Official Title: MiACLR: Michigan Initiative for Anterior Cruciate Ligament Rehabilitation

NCT Number: NCT03626857

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MiACLR: Michigan Initiative for Anterior Cruciate Ligament Rehabilitation

Protocol Number: HUM00144992

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Principal Investigator: Riann Palmieri-Smith, PhD, ATC

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.2, 1.3	**Study Schema**	Intervention visits split up to follow/ match the SOA. Testing visits corrected too
1.4	**Schedule of Activities**	Adjusted study visits and windows from weeks to days. Added footnotes for clarification of interventional visits. Added the clinical x-ray record review (approved in previous amendment).
4.1	**Overall Design**	Addition of *sex* as a stratification factor as requested by the DSMB.
8.1	**Efficacy Assessments**	Added clinical x-ray record review and language for SOC group. Both were approved in the last IRB amendment, but the wrong version of the protocol was used for submission.
8.3.3	**Events of Special Interest**	Addition of graft failure as an event of special interest as requested by the DSMB.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

  National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

  The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: MiACLR: Michigan Initiative for Anterior Cruciate Ligament Rehabilitation

Study Description: This study is a single-center, randomized, double-blind, placebo-controlled parallel-group clinical trial to examine the combined efficacy of neuromuscular electrical stimulation (NMES) and eccentric exercise (ECC) to promote the recovery of quadriceps strength, improve physical and biomechanical function, and reduce the risk of post-traumatic osteoarthritis (OA) after ACLR. Study interventions will begin immediately after ACLR at the first postoperative visit. The 2 arms of the trial include:

- NMES + ECC: NMES for 8 weeks followed by ECC for 8 weeks;
- NMES placebo + ECC placebo: NMES placebo for 8 weeks followed by ECC placebo for 8 weeks.

An additional group of participants will be enrolled, but not randomized and will receive neither study intervention; rather, they will serve as a Standard of Care (SOC) control group. All three groups of participants will receive standard of care ACL rehabilitation

Objectives: Primary Objective: To determine whether NMES+ECC will maximize quadriceps strength symmetry better than NMES placebo + ECC placebo at 9 months and 18 months post- anterior cruciate ligament reconstruction (ACLR).

Secondary Objective 1: To determine whether NMES+ECC will limit negative changes in cartilage health, defined as decreased proteoglycan cartilage density measured with T1rho or increased cartilage matrix degradation measured with T2 mapping in relation to the contralateral side, at 18 months following ACLR.

Secondary Objective 2: To determine whether NMES+ECC will maximize biomechanical symmetry and physical function better than NMES placebo + ECC placebo at 9 months and 18 months post-ACLR.

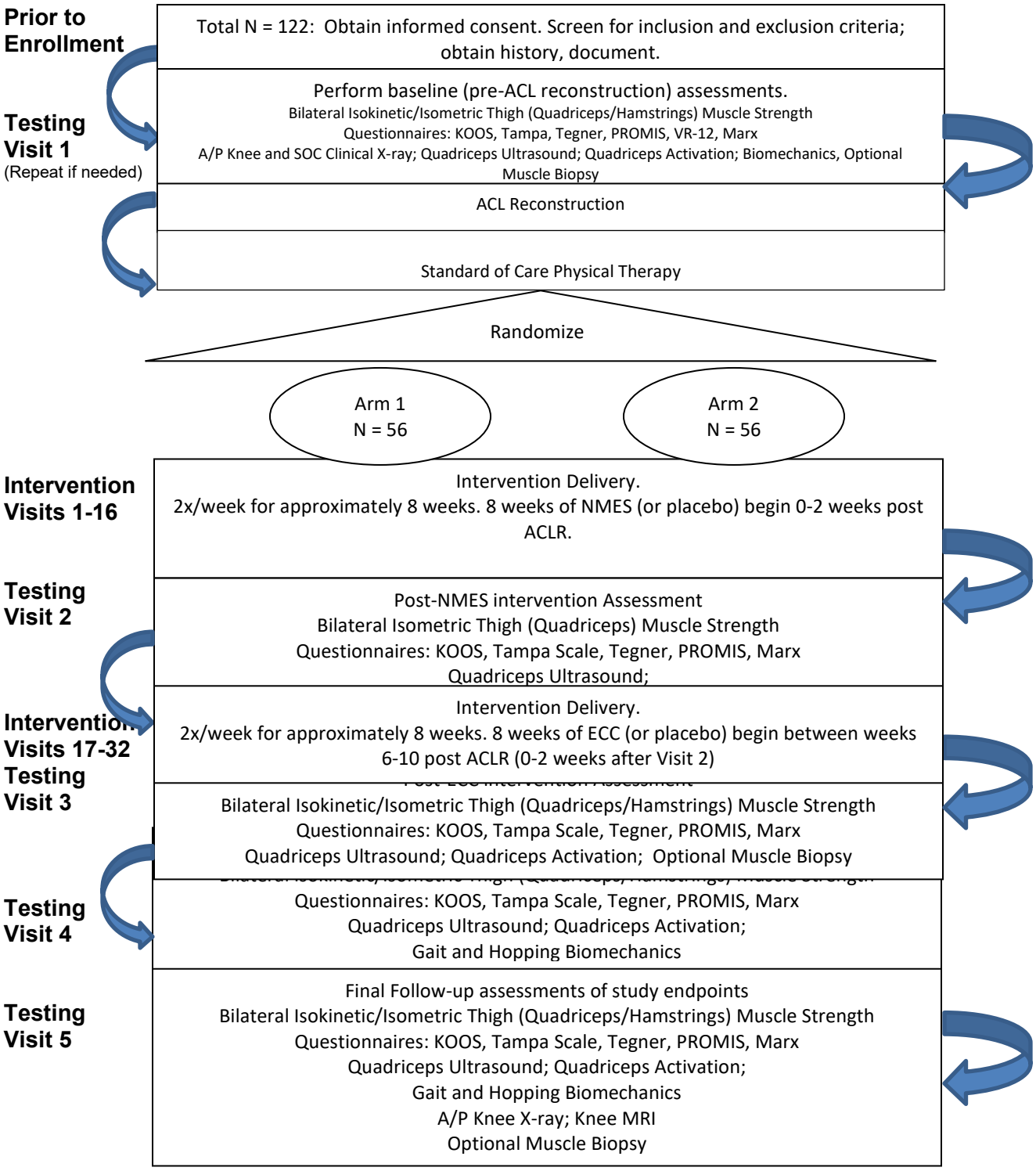
Exploratory Objective: We will compare the SOC control group to the placebo group to assess the ability of reducing the intensity of NMES and ECC to achieve a “true” placebo.

Endpoints: Primary Endpoints: change from baseline to 9 months and to 18 months post-ACLR in isokinetic quadriceps strength symmetry index

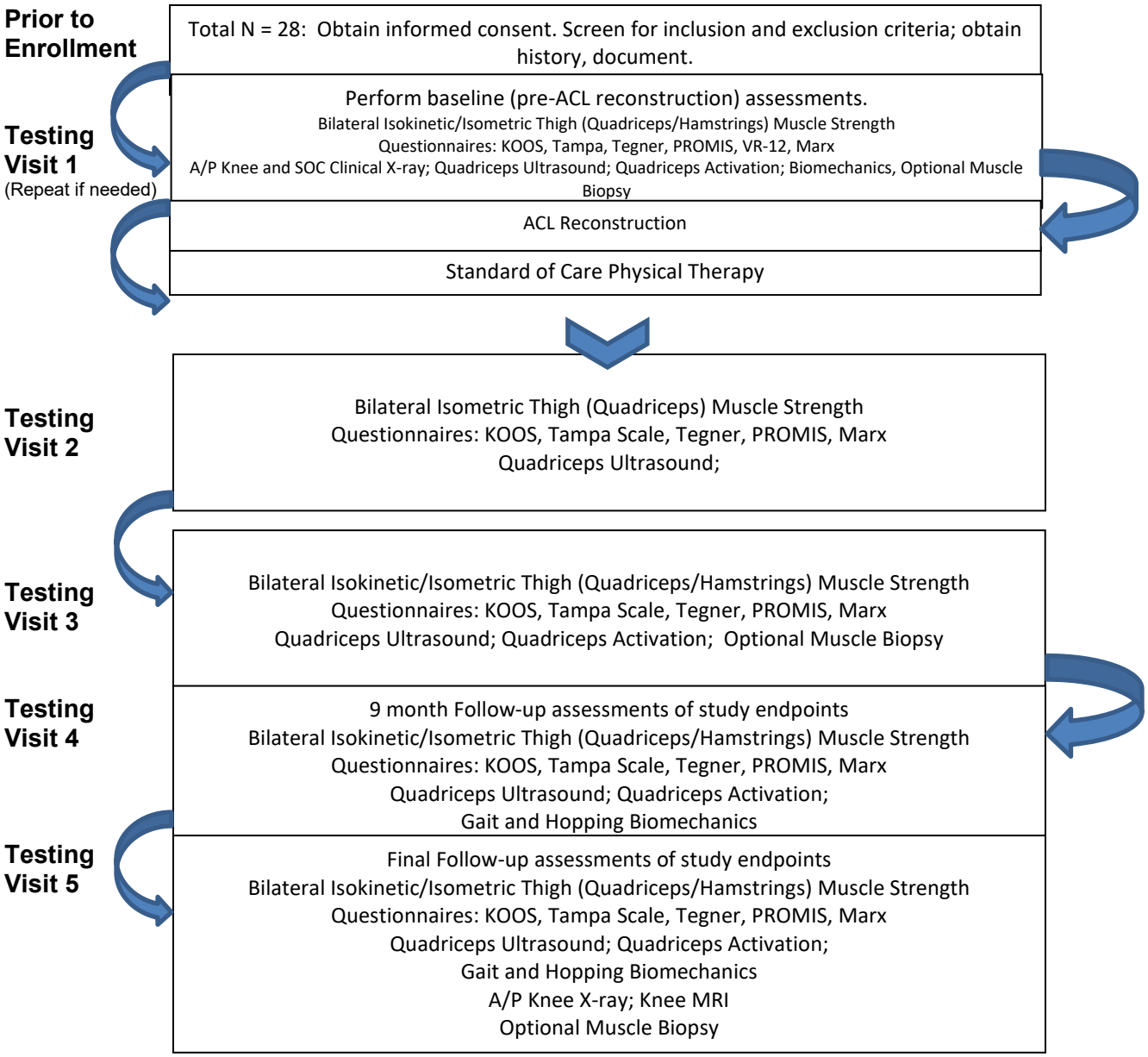
Secondary Endpoints: 1) T1 rho and T2 relaxation time symmetry

	scores for knee joint cartilage in the deep and superficial layers, 2) knee flexion angle/moment symmetry, 3) score on the Knee Injury and Osteoarthritis Outcome Score, 4) hop distance symmetry at 18 months Exploratory Endpoints: Change from baseline to 9 months and to 18 months post-ACLR in isokinetic quadriceps strength symmetry index.
Study Population:	150 patients who have sustained a unilateral ACL injury between the ages of 14-45 years will be consented. Of those, 122 participants will be randomized to one of the two treatment groups; 10 of those participants will be removed from analysis due to the COVID-19 pandemic. 28 participants will be enrolled (not randomized) to the SOC Control group. III
Phase:	
Description of Sites/Facilities Enrolling Participants:	The University of Michigan
Description of Study Interventions:	This study combines the following interventions into two randomized treatment arms: NMES: defined as delivery of high-intensity neuromuscular electrical stimulation (NMES) beginning in weeks 0-2 post-ACLR and continuing for 8 weeks. ECC: defined as delivery of eccentric exercise (ECC) at 70-90% of the 1 RM (repetition maximum) beginning between the 6^{th}-10^{th} week post-operative until the 14-18^{th} week post-operative. NMES placebo: defined as delivery of low-intensity neuromuscular electrical stimulation (NMES) beginning in weeks 0-2 post-ACLR and continuing for 8 weeks. ECC placebo: defined as delivery of eccentric exercise (ECC) at 15% of the 1 RM (repetition maximum) beginning between the 6^{th}-10thth week post-operative until the 14-18^{th} week post-operative. The treatment groups to which participants will be randomized are: (1) NMES + ECC (2) NMES placebo + ECC placebo There will also be a SOC Control group in which participants receive no study-related treatment or placebo outside of their standard of care physical therapy.
Study Duration:	5 years
Participant Duration:	Approximately 20 months from the time the consent form is signed
Estimated Time to Complete Enrollment:	5 years

1.2 SCHEMA – TREATMENT GROUPS



1.3 SCHEMA – SOC CONTROL GROUP



1.4 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Pre-Screening (Phone and Record Review)	Testing Visit 1 Enrollment/Baseline	Repeat Testing Visit 1[d]	ACL Surgery	Intervention or SOC PT Visits 1-16[f]	Testing Visit 2 Post-NMES Intervention	Intervention or SOC PT Visits 17-32[g]	Testing Visit 3 Post-ECC Intervention	Testing Visit 4 9 months post-ACLR[c]	12 months post-ACLR	15 months post-ACLR	Testing Visit 5 18 months post-ACLR[c]
Days on Study (relative to ACLR)	-365	-30	-30	0	1-70	N/a[h]	56-126	N/a[h]	270	365	455	540
Visit Window (days)- Treatment Group	0	0	0	0	0	+7	0	+7	+ 30	±14	±14	±30
Informed consent/assent	X											
Inclusion/Exclusion	X	X	X	X								
ACL Reconstruction Surgery				X								
Demographics		X										
Medical history		X	X									
Clinical Knee X-ray Record Review		X										
Pregnancy test[a]		X	X									
Randomization[b]					X							
Concomitant medication review		X----------										----------X
Administer NMES or placebo[e]					X							
Administer ECC or placebo[e]							X					
Standard of Care Rehabilitation monitoring					X----------				----------X			
Height & Weight		X	X						X			X
Assessment of Adverse Events		X----------										----------X
Knee X-ray		X	X									X
Knee MRI												X
Quadriceps/Knee Ultrasound		X	X			X		X	X			X
Thigh Muscle Strength Assessment		X	X			X		X	X			X
Quadriceps Activation Assessment		X	X			X		X	X			X
Physical Activity Monitoring										X	X	
Questionnaires		X	X			X		X	X			X
Gait Biomechanics		X	X						X			X
Clinical Hop Testing									X			X
Hopping Biomechanics									X			X
Optional Muscle Biopsy		X	X					X				X

[a] Urine pregnancy test for women of child bearing potential
[b] Randomization will occur after ACL reconstruction and implemented at first intervention visit. SOC control group will not be randomized.
[c] Testing procedures will be scheduled within a 30 day window before or after 9 and 18 month testing date
[d] Completed if Testing Visit 1 falls out of window
[e] Does not apply to SOC control group
[f] Intervention or SOC PT visits 1-16 can take place beginning on post-op day 1 but may begin up to 2 weeks after surgery.
[g] Intervention or SOC PT visits 17-32 can take place beginning on day of Testing Visit 2 but may begin up to 2 weeks following that visit.
[h] Testing Visit 2 and 3 will take place within 7 days of the last intervention or SOC PT visit.

2 INTRODUCTION

2.1 STUDY RATIONALE

Anterior cruciate ligament (ACL) injuries are both common and costly, with approximately 300,000 tears occurring annually, at a lifetime cost approaching $8 billion dollars. While ACL reconstruction (ACLR) successfully restores knee laxity to within normal limits, current ACL rehabilitation is, in part, inadequate, as it fails to restore quadriceps strength. These clinically significant reductions in quadriceps strength limit the patients' ability to engage in physical activity, lead to a decreased quality of life, and are an independent predictor of the development of post-traumatic osteoarthritis (OA) that plagues over 50% of limbs within 10-20 years of ACLR.

Current ACL rehabilitation paradigms are inadequate, as they do not effectively target the mechanisms, muscle inhibition and altered muscle morphology, that lead to muscle weakness after ACL injury and ACLR. ACL injury results in a reflexive, neurological shutdown of the quadriceps muscle (i.e. muscle inhibition), while simultaneously changes in protein and genetic expression lead to altered muscle morphology (i.e. atrophy). Further, as muscle inhibition prevents full voluntary quadriceps activation, it can perpetuate changes in muscle morphology. Failure to treat both causes of quadriceps weakness is a critical problem with ACL rehabilitation as doing so prevents full muscle strength from being restored.

2.2 BACKGROUND

Anterior cruciate ligament (ACL) injury is a significant US public health problem. ACL tears are common, particularly in younger, physically active individuals, with approximately 300,000 tears occurring each year in the US[1,2] at an annual lifetime cost of $7. 6 billion when treated surgically. While in the short-to-intermediate term, consequences range from reduced functional and academic performance due to time loss from school or work; the long-term consequences are much greater, with post-traumatic osteoarthritis (OA) affecting an estimated 50% of reconstructed limbs[3] between 10-20 years after injury[4]. Given the peak incidence for an ACL rupture is 16 years of age[5], a significant number of young to middle age adults will be effected by premature joint degeneration for which no cure exists. Innovative treatment options capable of reducing the incidence of post-traumatic OA following ACL injury and ACL reconstruction (ACLR) are desperately needed. If the risk for OA following ACLR could be halved, the cost to society could be reduced by $1. 1 billion dollars annually. [3]

Quadriceps weakness persists after ACL injury and reconstruction. Quadriceps weakness (e. g. , deficits or declines in peak torque during isokinetic testing) is a common consequence of ACL injury and ACLR. Despite restoration of anterior-posterior knee laxity through surgery and aggressive rehabilitation protocols designed to re-establish strength, quadriceps weakness remains. [6-8] Achieving quadriceps strength in the injured/reconstructed limb that is 90% of the uninjured side is considered important for realizing a successful outcome and reducing re-injury risk after ACLR[9]; however patients are often released from formal care and cleared to return to full activity at values much lower than this criterion. [12-14] Our own work illustrates this point, with quadriceps strength in the ACLR knee only averaging 71. 2% of the uninjured side at the time patients were cleared by their physician to return to full

activity. [10] Only 20% of our patients (13/66) were able to achieve the recommended 90% quadriceps strength symmetry criterion between limbs at time of clearance. While quadriceps strength does seem to improve over time after return to activity, deficits of 10-20% can persist at two years and beyond. [11] Furthermore, quadriceps strength early after ACLR (4 months post-surgery) is significantly associated with joint space width narrowing at 4 years after reconstruction, suggesting restoring early quadriceps strength deficits may be significant to combatting post-traumatic OA. [17]

Quadriceps weakness is a risk factor for post-traumatic OA after ACL reconstruction. Degeneration of articular cartilage along with a breakdown of subchondral bone, otherwise known as OA, is a common condition affecting more than 26 million Americans. [12] OA that results secondary to traumatic joint injury is referred to as post-traumatic OA and accounts for ~5. 6 million cases of OA annually. [13] Quadriceps strength is a risk factor for idiopathic OA[14] and more recently has been linked with post-traumatic OA. Work by Keays et al. [15] demonstrated that six years after ACLR weak quadriceps muscles and low quadriceps to hamstring strength ratios data were able to discriminate between participants without OA (Kellgren and Lawrence (K-L) grades 0 and 1) and those with OA (K-L grade ≥2). Oiested and colleagues[16] have also found a relationship between quadriceps strength loss that occurred 2 to 15 years following ACLR and knee OA (K-L grade ≥2).

Using a prospective study design, Tourville et al[17] noted that quadriceps strength loss measured within a few months following ACL injury was associated with joint space width narrowing by four years after ACLR. Given that quadriceps strength is a modifiable risk factor, targeting it with evidence-based interventions is a critical next step in combatting the post-traumatic OA resulting secondary to ACL injury (Aim 1). Further, identifying the magnitude of quadriceps weakness that predicts OA onset is unknown and also warrants study (Aim 2).

Mechanisms for quadriceps weakness after ACL injury and ACLR. Muscle inhibition and muscle atrophy are considered to be the primary mechanisms leading to quadriceps weakness. Together, inhibition and atrophy are able to account for > 60% of the variance in quadriceps strength loss associated with ACL injury. [18] Muscle inhibition is a reflexive shutdown of the quadriceps muscles that results from altered neural signaling[19,20], leads to a diminished ability to voluntarily contract the muscle, and overtime can result in muscle atrophy. Early after ACLR, the magnitude of inhibition is large (~25%) and can prevent effective strengthening, thus hindering rehabilitation. [21] In fact, despite resistance training in patients with knee injuries, quadriceps strength can remain unchanged or decline, an effect attributed to muscle inhibition. [21] Evidence also suggests that substantial inhibition (15%) can persist for 2 years after ACLR. [22]

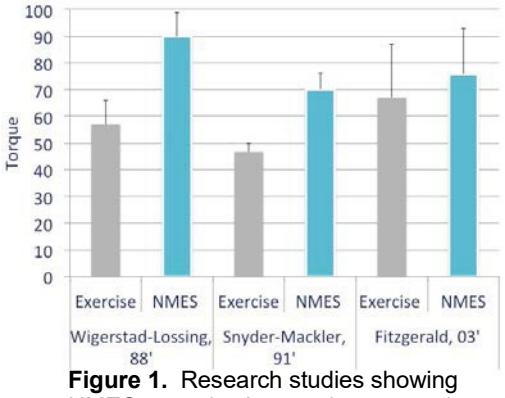


Figure 1. Research studies showing NMES+exercise is superior to exercise alone at improving quadriceps strength in patients with ligament/ACL injuries

Muscle atrophy, or the loss of quadriceps muscle mass, is the other leading mechanism proposed to contribute to quadriceps weakness. Muscle atrophy is a predictor of the strength deficits. [23] It is thought that adaptations in protein and genetic expression after ACLR can have a significant impact on muscle size and function. Evidence in support of this idea comes from studies

measuring molecular effects of muscle hypertrophic/atrophic pathways. These studies have found that hypertrophic pathways are depressed (insulin-like growth factor 1) and circulating biomarkers of muscle atrophy (myostatin and transforming growth factor-β) are significantly elevated after ACLR. [24] Work from our labs[25] and others[26] also indicates that quadriceps cross sectional area (CSA) is significantly reduced in the ACLR limb compared to the contralateral limb, and quadriceps muscle atrophy is a predictor of the strength deficits. Further, data from the NIH-funded Osteoarthritis Initiative has found that high quadriceps CSA is associated with greater quadriceps strength and less degenerative changes at the knee after ACLR. [27]

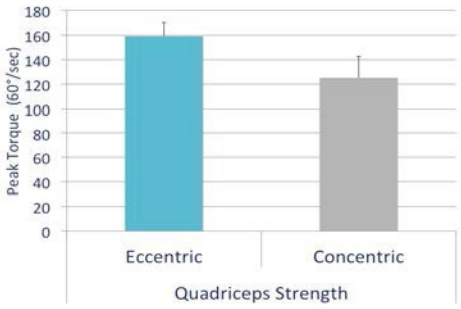


Figure 2. Data illustrating that eccentric exercise is superior to concentric exercise at improving quadriceps strength following ACLR. Data from Gerber et al (2007).

Neuromuscular electrical stimulation (NMES) and Eccentric Exercise (ECC) can restore quadriceps strength. NMES, when applied appropriately, can directly depolarize motor axons in the treated muscle leading to involuntary muscle contraction. [28] Therefore, use of NMES is an attractive therapy to override muscle inhibition as it can activate the quadriceps exogenously. Previous work has shown that NMES is superior to exercise alone at improving muscle strength (Figure 1). [29-31]

To target muscle atrophy, emerging evidence suggests that the higher mechanical stress achieved via ECC is critical to initiate strain-sensing molecules that mediate tissue growth. [8] Specifically, when a sarcomere is elongated by mechanical force, titin, the giant elastic protein that spans the entire length of a sarcomere, unfolds at the M- band, and removes its auto-inhibition. [8,32] This positive strain of the sarcomere allows phosphorylation of titin-kinase that begins a cascade of signaling events that ultimately promote muscle growth. [8,32,33] Given the unique capability of ECC to mechanically promote protein synthesis, the incorporation of ECC into ACLR rehabilitation after muscle inhibition is minimized via NMES is likely an effective therapeutic prescription to promote the optimal recovery of quadriceps strength. Indeed, ECC has been shown to improve muscle strength[34,35] and reduce atrophy[34,36] following ACLR in small research studies (Fig. 2). Hence, it is our contention that applying these therapies in succession will maximize muscle strength gains after ACLR.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The majority of risks associated with this study are the same as those for medical treatment of ACL injury, surgical reconstruction of the ACL, physical therapy associated with ACL injury/ACL reconstruction, and the activities that the typical ACL patient took part in prior to their injury. Risks for this study are minimal, and major issues would be uncommon. For this study, the risks

are as follows:

1. Any risk specific to physical therapy or the eccentric exercise intervention (these risks would be similar) including knee effusion/hemarthrosis, muscle pain, rupture of graft tissue, or injured muscle, ligament or tendons.
2. Any risk specific to the neuromuscular electrical stimulation (NMES) intervention, including muscle pain, rupture of graft tissue, fracture, or injury to muscle, ligaments or tendons. Discomfort during application of the electrical stimuli is common and to be expected during the NMES intervention.
3. Any risk specific to the biomechanical testing that requires the subjects to hop/land including knee effusion/hemarthrosis, muscle pain, rupture of graft tissue, or injured muscle, ligament, or tendons.
4. Any risk specific to musculoskeletal imagining (i.e., X-ray and MRI) including discomfort while lying in the scanner (MRI), exposure to radiation (X-ray), and exposure to loud noises (MRI). All appropriate precautions that occur with standard clinical imaging will take place, (i.e. use of lead vests for X-ray, ear plugs or equivalent for MRI) and, thus, the risk is mitigated.
5. Any risk related to topical application of tape or electrical stimulation pads, which could occur secondary to all study procedures, such rash or itching.
6. Any risk specific to the ACL reconstruction, including infection, cyclops lesion, deep vein thrombosis, damage to nerves/vessels, cellulitis, hemarthrosis requiring arthrocentesis, septic arthritis, pain not controlled with analgesics, as well as risks associated with anesthesia (e. g. , nausea and vomiting).
7. Any risk related to the muscle biopsy including discomfort, infection, bruising, and/or blood clot.
8. Any risk specific to loss of confidentiality.

2.3.2 KNOWN POTENTIAL BENEFITS

The study participants and those affected by ACL injuries at large could benefit significantly from this study. This study will help to identify interventions that will improve/maximize quadriceps strength after ACLR. Research has shown that patients with stronger quadriceps muscles have improved functional performance, biomechanical symmetry and are less likely to re-injure their knees. Further, quadriceps weakness has been linked to the post-traumatic OA that affects upwards of 50% limbs after ACLR. Therefore, improving muscle strength has the potential to lessen the risk of OA, a hypothesis the proposed study will directly evaluate. Based on our pilot data, we would expect participants randomized to receive NMES+ECC would see greater improvements in quadriceps strength than patients in NMES placebo + ECC placebo and thus may directly benefit from participation.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Risk 1: Any risk specific to physical therapy or the eccentric exercise intervention (these risks would be similar) including knee effusion/hemarthrosis, muscle pain, rupture of graft tissue, or injured muscle, ligament, or tendons.
Protection/Mitigation Risk 1: We will only initiate the eccentric exercise intervention once participants have good quadriceps control. Further, no participant will begin eccentric exercise until their physician has cleared them to start the intervention. The BLAST leg press device (the device used to complete eccentric exercise) has multiple safeguards built in that allow the participants or investigator to stop the activity at any time. Participants will be instructed to tell

study staff if the feel pain in their knee joint and if this were to occur, the intervention would be stopped. If an injury were to occur, the research laboratory is approximately 100ft from the MedSport clinic where athletic trainers, doctors, nurses, and physical therapists are present.

Risk 2: Any risk specific to the neuromuscular electrical stimulation (NMES) intervention, including muscle pain, rupture of graft tissue, fracture, or injury to muscle, ligaments, or tendons. Discomfort during application of the electrical stimuli is common and to be expected during the NMES intervention.
Protection/Mitigation of Risk 2: Injury to any musculoskeletal tissue would be rare, however all participants will be instructed to alert study staff if they feel pain in their knee joint. If joint pain were felt, the intervention would be stopped. There is no way to prevent the discomfort in the muscle on the skin when the NMES intervention is applied. Large electrodes will be used to deliver the stimuli which disperse the current and minimize discomfort.

Risk 3: Any risk specific to the biomechanical testing that requires the subjects to hop/land including knee effusion/hemarthrosis, muscle pain, rupture of graft tissue, or injured muscle, ligament or tendons.
Protection/Mitigation of Risk 3: In order to minimize this risk, no ACLR participant will be allowed to undergo biomechanical testing unless cleared by their physician to do so. If injury were to occur during test, the study team would manage the injury and will then refer the participant to Drs. Wojtys or Bedi for follow-up. It is important to note that volunteers will not be performing the landing procedures until 9 months after ACL reconstruction (i. e., when the volunteer would typically be released to return to sport or activity).

Risk 4: Any risk specific to musculoskeletal imagining (i.e. , X-ray and MRI) including discomfort while lying in the scanner (MRI), exposure to radiation (X-ray), and exposure to loud noises (MRI).
Protection/Mitigation of Risk 4: All appropriate precautions that occur with standard clinical imaging will take place (e. g. , use of lead vests for X-ray, ear plugs or equivalent for MRI).

Risk 5: Any risk related to topical application of tape or electrical stimulation pads, which could occur secondary to all study procedures, such rash or itching.
Protection/Mitigation of Risk 5: If irritation secondary to application of tape or electrical stimulation electrodes would occur, we would treat with topical hydrocortisone. If rash/irritation did not improve (or appeared to be something more severe), we would refer to Drs. Wojtys or Bedi for evaluation and treatment.

Risk 6: Any risk specific to the ACL reconstruction, including infection, cyclops lesion, deep vein thrombosis, damage to nerves/vessels, cellulitis, hemarthrosis requiring arthrocentesis, septic arthritis, pain not controlled with analgesics, as well as risks associated with anesthesia (e. g. , nausea and vomiting).
Protection/Mitigation of Risk 6: This risk/event will be captured as the ACLR will occur after participants
have signed informed consent/assent. If any of these were to occur or we noticed signs or symptoms of any of these conditions, we would refer to Drs. Wojtys or Bedi for immediate follow-up.

Risk 7: Any risk related to the muscle biopsy including discomfort, infection, bruising, and/or blood clot.
Protection/Mitigation of Risk 7: A sterile environment will be used to collect biopsy samples in

order to mitigate risk of infection. Participants will be sent home with instructions to care for their incision/wound after the biopsy. A study team member will follow-up with each participant opting to participate in the biopsy the day after the procedure.

Risk 8: Any risk specific to loss of confidentiality.
Protection/Mitigation of Risk 7: To ensure confidentiality of subject data, only members of the study team will have access to the link between personal identifiers and a study ID number. Information about study participation may also be noted in the medical record to ensure continuity of care and proper billing. This information is protected by HIPAA and only accessible to authorized faculty and staff of the University of Michigan.

Questionnaires and data files will be labeled with subject ID numbers. An encrypted file containing personnel identifiers will be stored on a secure server, and will only be accessible to the members of the study team. All electronic files will be stored on secure, encrypted servers within the University of Michigan system. Informed consents/assents, completed questionnaires, and other paper study source documents will be kept in locked files and stored in a room where key access is required.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	HYPOTHESES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary			
To determine if NMES+ECC will maximize quadriceps strength symmetry better than the NMES placebo + ECC placebo at 9 months and 18 months post-ACL reconstruction (ACLR).	Participants receiving NMES+ECC will have greater quadriceps strength symmetry at 9 and 18 months post-ACLR when compared with the other 2 study arms.	Co-primary endpoints: • Change from baseline to 9 months post-ACLR in isokinetic quadriceps strength symmetry index. Change from baseline to 18 months post-ACLR in isokinetic quadriceps strength symmetry index.	This trial is designed to evaluate whether NMES and ECC can improve quadriceps strength. Thus, quadriceps strength at 9 months (when participants return to activity) and 18 months (longer-term assessment) are the primary endpoints for the trial.
Secondary			
Secondary Objective 1: To determine if NMES+ECC will limit negative changes in cartilage health, defined as decreased proteoglycan cartilage density measured with T1rho or increased cartilage matrix degradation measured with T2 mapping in relation to the contralateral side, at 18 months following ACLR.	Participants in the NMES+ECC group will have significantly lower T1rho and T2 values in the injured knee compared to the uninjured knee, indicating greater proteoglycan density and less cartilage matrix degradation. Further, we would expect larger (injured/uninjured) ratios for both T2 and T1rho in the NMES placebo + ECC placebo group when compared to the NMES + ECC group. Ratios greater than 1. 0 would indicate less proteoglycan density (T1rho) and greater matrix degradation (T2).	• T1 relaxation ratio at 18 months post-ACL • T2 relaxation ratio at 18 months post-ACL	T1 and T2 relaxation times have been able to show early change in cartilage after ACLR and are sensitive to change in cartilage when the gold standard X-ray does not yet reveal change.

OBJECTIVES	HYPOTHESES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary Objective 2: To determine if NMES+ECC will maximize biomechanical symmetry and physical function better than the placebos at 9 months and 18 months post-ACL reconstruction (ACLR).	Participants receiving NMES + ECC will have greater knee flexion angle and moment symmetry, higher scores on the KOOS, and larger distances hopped on the crossover hop and single-legged hop at 9 and 18 months when compared with other 3 study arms.	• Knee joint flexion angle and moment at 9 months post ACLR • Knee joint flexion angle and moment at 18 months post ACLR • Score on KOOS at 9 months post-ACLR • Score on KOOS at 18 months post-ACLR • Distance hopped during cross-over hop and single-legged hop for distance at 9 months post-ACLR • Distance hopped during cross-over hop and single-legged hop for distance at 18 months post-ACLR	Knee flexion angle and moment are sensitive to changes in quadriceps strength. KOOS is a validated and reliable scale to assess patient-perceived function after ACLR. Distance hopped during the cross-over and single-legged hop for distance are standard clinical hop tests used after ACLR.
Exploratory Objective: To assess the ability of reducing the intensity of NMES and ECC to achieve a “true” placebo by comparing the NMES placebo + ECC placebo to the SOC Control group.	Participants receiving NMES placebo + ECC placebo will have similar change to those in the SOC control group from baseline to 9 months and to 18 months post-ACLR in isokinetic quadriceps strength symmetry index.	• Change in isokinetic quadriceps strength symmetry index from baseline to 9 months post-ACLR • Change in isokinetic quadriceps strength symmetry index from baseline to 18 months post ACLR	This trial is designed to evaluate whether NMES and ECC can improve quadriceps strength. Thus, quadriceps strength at 9 months (when participants return to activity) and 18 months (longer-term assessment).

4 STUDY DESIGN

4.1 OVERALL DESIGN

In order to determine whether high intensity NMES +ECC can improve quadriceps strength better than the current standard of care (NMES placebo + ECC placebo) (primary objective), we propose a comparative effectiveness, single-center, randomized, double-blind, parallel group, placebo-controlled trial of NMES and ECC in patients with ACLR. One-hundred twelve participants scheduled to undergo an ACLR will be randomized into one of two study arms: 1) NMES + ECC ; or 2) NMES placebo + ECC placebo. All participants regardless of assignment will undergo standard of care ACL rehabilitation that focuses on minimizing effusion, restoring range of motion, and improving muscle strength (standard concentric exercises). The study intervention duration is 14-18 weeks, with NMES and NMES placebo beginning within 2 weeks of the ACLR and is provided twice/week for 8 weeks and ECC and ECC placebo beginning 6-10 weeks post-ACL surgery and is provided twice/week for 8 weeks.

Additionally, 28 patients will be enrolled into a control group where no study interventions are provided and participants will be followed and tested as they undergo physical therapy as prescribed by their treating physician. These patients are eligible for the study but refuse to participate in the randomized controlled trial however, they are willing to participate in the study procedures to provide efficacy measures.

We will randomize participants to treatment groups using minimization, a method of unbiased allocation ensuring balance across stratification factors: meniscal injury and treatment, articular cartilage injury (described below), sex and graft type. (See section 6. 3 for details on the randomization method using the minimization method.)

Our primary outcomes will be examined at three study time points: 1) pre-ACLR (within 2 weeks prior), 2) 9 months post-ACLR (±2 weeks), and 3) 18 months post-ACLR (±2 weeks). MRI and X-ray measures will be collected at 18 months post-ACLR, biomechanical measures at 9 and 18 months post-ACLR, hop testing at 9 and 18 months post-ACLR and questionnaires at all 3 time points. Additionally, we will examine change in quadriceps muscle strength and muscle volume from baseline to post-NMES intervention (testing visit 2) and post-ECC intervention (testing visit 3) in an attempt to understand the effects of the individual components of the combined NMES+ECC intervention.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

A double-blind, parallel group, placebo-controlled randomized clinical trial assessing the superiority of NMES and ECC will be utilized. This design was chosen to assess whether our multimodal intervention (NMES + ECC) is superior to the placebo arm. An independent group of participants will receive only the standard of care rehabilitation therapy to assess the ability of reducing the intensity of NMES and ECC to achieve a “true” placebo. This additional non-randomized group strengthens the study by confirming the sham NMES & ECC procedures can serve as “true” placebos.

4.3 JUSTIFICATION FOR DOSE

Neuromuscular Electrical Stimulation: Patients randomized into the NMES + ECC arm will receive NMES 2x/week for 8 weeks following ACLR, beginning 0-2 weeks post-operative. For the first two weeks, NMES will be delivered in the clinic by the patients' physical therapist. The treatment intensity for the first two weeks will be set to the maximum tolerated by the patient, but all other stimulation parameters will remain the same as outlined for the remaining 6 weeks of NMES intervention. For the last 7 weeks of NMES treatment, patients will be positioned in a dynamometer with their hips flexed to 90° and ACL knee flexed to 60°. Stimulating electrodes will be placed over the vastus lateralis proximally and the vastus medialis distally. An electrical stimulator (Vectra Neo, DJO Global, Vista, CA) will be set to deliver a 2500 Hz alternating current, modulated at 75 bursts/s, with a ramp-up time of 2-seconds, followed by a 50-second rest period. Fifteen isometric actions lasting 10 seconds each will be elicited during each session. The treatment intensity will be set at a minimum intensity (higher if tolerated) to achieve a maximum voluntary isometric quadriceps contraction (MVIC) in the injured limb that is equivalent to an MVIC of 40% of the contralateral side. **Justification for Dose:** Dose response curves from previous work show that in order to recover ~70% of isometric quadriceps strength, NMES needs to be delivered at an intensity that will elicit 40% of the contralateral MVIC.

NMES placebo. Participants randomized to the NMES placebo + ECC placebo arm will receive an NMES placebo intervention 2x/week for 8 weeks following ACLR, beginning 0-2 weeks post-operative (to mimic the timing associated with the NMES intervention). The NMES placebo will utilize the same equipment and follow the same protocol as in the actual NMES intervention, with the exception that the intensity of the current will be set lower in the last 6 weeks of NMES intervention. The treatment intensity will be set at an intensity to achieve a MVIC of 15% of the injured side. In the first 2 weeks of NMES treatment will be delivered at max tolerated by the patient as is done for the NMES active arm. **Justification for Dose:** In our pilot work, electrical stimulation of this intensity does not result in improvements in muscle strength; as such it is a fitting placebo for the current trial.

High-Intensity Eccentric Exercise (ECC): Participants randomized into the NMES + ECC arm will receive ECC 2x/week for 8 weeks, beginning between 6 - 10 weeks post-ACLR when adequate quadriceps control is achieved, determined by the participant's physician. To perform the ECC, patients will be positioned in a BLAST!™Leg Press (BioLogic Engineering Inc.) with their ACLR knee range of motion limited to 20 to 60° of knee flexion. Patients will perform warm-up trials consisting of 10 concentric and eccentric quadriceps actions with the ACLR limb. Following the warm-up trial, each participant will perform 10 isokinetic eccentric quadriceps actions with the ACLR limb. In total for each session, patients will complete 4 sets of 10 leg press actions with 2-minute rests between each set. During the eccentric actions, patients will train at an eccentric intensity equal to 70-90% of their one-repetition maximum (this will be recorded at the beginning of each week), and a concentric intensity equal to 40% of their one repetition maximum. **Justification for Dose**: In our pilot work[35,37], using an eccentric intensity identical to that described above, patients, on average, realized a 90% improvement in quadriceps strength over 6 weeks (which is shorter than the 8 weeks implemented in this study).

ECC placebo: Participants randomized to the NMES placebo + ECC placebo arm will receive an ECC placebo intervention 2x/week for 8 weeks, beginning at 6 - 10 weeks following ACLR. ECC placebo will utilize the same equipment and follow the same protocol as in the actual eccentric intervention, with the exception that the eccentric training intensity the patients will use

is lower. Rather than training at 70-90% of their MVIC, patients will train at 40%. This intensity is too low to generate increases in muscle strength. **Justification for Dose:** In our pilot work, eccentric exercise of this intensity does not result in improvements in muscle strength; as such, it is a fitting placebo for the current trial.

Standard of Care: All participants, both those receiving study intervention (i.e. NMES + ECC or NMES placebo + ECC placebo) or in the SOC control group, will receive standard of care physical therapy post-ACLR. The SOC control group will provide confidence that the intensity of NMES and ECC in the placebo group is sufficiently low.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit (month 18) shown in the Schedule of Activities (SoA), Section 1. 4.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the last study participant.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this trial, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent/assent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 14-45 years
4. Have an ACL tear diagnosed by a physician
5. Scheduled to undergo ACL reconstruction with any type of autograft (i.e. , a graft of tissue from one point to another of the same individual's body)
6. Patient receiving physical therapy at a MedSport facility

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria below will be excluded from participation in this study.

Exclusion Criteria at time of screening:

1. Previous major surgery to either knee
2. Patients who experience a knee dislocation (prior to or in conjunction with the ACL tear)
3. Female volunteers who are pregnant or planning to become pregnant during the study period
4. Patients who cannot undergo an MRI (e. g. metal in body)

Exclusion Criteria at time of randomization:

1. Bony fracture accompanying the ACL injury
2. Multi-ligament reconstruction at the time of the ACLR

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

- **Patients:** Approximately 150 patients (10 of which will be removed from analysis due to the COVID-19 pandemic) will be recruited without regard to gender, race or ethnicity. Potential participants must be between 14-45 years of age.
- **Anticipated accrual rate:** 30 patients per year
- **Anticipated number of sites:** 1
- **Source of participants:** Our primary source of participants will come from Michigan Medicine (in particular MedSport).
- **Recruitment venues:** Michigan Medicine, Local Physical Therapy Clinics
- **How potential participants will be identified and approached:** To identify potential participants, we will examine electronic medical records from MedSport, which is part of Michigan Medicine, for patients who have been diagnosed with an ACL tear. Our study coordinator will examine the record for any exclusion criteria that may also be listed. Once a potential participant is identified, we will approach the patient at a scheduled appointment with their orthopedic surgeon. At this appointment, the coordinator will provide information about the study, gauge patient interest in participating, and confirm eligibility. If the potential participant expresses interest in participating we will follow-up with a phone call or email within 3 days to schedule a pre-operative/baseline testing session.
- **Recruitment strategies:** Direct recruitment through Michigan Medicine, Flyers in local physical therapy clinics in Ann Arbor, Flyers around University of Michigan campus, clinicaltrials.gov
- **Subject retention:** In regard to subject retention, every effort will be made by the study team to achieve optimal retention of participants. We deliver study interventions at a time that corresponds with participants' regularly scheduled physical therapy visits (i.e., they are already in our clinic for treatment and we simply schedule their intervention to coincide with their existing appointment). This has led to 100% subject retention in our pilot work. Retaining subjects at the 9-month data collection time point should not be problematic as patients are still reporting to the clinic at that time (or have just completed therapy and thus are easily accessible to study staff). We will schedule the 9-month appointment time at the time of their last intervention session and follow-up with an email and postcard reminder 2 weeks prior to that appointment. Additionally, we will follow-up with an email 2 days prior to the scheduled appointment to confirm attendance. Similar procedures led to 100% retention in our pilot trial. For the 18-month time point, phone and email contact will be made with subjects or their parents or guardians (if applicable) one month prior to our goal date for testing. When participants are reached, we will schedule a time for an MRI and a separate time to come to MedSport for X-rays, as well as strength/biomechanical testing. Reminders will be emailed and mailed to participants

10 days prior to their scheduled test dates and emailed again at 2 days prior to the test date. In our prior trial, we approached select patients in a similar manner at one-year post-ACLR and 95% agreed to return for follow-up.

- **Subject Incentives:** Participants will receive $25/week for undergoing study interventions (16 weeks x $25 = $400) (groups randomized to receive interventions only). All Participants (randomized and SOC control) will receive $10/hour for each hour of study testing in which they participate. If the participant is a minor, the minor will receive the incentive.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The following study interventions will be administered in addition to standard of care ACL rehabilitation as prescribed by the operating physician. Note that the SOC control group will not receive study interventions; they will only receive standard of care ACL rehabilitation.

Neuromuscular electrical stimulation (NMES): This study intervention will be delivered using the Vectra Neo (DJO Global, Vista, CA)

Eccentric Exercise: Eccentric exercise will be delivered using a BLAST!™ Leg Press System (BioLogic Engineering).

6.1.2 DOSING AND ADMINISTRATION

Neuromuscular Electrical Stimulation: Participants randomized into the NMES + ECC will receive NMES 2x/week for 8 weeks following ACLR, beginning 0-2 weeks post-operative. For the first two weeks, NMES will be delivered in the clinic by the participants' physical therapist. The treatment intensity for the first two weeks will be set to the maximum tolerated by the participant, but all other stimulation parameters will remain as outlined below for the remaining 6 weeks. For the last 6 weeks of NMES treatment, participants will be positioned in a dynamometer with their hips flexed to 90° and ACL knee flexed to 60°. Stimulating electrodes will be placed over the vastus lateralis proximally and the vastus medialis distally. A Chattanooga stimulator (Vectra Neo) will be set to deliver a 2500 Hz alternating current, modulated at 75 bursts/s, with a ramp-up time of 2-seconds, followed by a 50-second rest period. Fifteen isometric actions lasting 10 seconds each will be elicited during each session. The treatment intensity will be set at a minimum intensity (higher if tolerated) to achieve a maximum voluntary isometric quadriceps contraction (MVIC) in the injured limb that is equivalent to an MVIC of 40% of the contralateral side. **Dose Changes:** During the last 6 weeks of NMES treatment intensity will be set at the beginning of each week to ensure strength gains are considered throughout the study. The study team will consider a dose change if the participant cannot tolerate the electrical stimulation. If this were to occur, the physical therapist in charge of the intervention would determine the maximum amount of electrical stimulation tolerable by the participant and determine the percentage of the MVIC from the contralateral, uninjured limb this stimulation would produce.

NMES placebo. Participants randomized to NMES placebo + ECC placebo will receive an NMES placebo intervention 2x/week for 8 weeks following ACLR, beginning 0-2 weeks post-operative (to mimic the timing associated with the NMES intervention). Similar to the NMES intervention group, for the first two weeks NMES will be delivered in the clinic by the participants' physical therapist. The treatment intensity for the first two weeks will be set to the maximum tolerated by the participant, but all other stimulation parameters will remain as outlined below for the last 6 weeks. The NMES placebo will utilize the same equipment and follow the same protocol as in the actual NMES intervention, with the exception that the intensity of the current will be set lower. The treatment intensity will be set at an intensity to achieve a MVIC of 15% of the injured side.

High-Intensity Eccentric Exercise (ECC): Participants randomized into the NMES + ECC group will receive ECC 2x/week for 8 weeks, beginning between 6 - 10 weeks post-ACLR when adequate quadriceps control is achieved. To perform the ECC, participants will be positioned in a BLAST!™Leg Press (BioLogic Engineering Inc.) with their ACLR knee range of motion limited to 20 to 60° of knee flexion. Participants will perform warm-up trials consisting of 10 concentric and eccentric quadriceps actions with the ACLR limb. Following the warm-up trial, each participant will perform 10 isokinetic eccentric/concentric quadriceps actions with the ACLR limb. In total for each session, patients will complete 4 sets of 10 eccentric/concentric actions with 2-minute rests between each set. During the eccentric actions, participants will train at an intensity equal to or greater than 70-90% of their concentric one-repetition maximum (this will be recorded at the beginning of each week). Concentric intensity will be set at 40% of the one-repetition maximum. **Dose Changes:** Treatment intensity will be set at the beginning of each week to ensure strength gains achieved during the study period are reflected in the dose delivered. The timing of initiation eccentric exercise will be evaluated on a patient-by-patient basis. It will begin when the patient has adequate quadriceps control to handle this type of training and will be determined by the physical therapist working with the participant and their physician.

ECC placebo: Participants randomized to NMES placebo + ECC placebo will receive an ECC placebo intervention 2x/week for 8 weeks, beginning at 6 - 10 weeks following ACLR. ECC placebo will utilize the same equipment and follow the same protocol as in the actual eccentric intervention, with the exception that participants will train eccentrically at 10% of their MVIC.

6.2 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Procedures for assignment to a study group: Participants in the treatment groups will be allocated to one of the two study arms using minimization[38,39], a method of unbiased allocation ensuring balance across confounding factors. This approach is selected over the more common random permuted blocks within stratum design because of the importance of controlling for two known confounders (1) meniscal injury and treatment and (2) articular cartilage injury – that have 4 x 3 = 12 combinations. Some of these combinations are relatively rare (e. g. meniscal tear left intact) which would result in potential imbalance (and subsequent loss of power) among the study arms. We will also include graft type (bone-patellar tendon-bone or quadriceps tendon, hamstring,) and sex as minimization factors. A secure web-based application developed by the Data Coordinating Center will be used by the research coordinator to enter participant information (e. g. , participant ID, minimization factors) and to obtain the assigned study arm.

The following definitions of meniscal tears/treatment will be used for minimization: 1) normal/intact; 2) torn/excision (partial or complete); 3) torn/repaired; 4) torn/left intact. Articular cartilage injury will be defined using the Modified Outerbridge Scale and used in minimization as follows: 1) grade 1 (normal or soft); 2) grade 2 (partial thickness lesions; and 3) grade 3 and 4 (full-thickness lesions).

Blinding. Considerable care will be taken to ensure fidelity of blinding. Study staff will have standardized scripts to describe treatment arms to participants in order to prevent different treatment expectations. Study staff collecting outcome measures will not be aware of treatment assignment. Physical therapists, delivering the interventions, will be provided with treatment allocation from the DCC. Blinded data reviews will be done and the statistical analysis plan will be written prior to database lock and unblinding of statisticians.

6.3 STUDY INTERVENTION COMPLIANCE

Treatments will be delivered in person, so we will be able to directly monitor adherence. Adherence will be quantified as a count variable defined as the total number of sessions completed relative to the number of session prescribed (32 sessions total: 16 of both NMES and ECC or corresponding placebos). We will also use CRFs to record the percentage of MVIC that the NMES treatment produced in each session, as well as the intensity of the eccentric contractions during ECC sessions. We will then determine the number of session participants trained at the recommended dosage relative to the number of sessions recommended (32 total) to have an assessment of dosage adherence.

6.4 CONCOMITANT THERAPY

All participants in this trial will be undergoing standard anterior cruciate ligament rehabilitation as prescribed by their physician and delivered by their physical therapist. Physical therapists will record the components of the standard rehabilitation delivered at each visit for entry in the study database. Eccentric exercise will not be a part of standard of care rehabilitation. While low-intensity NMES is generally considered to be part of ACLR standard of care rehabilitation, all study participants will receive NMES regardless of group assignment and thus any additional treatment with NMES is not warranted. The experimental NMES intervention will be of high intensity, while the placebo NMES will be of low intensity. The low-intensity NMES used for our placebo is generally what is used in standard of care ACL rehabilitation.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION / WITHDRAWAL

It is critical to the integrity of this study that participants adhere to the visit schedule outlined in the protocol. As such, every reasonable effort should be made to convey the importance of remaining on the study to the participants.

7.1 DISCONTINUATION OF STUDY INTERVENTION

Study interventions may be discontinued for any of the following reasons:
• Unacceptable toxicity (this may include serious adverse events [SAEs] related to study treatment)
• Participant request or withdrawal of consent
• Pregnancy
• Investigator discretion

In instances where participants elect to discontinue NMES treatment per protocol, the following procedure will be followed:

1) Participant will be asked if they would be willing to continue with NMES at a maximum tolerated dosage. If yes, patient will continue trial with NMES at this revised dosage. If no, go to #2.
2) Participant will be asked if they would be willing to continue with the ECC treatment only (i.e., discontinue NMES, but remain in the study and only receive ECC per protocol). If yes, participant will continue in trial only completing ECC. If not, go to #3
3) Participant will be asked if they would be willing to continue being followed to gather study-related measurements, but receive no study-related interventions. If yes, participant will continue in trial only being measured as outlined in the study protocol. If no, participant will be withdrawn from the study as requested.

Consistent with the intention-to-treat principle, randomized participants who permanently discontinue study interventions will continue in the study, and remaining study procedures may be completed as indicated by the study protocol. The data to be collected at the time of study intervention discontinuation will include the following:

- In any participant who does not complete study interventions, we will attempt to gather MRI, strength, and biomechanical data at the 18 month study time-point. Of note, these participants will not be considered SOC control group participants for future study conduct or analyses.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance

- If any adverse event (AE), or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant is unable to tolerate study intervention dosage
- Due to COVID-19, or any other pandemic crisis

The reason for participant discontinuation of study intervention will be recorded on the Treatment Status Case Report Form (CRF). Withdrawal from the study will be recorded on the Final Status CRF. Participants who were discontinued from treatment due to COVID-19 or other pandemic crisis may be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for four scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant within one day of the missed visit and attempt to reschedule the visit. Study staff will counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Enrollment/Baseline/Testing Visit 1/Repeat Testing Visit 1
The following study procedures and evaluations will be performed at Testing Visit 1 (-30 days)) and may be repeated if the surgery is rescheduled making the visit fall outside of the 30 day window:

- Informed consent or assent (for participants under the age of 18 years of age)
- Urine pregnancy test for women of child bearing potential. Child bearing potential is defined as a premenopausal female capable of becoming pregnant.
- Concomitant medication review
- Height (cm) and weight (kg)
- Adverse event review and evaluation
- Anterior/Posterior (A/P)A knee X-ray. Briefly, participants will be positioned for the X-rays using a Synaflexer (BioClinica, Inc.) to ensure similar positioning across study time points. The X-ray tech at MedSport will be responsible for obtaining images.
- Clinical Knee X-ray - The knee x-rays that are gathered as part of standard clinical care for an ACL injury will be pulled from MiChart an used to determine patellar alignment. Specifically, from the lateral and patella "sunrise" views, we will use custom written MATLAB, semi-automatic software to determine measures of patellar alignment. From the sunrise view, edge detection will be used semi-automatically to identify the anterior margins of the femur and posterior margins of the patella. Anatomical landmarks required for the following measurements will be determined automatically and include the most anterior point of the medial and lateral femoral condyles, the deepest point of the intercondylar sulcus, apex of the patella, and the medial and lateral femoral and patellar facets. Measures reflecting trochlear

dysplasia will include sulcus angle and sulcus depth as described by Merchant et al.1 Measures reflecting trochlear sulcus and patellar tilt will include the lateral patellofemoral angle and congruency angle. Patellofemoral index, ratio of medial and lateral patellofemoral joint space, will also be determined. From the lateral view, patellar height will be measured manually which reflects the ratio of the length of the patellar articular surface to the length of the patella tendon measured from the lower pole of the patella to the insertion into the tibia tubercle

- Study specific testing. Specialized measurements will be recorded by study staff (post-doctoral fellow, graduate students). Thigh muscle strength and quadriceps activation will require the use of a Cybex dynamometer equipped with both an anti-shear and a knee attachment. Biomechanics will be recorded with standard procedures using a Vicon Nexus motion capture system. Quadriceps/Knee Cartilage ultrasonograpy will be used to gather measurements of quadriceps atrophy and femoral cartilage thickness. An optional muscle biopsy may be gathered to assess markers of inflammation, muscle damage, and hypertrophy/atrophy. Biopsy may also be used for muscle phenotyping.
- Questionnaires
    - PROMIS Physical Function computer adaptive test (PF CAT): The National Institutes of Health (NIH) Patient-Reported Outcomes Measurement Information System (PROMIS®) Roadmap initiative (www. nihpromise. org) is a cooperative research program designed to develop, evaluate, and standardize item banks to measure patient-reported outcomes (PROs) across different medical conditions as well as the US population. The PF CAT is a broad instrument evaluating physical function in both upper and lower extremities; by responding to the test taker's selections in real time, it is able to reduce the number of questions needed to accurately ascertain a patient's functional status. A patient typically answers between 4-12 questions from a bank of 121 possible questions, and, usually, the test is completed in less than 1 minute. This test has demonstrated good-to-excellent construct validity in patients who have undergone ACL reconstruction. [41]
    - The Knee injury and Osteoarthritis Outcome Score (KOOS) is a knee-specific measure intended to examine patient reported outcomes after injuries that may lead to post-traumatic osteoarthritis, like an ACL injury. KOOS consists of 5 subscales: 1) Pain, 2) Other Symptoms, 3) Function in daily living (ADL), 4) Function in sport and recreation (Sport/Rec) and 5) knee related Quality of life (QOL). The previous week is the time period considered when answering the questions. Standardized answer options are given (5-point Likertscale) and each question is assigned a score from 0 to 4. A normalized score (100 indicating no symptoms and 0 indicating extreme symptoms) is calculated for each subscale.
    - The Tegner Activity Scale is a numerical scale with each value (0–10) representing specific activities. An individual who participates in competitive sports including soccer, football, and rugby at the elite level is considered to have an activity level of 10. An individual participating in the above listed sports but at the recreational level is considered to have an activity level of 6. An individual on sick leave or disability pension due to knee problems is considered to have an activity level of 0. The Tegner scale has proven valid, reliable, and responsive to change. [42]

- The Marx scale is utilized to assess a person's level of physical activity. It focuses on four activities: running, deceleration, cutting, and pivoting. Participants are asked to indicate approximately how many times in the past 12 months they performed each of these activities while at their healthiest and most active state. The four knee functions are rated on a 5-point scale of frequency and scores are added up to a maximum of 16 points with a higher score indicating more frequent participation. This scale has been shown to be both valid and reliable. [43]
- The Tampa Scale of Kinesiophobia (TSK-11) is a patient reported outcome developed to assess 'fear of movement-related pain' in patients with musculoskeletal pain. Each item is scored on a 4-point Likert scale, ranging from 1 'strongly disagree' to 4 'strongly agree'; total scores vary between 11 and 44, with higher scores indicating higher levels of fear of movement-related pain. Validity, Reliability, sensitivity, and specificity have all been established and fall in an acceptable to excellent range. [44]

Intervention Visits 1-16 (For Randomized Participants Only)
The following study procedures and evaluations will be performed at Visit 1-16 (twice per week during Weeks 1-8):

- Randomization. At Intervention Visit 1, subjects will be randomized prior to the administration of the study intervention.
- Study Intervention. At Visits 1-16, NMES or placebo will be administered twice per week. Study intervention parameters will be recorded by the physical therapist.
- Concomitant medication review
- Adverse event review and evaluation
- Standard of Care Rehabilitation Assessment (to be collected until discharged from formal physical therapy)

Intervention Visits 17-32 (For Randomized Participants Only)
The following study procedures and evaluations will be performed at Visit 17-32 (twice per week during Weeks 9-16):

- Study Intervention. At Visits 17-32, ECC or placebo will be administered twice per week. Study intervention parameters will be recorded by the physical therapist.
- Concomitant medication review
- Adverse event review and evaluation
- Standard of Care Rehabilitation Assessment (to be collected until discharged from formal physical therapy)

Testing Visit 2
The following study procedures and evaluations will be performed at testing visit 2 (post-NMES intervention visit):

- Height (cm) and weight (kg)
- Adverse event review and evaluation
- Study-specific testing. Specialized measurements will be recorded by study staff (post-doctoral fellow, graduate students). Thigh muscle strength and quadriceps activation will require the use of a Cybex dynamometer equipped with both an anti-shear and a knee attachment. Quadriceps/Knee Cartilage ultrasonograpy will be used to gather measurements of quadriceps atrophy and femoral cartilage thickness.
- Questionnaires. The PROMIS questionnaire will be administered in order to assess participant perceived function. Knee injury and Osteoarthritis Outcome Score is a knee-specific measure intended to examine patient reported outcomes after injuries that may lead to post-traumatic osteoarthritis, like an ACL injury. Tegner and Marx scores will be assessed in order to examine participant's activity levels. The Tampa Scale of Kinesiophobia will be delivered to determine participant's fear of re-injury.
- For those in the standard of care group – patients will reach the testing visit 2-time point when they have completed 16 PT treatments – approximately 2x a week for 8 weeks.
- Special considerations will be made for those with insurance visit limitations such as patients will reach testing visit 2 when they have completed 8 PT treatments – approximately 1x a week for 8 weeks.
-

Testing Visit 3

The following study procedures and evaluations will be performed at testing visit 3 (post-ECC intervention visit):

- Height (cm) and weight (kg)
- Adverse event review and evaluation
- Study specific testing. Specialized measurements will be recorded by study staff (post-doctoral fellow, graduate students). Thigh muscle strength and quadriceps activation will require the use of a Cybex dynamometer equipped with both an anti-shear and a knee attachment. Quadriceps/Knee Cartilage ultrasonograpy will be used to gather measurements of quadriceps atrophy and femoral cartilage thickness. A muscle biopsy may be gathered to look at markers of inflammation, muscle damage, and hypertrophy/ atrophy. Biopsy may also be used for muscle phenotyping.
- Questionnaires. The PROMIS questionnaire will be administered in order to assess participant perceived function. Knee injury and Osteoarthritis Outcome Score is a knee-specific measure intended to examine patient reported outcomes after injuries that may lead to post-traumatic osteoarthritis, like an ACL injury. Tegner and Marx scores will be assessed in order to examine participant's activity levels. The Tampa Scale of Kinesiophobia will be delivered to determine participant's fear of re-injury.
- For those in the standard of care group – patients will reach the testing visit 3-time point when they have completed 32 total PT treatments – approximately 2x a week for 16 weeks.
- Special considerations will be made for those with insurance visit limitations such as patients will reach testing visit 3 when they have completed 16 total PT treatments – approximately 1x a week for 16 weeks.

Testing Visit 4

The following study procedures and evaluations will be performed at Testing Visit 4 (Week 36 [9 months post-ACLR]):

- Concomitant medication review
- Height (cm) and weight (kg)
- Adverse event review and evaluation
- Study specific testing. Specialized measurements will be recorded by study staff (post-doctoral fellow, graduate students). Thigh muscle strength and quadriceps activation will require the use of a Cybex dynamometer equipped with both an anti-shear and a knee attachment. Biomechanics will be recorded with standard procedures using a Vicon motion capture system. Clinical hop tests only require the use of a tape measure that will be attached to ground. Quadriceps/Knee Cartilage ultrasonograpy will be used to gather measurements of quadriceps atrophy and femoral cartilage thickness.
- Questionnaires. The PROMIS questionnaire will be administered in order to assess participant perceived function. Knee injury and Osteoarthritis Outcome Score is a knee-specific measure intended to examine patient reported outcomes after injuries that may lead to post-traumatic osteoarthritis, like an ACL injury. Tegner and Marx scores will be assessed in order to examine participant's activity levels. The Tampa Scale of Kinesiophobia will be delivered to determine participant's fear of re-injury.

Testing Visit 5

The following study procedures and evaluations will be performed at Testing Visit 5 (Week 72 [18 months post-ACLR]):

- Concomitant medication review
- Height (cm) and weight (kg)
- Adverse event review and evaluation
- A/P knee X-ray
- Magnetic Resonance Images. MRI images of the involved and uninvolved knees will be recorded to assess knee joint cartilage. Scans will be approximately 1 hour in length and will take place at University Hospital on the 3T Phillips Research Scanner. Positioning and sequences will be described in the MOP.
- Study specific testing. Specialized measurements will be recorded by study staff (post-doctoral fellow, graduate students). Thigh muscle strength and quadriceps activation will require the use of a Cybex dynamometer equipped with both an anti-shear and a knee attachment. Biomechanics will be recorded with standard procedures using a Vicon MX motion capture system. Clinical hop tests only require the use of a tape measure that will be attached to ground. Quadriceps/Knee Cartilage ultrasonograpy will be used to gather measurements of quadriceps atrophy and femoral cartilage thickness. An optional muscle biopsy may be gathered to look at markers of inflammation, muscle damage, and hypertrophy/ atrophy. Biopsy may also be used for muscle phenotyping.
- Questionnaires. The PROMIS questionnaire will be administered in order to assess participant perceived function. Knee injury and Osteoarthritis Outcome Score is a knee-specific measure intended to examine patient reported outcomes after injuries that may lead to post-traumatic osteoarthritis, like an ACL injury. Tegner and Marx scores will be assessed in order to examine participant's activity levels. The Tampa Scale of Kinesiophobia will be delivered to determine participant's fear of re-injury.

12 and 15 Month Physical Activity Assessments
In order to account for physical activity occurring between the 9 and 18 month testing visits, study staff will email participants a physical activity assessment questionnaire at 12 and 15 months post-ACLR. The assessment will include a modified Marx Questionnaire. The Marx Questionnaire will be modified to reflect a 3-month period rather than a 12-month period. This assessment will also inquire about specific sports/activities that study enrollees are participating in.

8.2 SAFETY AND OTHER ASSESSMENTS

Subject Screening: The lead study coordinator, graduate student, or post-doctoral fellow will examine medical records prior to approaching patients in clinic regarding enrollment. If the study staff finds that a patient meets any of the study exclusion criteria, that patient will not be approached for enrollment into the study. If after examining medical records the patient appears to be eligible for study enrollment, study staff will approach in clinic, describe the study, and determine interest for potential enrollment. If a patient expresses interest in enrollment, they will be sent home with the study consent form and study staff will make follow-up contact within 3 days. During this contact, the staff member will screen subjects using the subject inclusion/exclusion criteria case report form to confirm eligibility. If eligibility is confirmed, study staff will schedule the first testing visit. The following information will be collected during screening:

- Date of birth
- Injury history
- Type of ACL reconstruction patient will undergo
- Date of scheduled reconstruction surgery.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312. 32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Serious adverse events (SAE) are defined as any event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions;
- Results in a congenital anomaly/birth defect;
- Any other adverse event deemed to jeopardize the subject's health and results in either a medical or surgical intervention so as to prevent one of the outcomes listed above.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. [Consistent with 21 CFR 312. 32 (a)]

Exclusions to the SAE definition for purposes of this study will include:

- Planned admissions to the hospital or elective procedures for pre-existing conditions, which occur during the course of study participation, will not be considered as SAEs.

- Adverse events which result in admission or observation at a hospital for 23 hours or less, will not be considered serious unless they also meet one of the other SAE definition criteria (e. g. deemed to jeopardize the subject's health and results in either a medical or surgical intervention.

8.3.3 EVENTS OF SPECIAL INTEREST

A graft failure will be considered an Adverse Event of Special Interest (AESI) in the MiACLR trial. Graft failures will be diagnosed by the patient's physician using a combination of objective laxity (e.g. positive pivot shift) and/or MR imaging. Any such diagnosis, whether treated with an ACL reconstruction revision or not, will be characterized as an AESI. A graft failure is being considered as an AESI as the ACL graft could potentially be impacted by the interventions of interest in the study. As such, a higher than suspected number of ACL graft failures in either group could suggest the active or placebo interventions are compromising the graft tissue/ACL reconstruction and are

unsafe. It is expected that ~10% of ACL grafts will fail with autograft ACL reconstruction and standard ACL rehabilitation.

8.3.4 CLASSIFICATION OF AN ADVERSE EVENT

8.3.4.1 SEVERITY OF EVENT

The intensity of all AEs will be graded using a five-point grading scale in which the following descriptions of severity will apply. Note that for some AEs, Grades 4 and/or 5 may not be applicable. In those cases only 3 different grades (Grade 1-3) are to be considered:

- **Mild (Grade 1)** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate (Grade 2)** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe (Grade 3)** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. They are medically significant but not immediately life-threatening. Of note, the term "severe" does not necessarily equate to "serious".
- **Life-threatening (Grade 4)** – Events result in life-threatening consequences; urgent intervention indicated.
- **Fatal (Grade 5)** – Events result in death.

8.3.4.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related**: The AE is known to occur with the study interventions/procedures, follows an obvious sequence of time from the study intervention or procedure, the event ceases on discontinuation of the study intervention or procedure (and reoccurs on restarting), and/or the event includes data that was only collected for this study
- **Probably Related**: The AE is lesser known or suspected to occur with the study interventions/procedures, follows a reasonable sequence of time from the study intervention or procedure, and the event ceases or diminishes with discontinuation of the study intervention or procedure.
- **Possibly Related**: The AE is lesser known or a possible effect of the study interventions/procedures, occurs in sequence of time from the study intervention or procedure, for which the event may be attributed to the study intervention or procedure, and/or the event could be explained by the characteristics of the population of study (i.e. young or middle-aged adults, person who have suffered an ACL injury and underwent a subsequent ACL reconstruction)
- **Unlikely Related**: The AE is not a previously known or a suspected effect of the study intervention or procedure, does NOT follow a sequence of time from the study intervention or procedure, for which the event could be attributed to the administration of the study intervention or completion of any study procedure, and/or the event can be readily explained by the characteristics of the population under study.

- **Unrelated**: The AE is not known to be an effect of the study intervention or procedure, does NOT follow a sequence of time from the study intervention or procedure, for which the event could be attributed to the administration of the study intervention or completion of any study procedure, and/or the event can be readily and easily explained by the characteristics of the population under study. An event would also be considered unrelated, if a subject never received study drug, study device, or underwent research study procedure.

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8.3.4.3 EXPECTEDNESS

The Principal Investigator or designee will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention in the protocol or informed consent.

8.3.5 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

A consistent methodology for eliciting AEs will be used at all subject evaluation time points that includes open-ended questions such as:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. AEs may also be identified through medical record review or through physician evaluation.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

All reportable events with start dates occurring any time after informed consent is obtained until the last study visit (i.e. 18 month study visit). At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or until last study visit. In the event that an AE or SAE occurred at the last study visit it would be followed for 7 days (non-serious AEs) or 30 days (serious AEs) after said study visit.

8.3.6 ADVERSE EVENT REPORTING

All adverse events (AEs), whether observed by the investigator or reported by the participant, are recorded in the study database by study coordinators. For each AE, the date of onset, severity, and duration is recorded on the electronic case report form (eCRF). In addition, the

investigator or designee will assess whether the AE is serious or not, relationship to study intervention, and whether it is expected or not. All AEs will be monitored and followed until they are adequately resolved or until the end of the study (i.e. 18 month study visit). All AEs must be submitted to the database via the AE eCRF within 72 hours of first knowledge of the event. There will be a special form that is completed for serious adverse events; see Section 8. 3. 6 below.

8.3.7 ADVERSE EVENT OF SPECIAL INTEREST REPORTING

If a graft failure, the only AESI for the current study, is identified by the study team through patient reporting or review of medical records it will be monitored and reported using the guidelines discussed in section 8.3.5. If the graft failure, however, results in an ACL revision reconstruction this would result in an SAE which would be monitored and reported as discussed in section 8.3.7. Whether designated as an AE or an SAE the AESI will be flagged and specifically reported to the DSMB providing details regarding the number for each study group, and a breakdown of how the AESIs were thought to occur (traumatic or atraumatic). The AESIs will be followed for outcome information until resolution (e.g. date of revision surgery), stabilization (e.g. end of care for the AESI), or study completion, with stop dates recorded for resolution.

8.3.8 SERIOUS ADVERSE EVENT REPORTING

All SAEs will be mapped to system organ classes (SOC) using preferred MedDRA SOC terms, graded for severity and relationship to study drugs as detailed above, and recorded with start dates occurring any time after informed consent is obtained until 30 days after the last day of study participation. At each study visit, the investigator or designee will inquire about the occurrence of SAEs since the last visit and study participants, and their significant others will be asked in advance to notify the study investigators immediately regarding any hospitalization, serious change in their health or in the event of a death. Events will be followed for outcome information until resolution or stabilization, with stop dates recorded for resolution.

Regardless of whether the SAE is deemed related to use of the study interventions, the data for the SAE must be reported with the appropriate information by the study investigators or their designee to the Data Coordinating Center within 24 hours of learning of the event. In addition, new follow-up data must be reported within 24 hours of receipt. The designated study medical monitor, Tariq Awan, DO, or designee may be contacted at any time for immediate discussion regarding such an event.

Hospitalizations for the following reason, which are not related to an AE, do not require reporting:
• Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions.

When recording a death, the event, or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, the report will be identified as an “Unexplained Death”.

Investigators must report all SAEs to their governing IRB/IEC, as required by local regulations and guidelines.

A record of all SAEs, by study subject and preferred SOC terms will be recorded centrally by the DCC.

8.3.9 REPORTING EVENTS TO PARTICIPANTS

We will notify participants of any safety information that modifies the risk profile and may change their willingness to continue in the study. Changes in risk profile or study design affecting participant safety will be address via protocol amendment. This information would be reviewed with active subjects who would be asked to sign the amended consent to continue study participation.

8.3.10 REPORTING OF PREGNANCY

If a participant were to report a pregnancy during the study period the interventions would be stopped and follow-up would be discontinued.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812. 3(s)).

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures).

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

If an unanticipated problem occurs that places a participant at greater risk than originally anticipated, participants will be notified. Changes in risk profile or study design affecting participant safety will be address via protocol amendment. This information would be reviewed with active subjects who would be asked to sign the amended consent to continue study participation.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

The primary hypothesis is that quadriceps strength symmetry will be greater with the combination of NMES and ECC relative to the placebos at both 9 months and 18 months post-ACLR. Statistically, the following null and alternative hypotheses will test the superiority of the combination of NMES + ECC versus placebo arm in the change from baseline to 9 months and 18 months post-ACLR in isokinetic quadriceps strength symmetry index separate analyses:

H_{01}: $\Delta_{9mo\ NMES + ECC} \leq \Delta_{9mo\ NMES\ placebo + ECC\ placebo}$
H_{A1}: $\Delta_{9mo\ NMES + ECC} > \Delta_{9mo\ NMES\ placebo + ECC\ placebo}$
H_{06}: $\Delta_{18mo\ NMES + ECC} \leq \Delta_{18mo\ NMES\ placebo + ECC\ placebo}$
H_{A6}: $\Delta_{18mo\ NMES + ECC} > \Delta_{18mo\ NMES\ placebo + ECC\ placebo}$

where Δ reflects the adjusted mean change from baseline at 9 or 18 months post-ACLR in isokinetic quadriceps strength symmetry index.

9.2 SAMPLE SIZE DETERMINATION

For the main study objectives, the sample size of 112 participants (56 per each of the 2 treatment groups) allows sufficient power to test the primary hypotheses regarding two separate comparisons (each co-primary endpoint) of the combination NMES + ECC vs NMES placebo + ECC placebo. Each test will be conducted using a two-sided 0. 025 Type I error level (=0. 05/2, the Bonferroni adjustment for two comparisons). We hypothesize that the effect size (treatment difference / standard deviation) will be at least 0. 7 for the tests of NMES + ECC vs NMES placebo + ECC placebo. Assuming 20% drop-out (resulting in an effective sample size of 45 per group), we have at least 80% power to detect this effect size. Preliminary data from the

same site with a comparable patient population and primary endpoint demonstrated a 1. 14 effect size. Because of the small sample size and variability in measurements in this preliminary study, we conservatively reduced the effect size for this study. [35]

An additional cohort of 28 participants will be studied – the SOC control group. The sample size of 28 participants for this group is based on feasibility considerations.

Thus, the total sample size for the trial is 140 participants.

9.3 POPULATIONS FOR ANALYSES

Several analysis sets will be used in analyses:

- The main population for efficacy will be the modified intention-to-treat population (mITT), defined as all participants randomized and receiving at least one study intervention. Subjects will be analyzed by assigned treatment.
- A second analysis set will be used to assess the robustness of the primary conclusions in the subset of participants in the mITT analysis set. This set contains participants who complied with the protocol sufficiently to ensure that these data would be likely to represent the effect of treatment according to the underlying scientific model. The Per Protocol (PP) analysis set will consist of all subjects in the mITT population who do not have a major protocol violation, inclusive of violation of entry criteria.
  The Safety Population is defined as all participants who are randomized and receive at least study intervention. The Safety Population will be used for all safety analyses. Subjects will be analyzed by assigned treatment.
- All participants in the SOC control group will be included in the exploratory analyses.

9.4 STATISTICAL ANALYSES

A statistical analysis plan (SAP) will be written for the study that contains detailed descriptions of the analyses to be performed. The SAP will be finalized prior to the database lock and unblinding of the data. The approach to analyses affected by the COVID-19 or other pandemic crisis will be described in the SAP along with the analyses for the exploratory objective.

9.4.1 GENERAL APPROACH

The design of this randomized controlled study is a placebo-controlled investigation of the combination of NMES and ECC vs NMES placebo + ECC placebo. Continuous variables will be summarized using descriptive statistics including n, mean, median, standard deviation, range (e. g. , minimum and maximum). Qualitative variables will be summarized using counts and percentages. Summaries will be provided by treatment group and overall. Unless otherwise specified, statistical analyses will be performed using SAS Version 9 or higher. For the co-primary endpoints, the nominal significance level is 0. 025 (two-sided test with a Bonferroni adjustment for two comparisons); for all other statistical tests, the nominal significance level is 0. 05 (two-sided). p-values and confidence intervals will be reported.

Linear models will be used for most analyses (primary and key secondary endpoints that are

continuous), with terms for treatment group, type of graft, surgeon, age, gender and baseline in all models.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINTS

The primary hypotheses will be tested at the two-sided 0. 025 level, adjusting for the multiple comparisons of 2 co-primary endpoints. Separate linear models will be fit for each of the two co-primary endpoints, one for the 9-month and one for the 18-month changes from baseline in isokinetic quadriceps muscle strength. As described above, the model will include treatment group, type of graft, surgeon, age, gender, and baseline isokinetic quadriceps strength symmetry index. Least-squares (LS) mean change from baseline, along with associated standard errors, will be presented for each intervention group. For the comparisons of combination NMES + ECC to the placebo group, group LS mean differences in the change from baseline will be presented, along with associated 97. 5% upper confidence bounds and p-values. The primary analysis of the primary endpoints will use the mITT analysis set. A secondary analysis of the primary endpoints will use the PP analysis set. Appropriate non-linear parametric or non-parametric tests may be applied if the assumptions of the model are not satisfied. Details will be presented in the SAP.

Assessment of the extent of missing data and its mechanism will be explored for the primary endpoints using the mITT analysis set. Graphical and numerical descriptive methods will be used to summarize and the primary outcome over time by intervention group and overall. We will begin by assuming missing at random, and employ multiple imputation methods[45,46] to handle missing data. Other more complicated methods may be employed if there are concerns about differential follow-up and nonignorable missing (where the stochastic process that generates missingness is explicitly modeled such as a pattern mixture model[47]) as a sensitivity analysis. The model will include the same covariates that are included in the primary analysis.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

All tests of secondary endpoints (e. g. , T1 relaxation ratio at 18 months post-ACL, score on KOOS at 9 months post-ACLR) will be conducted at the two-sided 0. 05 level, without adjustment for multiplicity, using comparable models as for the primary endpoints. The mITT analysis set will be used. Appropriate non-linear parametric or non-parametric tests may be applied if the assumptions of the model are not satisfied. Details will be presented in the SAP.

In addition, for endpoints used to assess secondary objective 1 (i. e,. T1 relaxation ratio at 18 months post-ACL and T2 relaxation ratio at 18 months post-ACL), we will include articular cartilage injury and meniscus injury and treatment as covariates in the linear model.

9.4.4 SAFETY ANALYSES

Safety analyses will be performed on the safety analysis set. Safety data, including frequency of events and proportion of participants experiencing events (e. g. , serious adverse events, adverse events leading to early discontinuation of study intervention), will be summarized descriptively overall and by treatment group. For categorical safety outcomes, numbers and percentages will be used. For continuous safety outcomes, number, mean, standard deviation, median, interquartile range, minimum and maximum will be used to summarize changes from baseline to each study visit in vital signs.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

The treatment groups will be compared descriptively with respect to demographic and baseline variables (e. g. , age, race, quadriceps strength symmetry index). No statistical tests will be used to compare the treatment groups. In addition, graphical and numerical descriptive methods will be used to summarize demographic and baseline characteristics by intervention group and overall.

9.4.6 PLANNED INTERIM ANALYSES

Not applicable. There will be one final analysis that will occur after the last participant's last visit and the database is locked and unblinded.

9.4.7 SUB-GROUP ANALYSES

Age at time of ACLR and BMI are other biological factors that can influence ACLR outcomes and thus will be considered as covariates and adjusted for in our analyses, as described above. Evaluation of model diagnostics will be included to avoid over-fitting because of the number of parameters that are being assessed.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Key efficacy and safety data will be listed by individual participant. Details will be in the SAP.

9.4.9 EXPLORATORY ANALYSES

Several planned exploratory analyses will be conducted:

- For the primary endpoints, we will incorporate articular cartilage injury and meniscus injury and treatment as covariates in the linear model, with attention to fit statistics to avoid over-fitting.
- For endpoints used to assess the secondary objective 1 (i.e. , T1 relaxation ratio at 18 months post-ACL and T2 relaxation ratio at 18 months post-ACL), we will also explore activity during the return to activity period, activity level prior to injury, BMI and other interaction effects to assess effect modification, with caution to avoid over-fitting
- We will also examine the ability of quadriceps strength at 9 months post-ACLR to predict changes in cartilage health at 18 months post-ACLR. To do this we will employ linear models with several parameterizations of strength (e. g. , continuous, quartiles, binary threshold), assessing the discrimination and calibration for the prognostic models (will include other potential factors in addition to 9-month post-ACLR strength). Additional relationships among variables, such as the correlation of T2 and T1rho MRI measures of OA to X-ray measure of OA, will be explored graphically, summarized descriptively, and modeled using multivariable linear or logistic models.
- We will examine change in quadriceps muscle strength and muscle volume from baseline to post-NMES intervention (testing visit 2) and post-ECC intervention (testing visit 3) in an attempt to understand the effects of the individual components of the combined NMES+ECC intervention.

Several exploratory analyses will be performed to assess the ability to reduce the NMES and

ECC intensity in the placebo treatment group so that it reflects a “true” placebo condition. The SOC control group is a group of participants who were eligible for the randomized controlled trial but refused and were willing to consent to an observational arm.
Specifically,

- The demographic and baseline variables will be summarized in the SOC control group (no NMES or ECC treatment) using equivalent tabular and graphical methods to those described in Section 9. 4. 5.
- The co-primary and key secondary endpoints will be summarized in the SOC control group for all observed cases.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The consent materials are submitted with this protocol. There is a separate consent document for those who wish to be randomized, and those that do not (SOC Control Consent).

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual’s agreeing to participate in the study and continues throughout the individual’s study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator or designee will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant’s comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Participants that are under the age of 18 will be asked to sign an assent form (in this study the consent form is the same form as the assent form) following the same procedures listed above.

In addition to the minor signing the consent form, one parent/legal guardian of the minor will be required to consent to participation.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, and funding agency. If the study is prematurely terminated or suspended, the Principal Investigator (PI) or designee will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).]

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigator, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover all types of tests (e. g. , MRI, X-rays) in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant’s contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements, whichever is longer.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the University of Michigan. This will not include the

participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the University of Michigan research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the University of Michigan.

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the University of Michigan. No biological specimens will be collected for this study.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

The Principal Investigator, Dr. Riann Palmieri-Smith, is responsible for the overall management and day-to-day operational oversight for the MiACLR study. She will be supported by the University of Michigan Statistical Analysis of Biomedical and Educational Research (SABER) Data Coordinating Center, under the direction of Dr. Cathie Spino, which is responsible for data collection, monitoring, and statistical activities. Dr. Palmieri-Smith and the SABER group will meet routinely throughout the study to administratively direct and monitor the progress of the clinical trial and to respond to any design, implementation or administrative issues that arise during the study

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including orthopaedics, physical therapy, and statistical/data analysis. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the National Institute of Child Health and Human Development.

10.1.7 CLINICAL MONITORING

Throughout the course of the study, data will be monitored for accuracy and completeness and study procedures will be monitored for adherence to the protocol and Good Clinical Practices (GCP). In addition to frequent contacts through e-mail and telephone, on-site monitoring visits will be coordinated by the Statistical Analysis of Biomedical and Educational Research (SABER) unit at the University of Michigan. SABER (the Data Coordinating Center for this study) will be responsible for operational aspects and monitoring of the trial, including at least annual monitoring visits and/or remote source data verification.

The clinical monitor will ensure that:

- Data collected and entered into the database are verifiable against source documents for the participants. The clinical monitor will need access to subject medical records and other study-related records needed to verify the entries on the electronic case report forms.
- Appropriate consent is obtained for each participant prior to study procedures.
- The rights and well-being of participants are being protected.
- The study is conducted in accordance with the currently approved protocol (including study treatment being used in accordance with the protocol), with any other study agreements, with GCP and with applicable regulatory requirements.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

DCC staff will prepare a data management plan. The data management plan will describe the front-and back-end edit checks, as well as forms tracking procedures, that will be implemented to ensure timely and high-quality data collection. It will also define the periodic reports that will be shared with site coordinators and PIs that summarize site performance. The data management procedures will be consistent with the International Conference on Harmonisation (ICH E6) standards for Good Clinical Practice (GCPs).

Monitoring of site performance will be carried out under the direction of the DCC. The Executive Committee will meet approximately monthly to review enrollment and retention reports, interval AEs and SAEs, compliance with the interventions, protocol compliance including interval study withdrawals, and delinquency reports as available.

10.1.9 DATA HANDLING AND RECORD KEEPING

Comprehensive data coordinating center (DCC) functions for this clinical trial, including database development, web-based data entry and management, as well as the creation and export of study reports for the DSMB will be provided by the University of Michigan Statistical

Analysis of Biomedical and Education Research (SABER) group. Housed in the top nationally ranked Department of Biostatistics, SABER, in its 17-year existence, has served as the DCC for over 50 studies, including multiple NIH-sponsored networks.

The DCC will use OpenClinica® (OpenClinica Clinical Trial Software; OpenClinica, LLC, Waltham, MA), a clinical trial software platform for electronic remote (i.e., site-based entry) data capture and clinical data management, as the basis for our custom-designed data entry and management system. The majority of data will be collected via electronic Case Report Forms (CRFs); however, other data sources, such as laboratory data from the central laboratory, may be used. In these circumstances, the DCC will also utilize electronic data transfer. Protocols for the transfer of data, with careful attention to data integrity, will be written by experienced programmers and stored in the OpenClinica database or data mart.

The DCC has established a set of standard operating procedures (SOPs) governing the processes used to ensure patient privacy and data confidentiality, including the use of anonymous participant IDs on CRFs and in reports. OpenClinica® enables compliance with Good Clinical Practice (GCP) and regulatory requirements by providing differentiated user roles and privileges, password and user authentication security, electronic signatures, SSL encryption, and comprehensive auditing to record and monitor access and data changes.

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. In addition to the protocol, Dr. Palmieri-Smith will prepare a Manual of Operations will provides additional detail on data collection procedures, including source documentation, eCRF completion guidelines, data handling procedures and procedures for data monitoring and quality control that ensure these data are accurate, consistent, complete and reliable and in accordance with ICH 36.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into OpenClinica a 21 CFR Part 11-compliant data capture system provided by the Data coordinating Center The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until

there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation or scheduled protocol-required activity. The DCC will summarize protocol deviations in site performance. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 2 years after the completion of the primary endpoint by contacting Riann Palmieri-Smith, PhD, ATC.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NICHD has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

There are no additional considerations identified at this time.

10.3 ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e. , abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
ANCOVA	Analysis of Covariance
ACL	Anterior Cruciate Ligament
ACLR	Anterior Cruciate Ligament Reconstruction
A/P	Anterior/Posterior
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
ECC	Eccentric Exercise
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
KOOS	Knee Injury and Osteoarthritis Outcome Score
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities

MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
MVIC	Maximum Voluntary Isometric Contraction
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NMES	Neuromuscular electrical stimulation
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
TSK-11	Tampa Scale of Kinesiophobia
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

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