Statistical Analysis Plan

Multimodality Intervention to Improve Function and Metabolism in Spinal Cord Injury

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Principal Investigator: Shalender Bhasin, MB, BS
Brigham and Women's Hospital

Chief Biostatistician: Karol M. Pencina, PhD
Brigham and Women's Hospital

Co-Investigators

Thomas W. Storer, PhD
Brigham and Women's Hospital

Kieran F. Reid, PhD
Brigham and Women's Hospital

Nancy K. Latham, PhD, PT
Brigham and Women's Hospital

Ross Zafonte, DO
Spaulding Rehabilitation Hospital

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Signed:

Principal Investigator: Shalender Bhasin, MB, BS
Brigham and Women's Hospital

Chief Biostatistician: Karol M. Pencina, PhD
Brigham and Women's Hospital

Introduction

This statistical analysis plan (SAP) provides details of the statistical methods for data collected as outlined in the protocol for the project "Multimodality Intervention to Improve Function and Metabolism in Spinal Cord Injury" and describes the analysis conventions to guide the statistical programming work. The scope of this SAP is limited to only the parent trial. The statistical analysis plan for the bone sub study is described in a separate document.

All analyses will be performed using SAS Version 9.4 or higher (SAS Institute, Inc., Cary, NC 27513) under the UNIX operating system or R version 4.4.1 or higher. The SAP will be signed off by the Principal Investigator and the Chief Biostatistician before the study database is locked.

The sample size estimation and the statistical analyses were guided by the consideration that spinal cord injury (SCI) is an orphan condition and drugs and interventions for use in people with SCI have received orphan drug designations from both the FDA and the European Medicines Agency.

This SAP will *not* be updated in case of future (administrative or minor) amendments to the protocol unless the changes have impact on the analysis of study data described here.

2.0 Study Background

Spinal cord injuries (SCI) impose a heavy burden on the affected person, the caregivers, and society. Over 288,000 Americans are living with paralysis resulting from

SCI. Approximately, 18,000 Americans incur a SCI each year; people who sustain a SCI are typically young. Today, spinal cord injuries cost $40.5 billion annually. The limitations imposed on a person's health, mobility, and community integration exact a heavy toll on overall quality of life and the cost of caring for persons with SCI. Persons with SCI consistently report function and activity as important to quality of life. Unsurprisingly, NCMRR has designated "*pharmaceutical, stimulation, and exercise…strategies to improve motor function and health of SCI patients"* a priority area of research.

Scientific Premise for the Home-Based Multimodality Intervention

Poor Physical, Metabolic, and Wellbeing Outcomes in Persons with SCI. SCI causes a profound loss of muscle mass and mobility. Physical inactivity, recurrent infections, and chronic inflammation contribute to whole body and visceral fat accumulation, attenuation of cardiovascular fitness and insulin sensitivity, and the development of pro-atherogenic dyslipidemia. Two-thirds of SCI patients are overweight, one third obese, nearly 40% have insulin resistance, and 12-20% have diabetes[6]. Thus, there is clustering of cardiometabolic risk factors in persons with SCI. Even as the mortality during the first year after SCI has decreased due to improved acute care, mortality rates after the first year have not changed appreciably. Heart disease and metabolic disorders have now emerged as the leading contributors to long term excess morbidity and mortality. Depressive symptoms, anxiety, and chronic pain contribute to poor wellbeing.

Rationale for Multi-Modality Intervention. Single interventions, such as arm cycle ergometry and electrical stimulation, although individually efficacious, have had limited impact on overall function and health outcomes. Systematic reviews by Hicks[18],

Valent[20], and others have highlighted the low quality of much of the evidence; most intervention trials have not been randomized, or have not included a matched control group, or have been limited by a small sample size. Furthermore, most intervention trials have been conducted in a Spinal Cord Center or a Rehabilitation unit, constraining the ability of mobility-limited subjects to adhere to the exercise regimen and scalability of these regimens into clinical practice. Recognizing the limited efficacy of single interventions and the difficulty of scaling laboratory-based interventions into clinical practice, we propose here a randomized, controlled trial of a Home-Based Multimodality Functional Recovery and Metabolic Health Enhancement Program, in alignment with the research priorities established by NCMRR,

Arm ergometry exercise training has been shown to be feasible, but only modestly efficacious in improving musculoskeletal health and metabolic outcomes. Current guidelines for healthy individuals –recommend that exercise should be performed at moderate (3-6 metabolic equivalents, METs) to vigorous (>6 METs) intensity to achieve benefits in exercise tolerance, metabolic profile, cardiovascular risk, and body composition.

Functional Electrical Stimulation (FES) training programs have been shown to be modestly efficacious in reversing muscle atrophy after SCI and improving mobility and general health. The quadriceps and the tibialis anterior adapt to electrically stimulated cycling by induction of muscle fiber hypertrophy and muscle regeneration. A recent report demonstrated the feasibility of a home-based FES – leg cycling (FES-LC) program for people with SCI. The major limitation of the FES-LC is the relatively low work rate intensities that are generated by FES-LC alone. However, substantially

greater work rates can be generated when FES-LC is combined with arm ergometry, than with either intervention alone.

Circulating testosterone levels decline after SCI and nearly half the men with SCI have testosterone levels below those expected for age. The use of opioids, chronic illnesses, inflammation, and denervation of the testis contribute to low testosterone levels[44,80,81]. Androgen deficiency contributes to the loss of muscle mass and strength, bone loss, sexual dysfunction, low mood, fatigue, and metabolic dysregulation. The lowering of testosterone levels in men either experimentally or by androgen deprivation therapy for prostate cancer is associated with muscle loss, insulin resistance, and increased risk of diabetes and cardiovascular disease. Androgens exert potent anabolic effects on muscle mass and strength even in patients with SCI. Our dose response studies have shown that the anabolic effects of testosterone on muscle mass and strength are related to testosterone dose and increments in testosterone levels. Thus, raising testosterone levels in men whose levels are low, low normal, or even normal induces further gains in muscle mass and strength. Androgens induce hypertrophy of type I and II fibers. Testosterone promotes myogenic differentiation of mesenchymal progenitors by activating Wnt β-catenin pathway. Two nonrandomized trials have reported significant gains in lean body mass in patients with SCI after testosterone treatment.

We will use testosterone undecanoate in this trial because: 1) it is approved by the FDA; 2) It is long acting, so only 3 injections will be required during the entire 16-week intervention period; 3) The safety of testosterone has been established in large trials in men and women.

We and others have shown that the anabolic effects of testosterone on the muscle are augmented by exercise training. Accordingly, combined administration of testosterone with FES-LC + AE exercise would be expected to induce greater gains in muscle mass and strength than can be achieved with FES-LC + AE exercise alone. Testosterone increases hemoglobin, 2,3 BPG, tissue capillarity, mitochondrial biogenesis and mitochondrial quality, all of which should improve tissue O2 delivery and cellular uptake leading to increased aerobic capacity. Additionally, testosterone improves mood in hypogonadal men, ameliorates depressive symptoms in HIV-infected men, and improves pain sensitivity in opioid-induced androgen deficiency.

Reflecting a nationwide trend away from extended in-patient rehabilitation, from 1973 to 2008, the median length of stay declined from 84 to 42 days for persons with complete paraplegia, and from 142 to 59 days for people with complete tetraplegia. Outpatient rehabilitation that includes physical exercise is fraught with barriers for individuals using wheelchairs. Access to rehabilitation facilities, proper equipment, transportation, and caregiver assistance are some of these barriers. Home-based exercise for SCI can overcome some these barriers and is likely to enhance adherence and patient convenience, and lower overall cost than hospital-based interventions.

Summary: Inter-linked deficits in multiple physiologic organ systems contribute to a vicious cycle of progressively declining musculoskeletal and metabolic health in patients with SCI. Each of the interventions – FES-LC, arm cycle ergometry, and androgen - is capable of increasing aerobic capacity, and muscle mass and strength, which would improve physical function and mobility, and thereby measures of wellbeing. Each intervention would also enhance metabolic adaptations through effects on muscle mass

and contractility mediated via myokines, fuel utilization, and other mechanisms, and indirectly via increased activity. The effects of exercise are augmented by androgen administration. Further, androgen may have additional beneficial effects in improving mood, wellbeing, pain sensitivity, and metabolic outcomes. Consequently, we hypothesize that a multi-modality intervention that includes hybrid exercise (FES-LC combined with concurrent arm ergometry) plus testosterone and targets deficits in multiple physiologic organ systems will be more efficacious in improving musculoskeletal and metabolic health than hybrid exercise plus placebo.

2.1 Objective

Primary Objective:

- To determine whether the multimodality intervention that includes 16 weeks of a home-based functional electrical stimulation - leg cycling (FES-LC) plus arm ergometer exercise (AE) (together abbreviated as FE-LC+AE) and testosterone treatment is more efficacious than placebo plus FES-LC + AE in improving peak aerobic capacity (VO_{2peak}) achieved during arm ergometry exercise alone.

Secondary Objectives:

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving peak aerobic capacity (VO_{2peak}) achieved during FES-LC exercise.

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC in improving peak aerobic capacity (VO_{2peak}) achieved during the combined FES-LC + AE exercise.

- To quantify a broader spectrum of aerobic performance outcomes beyond the primary endpoint of peak oxygen uptake (VO_2peak), additional supportive submaximal and integrative measures of aerobic performance will be assessed across all exercise modalities. Accordingly, we will determine whether the multimodal intervention results in superior improvements in additional cardiorespiratory performance parameters, including the metabolic threshold ($\dot{V}O_2\theta$), oxygen uptake efficiency slope $\Delta\dot{V}O_2/\Delta \log_{10} \dot{V}_E$, ventilatory efficiency slope ($\Delta\dot{V}_E/\Delta\dot{V}O_2$) and total work, assessed during arm ergometry alone, FES-LC alone, and combined FES-LC+AE testing modalities. These additional endpoints will provide a more comprehensive physiological profile of the intervention's impact on aerobic function, which is especially important in people with SCI, a designated orphan condition. Given the clinical heterogeneity and limited treatment options in this population, capturing a fuller range of cardiorespiratory responses is critical to understanding the efficacy and translational relevance of the multimodal intervention. The inclusion of these additional performance metrics will likely enhance the interpretability and generalizability of the findings.

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving one repetition maximum (1-RM) voluntary strength in the chest press exercise

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving muscle power during the chest press exercise

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving muscle endurance in the chest press exercise

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing whole body lean mass measured using dual energy X-ray absorptiometry (DXA)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing upper extremity lean tissue mass measured using DXA

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing lower extremity lean tissue mass measured using DXA

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing trunk lean tissue mass measured using DXA

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing appendicular lean tissue mass measured using DXA

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in increasing thigh muscle volume, measured using the Dixon technique with magnetic resonance imaging (MRI).

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing abdominal fat mass measured using Dixon MRI

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing visceral fat mass measured using Dixon MRI

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing liver fat mass measured using Dixon MRI

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving fasting glucose, hemoglobin A_{1C}, and HOMA-IR index of insulin sensitivity

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving plasma lipids (total cholesterol, LDL cholesterol, non-HDL cholesterol, HDL cholesterol, triglycerides) and apolipoproteins (apolipoprotein B and apolipoprotein A1)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing the circulating concentrations of inflammation markers (hsCRP, IL-6 and TNF-alpha)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving self-reported function and mobility using Spinal Cord Injury Functional Index (SCI-FI)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving depressive symptoms, ascertained using the Patient Health Questionnaire - 9 (PHQ-9)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing pain and its impact on life, ascertained using the Brief Pain Inventory (BPI)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC+ AE in improving loneliness, using a 3-item loneliness questionnaire

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in improving satisfaction with life, ascertained using the Satisfaction with Life Scale (SWLS)

- To determine whether the multimodality intervention is more efficacious than placebo plus FES-LC + AE in reducing anxiety ascertained using the 7-item Generalized Anxiety Disorder - 7 scale

Exploratory Objectives:

- Evaluation of the relationship between changes in testosterone levels and changes in outcomes in patients randomized to the testosterone arm.
- Compare the changes in aerobic capacity associated with AE, FES-LC, and FES-LC + AE
- Evaluate the role of adherence to FES-LC and AE as a potential modifier of treatment response across primary and secondary outcomes. We will evaluate several approaches to defining and quantifying adherence including accounting for factors such as medical events, COVID-19, equipment issues, and depressive symptoms.

2.2 Study Design

2.2.1 Study Design and Design Diagram

The proposed phase 2 trial is a randomized, placebo-controlled, parallel group trial in persons with C3-T12 motor level SCI, AIS grade A, B, C, or D 6 months or later after injury. The trial will test the hypothesis that a Home-Based Multimodality Functional Recovery and Metabolic Health Enhancement Program that addresses multiple pathophysiologic factors in SCI and includes functional electrical stimulation during leg cycling (FES-LC) plus arm ergometry and an androgen will be more efficacious than functional electrical stimulation during leg cycling (FES-LC) plus arm ergometry plus placebo in improving aerobic capacity, function, metabolism, bone health, and

wellbeing. The planned study enrollment is 84 subjects based on the initial assumptions of the hypothesized treatment effect and study discontinuation rate. The study will be conducted at a single trial site at the Brigham and Women's Hospital. An Interactive Response Technology (IRT) system will be used to randomize subjects to receive either the multimodality intervention including testosterone undecanoate injections or FES-LC and arm ergometry plus placebo injections in a 1:1 ratio.

2.2.2 Variables Used for Stratification at Randomization

Randomization will be stratified by sex (male, female), and age (19 to 44, 45 to 70 years). The intervention duration is 16 ± 2 weeks. We anticipate that approximately 10 to 20% of the study participants will be women.

2.3 Efficacy Endpoints

2.3.1 Primary

We selected peak aerobic capacity (VO_{2peak}) during arm ergometry as our primary endpoint because it can be measured accurately in people living with SCI in contrast to FES-LC alone which tends to have higher variability and considerably shorter test durations in people with SCI who are paraplegic. Additionally, some people with longstanding paraplegia or more complete lesions may need more or less motor assistance for leg cycling than others rendering it difficult to accurately measure the intrinsic aerobic capacity without the motor assistance. Furthermore, VO_{2peak} associated with arm ergometry in people with SCI and paraplegia who are ambulating using a wheelchair accounts for more than 90% of the whole body VO_{2peak} during the combined FES-LC + AE exercise. Peak aerobic capacity is an excellent marker of overall health,

physical function, and mortality and is closely related to metabolic health, insulin sensitivity and cardiovascular outcomes.

Secondary endpoints

- Change from baseline in VO_{2peak} achieved during FES-LC exercise alone
- Change from baseline in VO_{2peak} achieved during combined FES-LC + AE exercise
- Change from baseline in additional cardiorespiratory performance parameters, including the metabolic threshold ($\dot{V}O_2\theta$), oxygen uptake efficiency slope $\Delta\dot{V}O_2/\Delta\log_{10}\dot{V}_E$, ventilatory efficiency slope ($\Delta\dot{V}_E/\Delta\dot{V}O_2$) and total work, assessed during arm ergometry alone, FES-LC alone, and combined FES-LC+AE testing modalities.
- Change from baseline in the one repetition maximum (1-RM) voluntary strength in the chest press exercise
- Change from baseline in the muscle power in the chest press exercise
- Change from baseline in the muscle endurance in the chest press exercise
- Change from baseline in the whole body lean mass measured using dual energy X-ray absorptiometry (DXA)
- Change from baseline in the upper extremity lean tissue mass measured using DXA

- Change from baseline in the lower extremity lean tissue mass measured using DXA
- Change from baseline in the trunk lean tissue mass measured using DXA
- Change from baseline in the appendicular lean tissue mass measured using DXA
- Change from baseline in the thigh muscle volume, measured using Dixon magnetic resonance imaging (MRI). Thigh muscle mass will be assessed by MRI, using the Dixon method for separation of water and fat signals
- Change from baseline in the abdominal fat mass measured using Dixon MRI. MRI scanning using Dixon technique is noninvasive and offers greater accuracy and precision in assessing abdominal fat mass than DXA.
- Change from baseline in the visceral fat mass measured using Dixon MRI. MRI scanning using Dixon technique is noninvasive and offers greater accuracy and precision in assessing abdominal fat mass than DXA.
- Change from baseline in the liver fat mass measured using Dixon MRI. MRI scanning using Dixon technique is noninvasive and offers greater accuracy and precision in assessing abdominal fat mass than DXA.
- Change from baseline in fasting glucose, hemoglobin A_{1C}, and HOMA-IR index of insulin sensitivity.

- Change from baseline in plasma lipids (total cholesterol, LDL cholesterol, non-HDL cholesterol, HDL cholesterol, triglycerides) and apolipoproteins (apolipoprotein B and apolipoprotein A1)

- Change from baseline in the circulating concentrations of inflammation markers (hsCRP, IL-6 and TNF-alpha)

- Change from baseline in self-reported function and mobility using Spinal Cord Injury Functional Index (SCI-FI). SCI-FI is a multidimensional measure, specific for persons with SCI that assesses functional capacity in basic mobility, ambulation, self-care, and fine motor function, including wheelchair ambulation. The SCI-FI is psychometrically robust across a range of disability levels, and has high test-retest reliability, internal consistency, and construct validity, and excellent precision (113).

- Change from baseline in depressive symptoms, ascertained using the Patient Health Questionnaire - 9 (PHQ-9). PHQ-9 is a 9-item, validated, widely used measure of mood and depressive symptoms.

- Change from baseline in pain and its impact on life, ascertained using the Brief Pain Inventory (BPI). The BPI is a validated measure of pain that assesses pain intensity (sensory dimension) and interference with function (reactive dimension).

- Change from baseline in loneliness, using a 3-item loneliness questionnaire. The UCLA loneliness scale consists of 3 questions that measure three dimensions of loneliness: relational connectedness, social connectedness and self-perceived isolation.

- Change from baseline in satisfaction with life, ascertained using the Satisfaction with Life Scale (SWLS). SWLS is a 5-item scale that assesses a person's overall satisfaction with life. It's one of the most commonly used tools for measuring life satisfaction.

- Change from baseline in anxiety ascertained using the Generalized Anxiety Disorder - 7 scale (GAD-7). The GAD-7 is a screening tool and self-report scale used to assess the severity of generalized anxiety disorder (GAD); the seven-item survey asks how often a person experienced certain anxiety symptoms over the preceding two weeks.

2.3.4 Exploratory Endpoints

- The association between changes in testosterone levels and changes in aerobic capacity and other endpoints in patients randomized to testosterone arm.

2.4 Sample Size Justification

We plan to recruit 84 subjects, based on the following considerations: 1. type-I error probability 0.05; 2. randomization in a 1:1 ratio and stratification by sex (male, female) and age (19-40, 41-60); 3. a loss-to-follow-up rate of 20% resulting in 70 subjects completing the intervention. This assumption of 20% loss to follow up rate was based on experience from intervention trials conducted by co-I Zafonte in people living with SCI. There are no published trials of the combined administration of testosterone, arm cycle ergometry, and FES-LC in people with SCI. Therefore, the sample size estimates were guided by data on the effects of FES-LC and cycle ergometry in people with SCI,

and the effects of testosterone in hypogonadal men and in people with SCI. This study was powered to detect clinically meaningful differences in within-subject change in the primary outcome – aerobic exercise capacity - attributable to randomization to multi-modality intervention vs. FES-LC+ AE plus placebo injections. Based on previous studies, we hypothesize that there will a significant improvement in aerobic exercise capacity (+10%) in participants receiving FES-LC + AE plus placebo. Subjects receiving multimodality intervention will exhibit greater gains in aerobic capacity associated with AE than those associated with FES-LC+ AE exercise. The gain attributable to FES-LC plus arm cycle ergometry have averaged around 20% in previous studies, although there is considerable heterogeneity (16-27). Therefore, we hypothesize that addition of testosterone to FES-LC + AE will augment the treatment effect such that the mean gain in peak oxygen uptake attributable to multimodality intervention will be 25% (SD = 20%). Thus the treatment effect will be 25% improvement in peak aerobic capacity (SD no greater than 33%). Under the assumed effects described above, simulation studies estimate that the proposed primary mixed-effects analysis will have 90% power to detect an effect size $f = 0.75$ SD with conservative assumption of high within subject autocorrelation. These changes are clinically meaningful because improvements in aerobic exercise capacity of 8-10% have been associated with improvements in outcomes in people with SCI (16-20) and chronic obstructive lung disease.

We anticipate that multimodality intervention will increase lean body mass by an average 2.4 kg whereas the FES-LC + AE exercise alone will increase lean body mass by 0.5 kg. A sample size of 84 has more than 90% power to detect the hypothesized difference of 1.9 kg (SD 2.5 kg) between the two arms.

We anticipate that multimodality intervention would improve HOMA-IR insulin sensitivity index by ~30%, and the FES-LC + AE alone by ~ 10%. A sample size of 84 subjects has 80% power to detect this difference of 20% between intervention arms (SD no more than 30%). These assumptions are reasonable because in previous studies, FES and cycle ergometry have been shown to improve HOMA-IR by ~25% (26, 29). Also, testosterone administration to hypogonadal men has been associated with an average 20% improvement in HOMA-IR index; conversely, sex-steroid withdrawal in men was associated with 25-30% decrease in insulin sensitivity (54).

Similarly, the trial has sufficient power to detect effect sizes of 0.75 SDs or larger associated with the administration of multimodality intervention relative to FES-LC + AE for all secondary outcomes.

2.5 Interim Analysis

No interim analysis is planned for this sub-study.

2.6 Multiplicity Testing Procedures for Type-I Error Control

Type I error adjustments for multiple comparisons are not planned for efficacy endpoints, subgroup analyses, supportive analyses or sensitivity analyses for this sub-study. Spinal cord injury is an orphan condition and the sample size in randomized trials of complex interventions is limited by the low prevalence of the condition and the other life barriers that render participation in complex intervention trials challenging for people living with spinal cord injury.

2.7 Missing Data

If required (e.g. by referees in publication), multiple imputation of endpoints and covariates by the MICE methodology[12,13] may be considered where appropriate (i.e. for data reported in the manuscripts). This method is notable for being able to handle clustering of repeated measures at the participant level, a feature of the design of this trial and sub-study. Under such circumstances, we would consider whether to impute data for all sub-studies simultaneously.

3.0 Analysis Populations and Important Subgroups

3.1 Analysis Population

The Full Analysis Set (FAS) will include all randomized subjects. Subjects will be categorized according to treatment assigned at randomization. The FAS will be used for the summary of subjects' disposition and demographics and baseline characteristics. The FAS will be used for assessment of primary and secondary efficacy endpoints.

The Safety Set will include all randomized subjects who received at least one dose of the study medication.

Statistical analyses of treatment effect over time (e.g., change from baseline in VO_{2peak}) will comprise all randomized subjects, who have baseline and at least one post-randomization measurement.

A modified analysis set for participants censored at any time (and subsequently) that they are deemed treatment noncompliant per protocol, will be also considered and used for sensitivity analyses of efficacy endpoints.

Data from eligible measurements will be included, where eligible is defined as being obtained from questionnaires or scores for which at least 70% of items are non-missing.

3.2 Subgroup analyses

The following subgroup analysis will be carried out for the main study and may be also considered for the bone sub-study:

- By sex recognizing that the number of women is expected to be small
- By age (18 to 44, 45 or older)

Additional sensitivity analyses of primary and some secondary endpoints adjusting for adherence and fidelity measures (number of exercise sessions, total work done during the exercise, and effort level during training) may be undertaken, recognizing that the collection of the adherence measures by study participants during home-based exercise may be variable, especially during the COVID-19 pandemic.

4.0 Analysis Conventions

4.1 Definition of Baseline

Baseline on each outcome measure will be defined as the last available measurement obtained prior to the first dose of study drug (defined as on or before Day 1)

4.3 Definition of Visit Windows

Definitions of the visit windows (baseline and on-treatment) are described in the study protocol.

5.0 Demographics, Baseline Characteristics, Medical History and Study Drug Exposure

5.1 Baseline Characteristics

Descriptive analyses of baseline characteristics will be conducted in the Full Analysis Set that includes all randomized participants. Safety analyses will be conducted in the Safety Set that included all randomized participants who received at least one dose of study drug. We will assess the distributional characteristics of outcomes and covariates. Data collected in this study will be documented using summary tables. Statistics for continuous variables will include mean, standard deviation, median, quartile range, minimum, maximum, and sample size for each treatment group, and two-sided 95% confidence intervals of the mean difference between the treatment groups. Binary variables will be described with frequencies, percentages, and two-sided 95% confidence intervals of the difference in percentages between treatments. Where appropriate, transformation of variables to combat skew, or other irregularities will be employed. Comparability of groups will be assessed using graphical methods.

5.2 Treatment Exposure and Adherence

Adherence with the study drug will be computed by dividing the total number of doses that were administered, expressed as a percent of the total number of doses that should have been administered per protocol during the intervention period.

Adherence with the exercise intervention will be expressed in terms of 1) the number of days engaged in the prescribed exercise expressed as a percent of exercise sessions that should have been conducted; and 2) total work done during the home-based exercise.

6.0 Analysis of Endpoints

6.1 Primary Analysis

The effect of the multimodality intervention on the change over time in the VO_{2peak} versus placebo plus FES-LC + AE control will be analyzed using the mixed model repeated measures (MMRM) regression. Change will be defined as the difference from the baseline value. Models will consider visit, treatment effect, visit-by-treatment interaction, stratification factor (age) and baseline value as fixed effects. Repeated measure effect will be considered at participants' level. Unstructured covariance matrix will be assumed, however if convergence of the model is not achieved, then a compound symmetry structure will be utilized. Effects of intervention over the treatment period will be calculated as an average score from 8 and 16-week visits, and will be extracted, along with two-sided 95% confidence intervals and P values, from the mixed-model framework.

Sensitivity analyses of treatment effect may also be performed where appropriate after including in the model other fixed effects such as stratification factor (sex), and adherence measures.

6.2 Analysis of Secondary Endpoints

The intervention effects on secondary outcomes (e.g., 1-RM strength, SCI-FI scores; wellbeing measures; plasma lipids, apolipoproteins and lipoprotein particles; insulin sensitivity; and inflammation markers) will be analyzed using an approach similar to that for Aim 1, using mixed effects linear regression model controlling for stratification factor and baseline values, accounting for repeated measures at participant's level. Lean and fat mass, abdominal and liver fat, and insulin sensitivity will be examined by ANCOVA model with treatment and baseline value included with an approach similar to that for Aim 1.

Sensitivity Analyses will determine if pre-specified covariates influence the effect estimates. We will use a dimensionality-reduction strategy, using propensity scoring as a global adjustment factor. The following variables will be considered: sex; age (19-45, >45); baseline function; and adherence. Additional covariates may be incorporated based on exploratory analyses. We will perform per-protocol analyses on completers. Also, per-protocol analysis of subjects, who complete the trial, adjusting for adherence will be performed.

Treatment Adherence will be assessed as the proportion of scheduled injections actually administered, the number of home-based exercise sessions undertaken, and the total work done during the home-based exercise. A sensitivity analysis will employ

adjustment for adherence.

Analyses will not be adjusted for multiple comparisons because the hypotheses being tested are pre-specified; furthermore, the outcomes are highly correlated, making such adjustments overly conservative. This approach also recognizes that SCI is an orphan indication.

Safety Analyses

The Safety Assessment will involve tabulation of Adverse Events (AEs) and Serious Adverse Events (SAEs), and assessment of differences in event rates as appropriate. Events will be classified according to MedDRA and SOC coding system and tabulated as absolute number of events and number and proportion of participants experiencing one or more event. Testing of differences between the proportions of individuals with one or more events across arms will use multiple regression analyses with control for design effects.

Sub-Group Analyses

We will performed sub-group analyses separately for women and men and in both age groups (stratification factors in the design). Additionally, primary analysis will be assessed for robustness to control for age, sex, baseline function, and comorbid conditions. We will also consider the potential for sex to modify the effect of intervention on outcomes using interaction terms. However, this proof-of-principle study does not have sufficient power to determine sex differences. Potential sex-specific adverse effects (e.g., hair growth or menstrual irregularity in women, and prostate-related

adverse events in men) will be recorded. Additional sensitivity analyses of primary and some secondary endpoints adjusting for adherence measures (number of exercise session, total work done during the exercise, and effort level during training) may be undertaken, as described earlier.