VML pathology. For example, day 7 is typically the period when the inflammatory response is most pronounced. By day 21, fibrosis and scar tissue formation become the priority. Finally, on day 42, muscle fiber morphology, innervation, and mitochondrial function are top priorities for evaluating the overall impact of the treatment, as these markers have been shown to progressively degrade over longer periods of time. Total number of rats = 65 (2 groups, 10 rats/group, 3 time points, 5 provisional rats for training).

To evaluate these markers of secondary damage, the TA muscle will be sectioned into 3 portions (**See Fig. 6 for details**). The upper half will be used for histological analysis of inflammation and fibrosis, satellite cell content, and innervation. The bottom half of the TA will be sectioned into lateral and medial portions, with the medial portion being used to test mitochondrial function and the lateral portion for protein content related to Gal-1, inflammatory cytokines, and muscle atrophy markers.

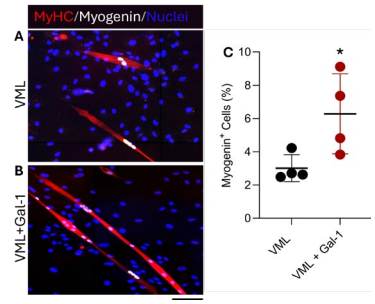

Fig. 3 – Gal-1 Treatment Improves VML Myoblast Differentiation: Myoblasts isolated from VML injured muscle were cultured for 3 days. Representative images show **(A)** VML and **(B)** VML+Gal-1 treated myoblasts differentiating into myotubes. **(C)** Gal-1 treated myoblasts increased nuclear expression of myogenin, the transcription factor that directs the cells to differentiate, fuse, and form myotubes. Scale bar = 100μm. * Significant difference, $p<0.05$.

Histology: Muscle cross-sections from the upper portion of the TA will be examined for markers of inflammation (macrophages: CD11b, CD206, and CD86, counterstained with DAPI), satellite cell activity (Pax7, MyoD, and Myogenin counterstained with laminin and DAPI), innervation (α-bungarotoxin colocalization with synaptic vesicle and neurofilament at the neuromuscular junctions), and myofiber morphology and fibrosis (Masson's Trichrome). Tissue samples will be imaged using the Echo Revolution microscope in Dr. Sorensen's lab, or the Olympus FV3000 confocal microscope in the Life Sciences Building for z-stack images of neuromuscular junctions, which represent innervation.

Mitochondrial respirometry: Muscle fiber bundles from the medial portion of the lower TA will be permeabilized using saponin and incubated in an oxygen-saturated respiration buffer (e.g., MiR05) at 37°C in a calibrated respirometer. We will sequentially add glutamate/malate for Complex I (LEAK respiration), ADP for oxidative phosphorylation (OXPHOS), succinate for Complex II and max coupled respiration, FCCP for maximal electron transport system

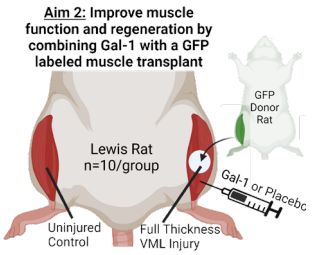

Fig. 4 - Aim 2 Study Design: In addition to weekly Gal-1 treatment or Placebo, a GFP labeled muscle transplant will be inserted at the time of surgery to facilitate regeneration of new muscle tissue. After 42 days, markers of secondary damage will be measured in conjunction with muscle function and the formation of new GFP fibers.

capacity (uncoupled respiration), and rotenone/antimycin A to assess residual oxygen consumption. Oxygen consumption rates will be normalized to tissue mass and analyzed to evaluate mitochondrial efficiency and dysfunction.

Protein content: The lateral portion of the lower TA will be homogenized and used to analyze protein content. Samples will be homogenized and the protein concentration quantified using a BCA assay. Western blot analysis will be used to measure the expression of atrophy markers (e.g. MuRF1, Atrogen-1) and Gal-1. For inflammatory cytokine analysis we will use the Luminex MAGPIX multiplex assay kit to measure the expression of IL-6, TNF-α, and TGF-β.

Surgeries will be completed in 8 months, with tissue analysis completed by one year. The expected outcome is that Gal-1 treatment will reduce inflammation and fibrosis, preserve mitochondrial function, innervation, and muscle morphology, and increase satellite cell content, ultimately creating an enhanced environment for muscle regeneration.

Aim 2: Assess the synergistic effects of combining Gal-1 treatment with a muscle transplant to address both primary and secondary VML injuries **(Fig. 4)**.

Muscle transplants are a promising solution for treating VML because they contain all the cellular and structural components necessary to build new muscle, but their success is often hindered by poor integration with the host muscle and the persistence of secondary damage **(Fig. 5)**. To address this limitation, we will combine Gal-1 treatment with a muscle transplant from ubiquitous GFP muscle donors. GFP labeling allows us to visualize and quantify the efficacy of the transplant. VML injuries will be induced as described in Aim 1, and fresh muscle grafts will be transplanted into the injury site immediately following surgery, replacing 100% of the removed tissue. Rats will be divided into two groups: one receiving saline as a placebo and the other receiving weekly Gal-1 injections. Total number of rats = 28 (2 groups, 10/group, 5 ubiquitous GFP donor rats, 2 wild type and 1 provisional GFP donor rat for training).

Following a muscle transplant, the contractile tissue from the transplant slowly degrades, leaving behind cells (e.g. satellite cells) and extracellular matrix. With these simple ingredients, new muscle fibers begin to form within the VML defect area. This process takes several weeks for the new muscle fibers to form and mature. As such, the therapeutic effects of this combined approach will be evaluated six weeks post-injury.

In addition to evaluating markers of secondary damage, our primary outcome will be focused on the functional recovery of the VML injured muscle, which we will measure using in-vivo electrical stimulation and force transduction. We will also focus on the regeneration and formation of new muscle tissue, which will be quantified through histological analysis of myofibers, with particular interest in the GFP expressing donor-derived fibers within the transplant site. Mitochondrial health will be assessed through high-resolution respirometry as described in aim 1. Likewise, neuromuscular junction

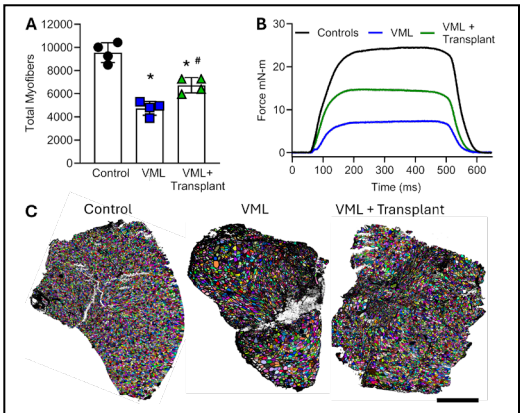

Fig. 5 – Muscle Transplants: A muscle transplant partially restores **(A)** myofiber numbers and **(B)** strength after VML injury. **(C)** Whole cross-sections of the tibialis anterior muscle from control, VML, and VML+transplant treatment groups. Each color within the sample represents a myofiber. * Different from control. # Different from VML. Significance set at $p<0.05$. Scale bar = 1mm.

morphology will be evaluated using confocal imaging to determine innervation integration with the donor tissue and to measure density, alignment, and synaptic integrity in the existing tissue.

Surgeries will be completed by 18 months with tissue analysis by the end of 2nd year. This aim is expected to demonstrate superior functional recovery, enhanced transplant tissue integration, and greater myofiber regeneration when using a combined approach compared to muscle transplants or Gal-1 treatment alone.

COLLABORATIVE EXPERTISE AND STUDENT TRAINING PLAN

The success of this project is built on the complementary expertise of our collaborative team, each contributing unique skills and extensive experience in areas critical to addressing the complications of VML. We have been meeting bi-weekly for roughly 6 months to develop the project and run experiments in each other's labs. Going forward, these meetings will be used to ensure clear communication, facilitate experimental troubleshooting, and enable the integration of findings from our diverse methodologies. These discussions will ensure that the project remains cohesive and adaptable to emerging challenges or insights and facilitate work towards our external funding applications.

Interdisciplinary Team Members

- **Dr. Jake Sorensen** is an expert in skeletal muscle physiology and has over seven years of VML research experience, with expertise in skeletal muscle histology and the rat VML model. His foundational work on the pathological progression of primary and secondary VML damage and the regenerative potential of various therapies, including muscle transplants, will guide the surgical creation of the VML injuries, histological evaluations, and functional assessment of the treatments.
- **Dr. Pam Van Ry** is a leading expert in the therapeutic applications of Gal-1, particularly in the context of muscular dystrophy, inflammation, and fibrosis. Her extensive knowledge of Gal-1 enables us to know the appropriate volume and method of delivery for administering Gal-1 successfully, eliminating the need for costly and time-consuming preliminary studies. She also brings extensive insights related to regenerative properties of Gal-1, which underpins the experimental design of this project. Importantly, her lab will produce and purify the human recombinant Gal-1 protein and administer the Gal-1 or placebo to our experimental rats. Her lab will also assist in histological staining, and protein content measurements.
- **Dr. Chad Hancock** specializes in evaluating muscle mitochondrial function and metabolism and how mitochondrial function changes with recovery and performance. He will lead advanced analyses of mitochondrial health and function in VML-injured muscle, which is a critical marker of secondary damage and a key focus of this study.

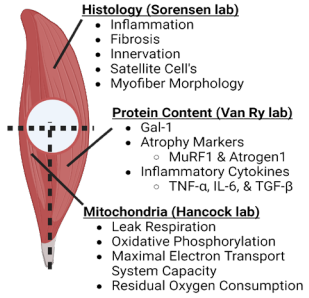

Fig. 6 - Collaborative Expertise: This illustration represents the interdisciplinary skills and expertise that will be used between our labs to address Gal-1's impact on VML injured skeletal muscle.

Student Engagement and Training

Undergraduate students will play an active role in this project. Our collaborative plan emphasizes providing students with hands-on research experience. Students will explore key aspects of muscle biology, including physiology, regenerative medicine, and molecular biology, all centered on addressing VML. By participating in this hands-on lab work, students will develop technical expertise in aseptic surgery, in-vivo muscle function testing, histological evaluation of muscle and nerves, protein quantification, and mitochondrial function assays. These skills are highly marketable and transferable to both clinical and basic science applications, enabling career readiness and innovation in the fields that our students are interested in pursuing. We believe this approach aligns with BYU's mission to cultivate the next generation of scientists by offering an immersive, mentorship-driven research environment.