Statistical Analysis Plan

An Open-Label Pilot Study of Losmapimod to Evaluate the Safety, Tolerability, and Changes in Biomarker and Clinical Outcome Assessments in Subjects with Facioscapulohumeral Muscular Dystrophy 1 (FSHD1)

PROTOCOL NO.	FIS-001-2019
Sponsor:	Fulcrum Therapeutics
CRO:	[redacted]
Version:	6.0
Date:	12DEC2023

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9DA02ABFF5A4477CBE9C10FF32C8CD42

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74A107B27616439DA97C0E01E3D31DFF

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Signing Time: 1/5/2024 | 9:52:11 AM EST
17F80896841A423799B690C9F322916E

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Signing Reason: I approve this document
Signing Time: 12/13/2023 | 3:01:59 PM EST
3A430D4441A640CE930CFE93A3751B65

Signature
Date: 12/13/2023

DOCUMENT REVISON HISTORY

VERSION	DATE	AUTHOR/ UPDATED BY	COMMENTS
V1.0	11-MAR-2020	NA	NA
V1.1	20-JUL-2020	[redacted]	1. Added three exploratory endpoints in section 1.2.3: 3. Skeletal muscle tissue replacement by fat using whole-body MRI • Lean Muscle Volume (LMV) • Muscle Fat Fraction (MFF) • Muscle Fat Infiltration (MFI) 2. Added some endpoints in section 4.1 IA#2 analysis, see below: • Summary tables of classic TUG and FSHD-optimized TUG. • PK Summaries • Summary table of muscle strength by quantitative dynamometry • Summary table of distance of walking measured by 6-MWT • Summary table of reachable work space (RWS) • Summary table lung ventilator function as measured by Spirometry • Summary table of physical function by MFM • Summary table of FSHD-HI total score • Summary table patient global impression of change • Summary table of FSHD-RODS total score Updated "Lean Muscle Mass" to "Lean Muscle Volume" in section 4.1 IA#2 analysis 3. Added more details for derivation of composite MRI endpoints in Appendix 6

			4. Added muscle PK parameters of losmapimod in section 1.2.2 and section 3.3 5. ~~Added "ratio to baseline" analysis for pHSP27/total HSP27~~ 6. Added missing data handling rule for missing DUX4 composite score in section 2.3.5 7. Deleted the summary of "the reasons for screen failure" in section 3.1.1 8. Deleted "number of years the subject had the disease" and "duration of time from diagnosis to the start of losmapimod" and updated "number of D4Z4 repeats" to "number of FSHD repeats" in section 3.1.3
V1.2	13-AUG-2020	[redacted]	1. Added "median imputation" in section 2.3.5 for "DUX4 COMPOSITE SCORE"
V2.0	25-SEP-2020	[redacted]	1. Removed muscle PK parameters of losmapimod in section 1.2.2 and section 3.3 in order to align with protocol version 3. 2. Removed muscle fat fraction (MFF) and muscle fat infiltration (MFI) parameters in section 1.2.3 in order to align with protocol version 3. 3. Updated appendix 4 to add 6th gene for the subset and steps for determining the top 2 transcripts.
V2.1	17-MAR-2021	[redacted]	1. Added open-label extension objectives and endpoints to align with protocol version 5. 2. Updated figure 1 in order to align with protocol version 5. 3. Added visit windows in table 1 to capture additional visits specified in protocol version 5. 4. Added muscle PK parameters of losmapimod in section 1.3.2 and section 3.3 in order to align with protocol version 5. 5. Updated dynamometry section 3.4.8 and added appendices 7, 8, 9, and 10 to clarify analysis. 6. Added manual muscle testing section 3.4.9 to align with protocol version 5. 7. Updated reachable work space section 3.4.4 to clarify descriptive statistics. 8. Updated MedDRA version to version 23.0 9. Added sensitivity analysis section 3.6.
V2.2	26-MAR-2021	[redacted]	1. Updated secondary endpoint description in section 1.3.2. 2. Updated visit windows in table 1. 3. Clarified dynamometry analysis in section 3.4.8.
V2.3	23APR2021	[redacted]	1. Added study extension disposition details to section 3.1.1. 2. Added additional demographics detail to section 3.1.3.

			3. Add treatment compliance definition to section 3.1.6. 4. Added exposure detail to section 3.2.1. 5. Added clarifying text for cohorts in section 3.3. 6. Updated dynamometry normalization calculation in section 3.4.8. 7. Changed MMT statistics from change from baseline to change from first observation.
V2.4	30APR2021	[redacted]	1. Removed 'optimized' from FSHD TUG. 2. Updated treatment compliance section 3.1.6 to clarify dose for main study. 3. Exclusion details added to MRI section 3.4.2. 4. Text added to dynamometry section 3.4.8 to clarify presentation of dominant vs non-dominant. 5. Text added to summary of major changes section 5 for presentation of FSHD-RODS and FSHD-HI data.
V3.0	10JUN2021	[redacted]	1. Clarified protocol deviation section 3.1.2 by adding 'significant' to description. 2. Text added to summary of major changes section 5 for presentation of MRI data. 3. Clarified in section 3.4.8 that both normalized and raw hand-held data will be analyzed. 4. Removed item 5 (ratio to baseline) from document revision history for version 1.1 5. Added correlation analyses to section 3.4.2 6. Added details about visit observation calculation to section 3.4.2
V4.0	20OCT2021	[redacted]	1. Updated SAP adjusted protocol windows to reflect error with EOS visit day 2. Add (Q1+Q2+Q3+Q4) to section 3.4.4. 3. Add percent change from baseline to manual dynamometry in section 3.4.8. 4. Appendix added for MMT mapping rules 5. Add MMT individual muscles to section 3.4.9.
V4.1	30SEP2022	[redacted]	1. Removed study assessments for extension: MRI, discovery biomarkers, RWS, TUG, muscle ultrasound, MMT, MFM, dynamometry, FSHD-RODS, FSHD-HI, PGIC, 6-MWT, and spirometry 2. Updated study objectives and endpoints for the extension to reflect removed study assessments 3. Removed language specifying that the 15 mg dose of study drug is administered as two 7.5 mg tablets 4. Updated analysis windows for new extension visit schedule
V6.0	07DEC2023		1. Updated analysis window in section 2.3.5 by adding week 216, 228, 240, 252, 264, and 276

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ABBREVIATIONS

Abbreviation	Term
AE	Adverse Event
C_{max}	Maximum observed plasma concentration
CRF	Case Report Form
CSR	Clinical Study Report
DUX4	Double homeobox 4
ECG	Electrocardiogram
EOS	End of study
EOT	End of treatment
FEV_1	Forced expiratory volume in 1 second
FSHD	Facioscapulohumeral muscular dystrophy
FSHD-HI	Facioscapulohumeral muscular dystrophy – Health Index
FSHD-RODS	FSHD Rasch-built Overall Disability Scale
FVC	Forced vital capacity
HSP27	Heat Shock Protein 27
MedDRA	Medical Dictionary for Regulatory Activities
MFM	Motor Function Measure
MMT	Manual muscle testing
MRI	Magnetic resonance imaging
PD	Pharmacodynamic(s)
PGIC	Patient Global Impression of Change
pHSP27	Phosphorylated HSP27
PK	Pharmacokinetic(s)
PT	Preferred
qPCR	Quantitative polymerase chain reaction
QTcF	QT interval by Fredericia
RWS	Reachable Work Space
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
SI	Système International
SE	Standard error
TEAE	Treatment-emergent Adverse Event
T_{max}	Time to reach maximum observed plasma concentration
TUG	Timed Up and Go
QMT	Quantitative myometry testing

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used to analyze, and report results for Protocol FIS-001-2019.

1.1 MAIN STUDY OBJECTIVES

1.1.1 Primary Objective

The primary objective of this study is to evaluate the safety and tolerability of long-term dosing of losmapimod tablets in subjects with facioscapulohumeral muscular dystrophy 1 (FSHD1).

1.1.2 Secondary Objectives

The secondary objectives of the study are:

1. To assess target engagement of losmapimod tablets in blood and skeletal muscle over long-term dosing

2. To evaluate repeated dose pharmacokinetics (PK) of losmapimod tablets in subjects with FSHD1 over long-term dosing

1.1.3 Exploratory Objectives

Exploratory objectives of the study are:

1. To evaluate on-treatment change in target engagement and DUX4 activity and other disease transcripts in skeletal muscle needle biopsy

2. To evaluate on-treatment change in skeletal muscle by [redacted]

3. To evaluate on-treatment change in skeletal muscle function by clinical outcome assessments

4. To evaluate on-treatment change in upper and lower limb mobility in the outpatient setting

5. To evaluate on-treatment change in lung ventilatory function

6. To evaluate on-treatment change in circulating proteins associated with DUX4 expression or muscle injury or repair

1.2 EXTENSION OBJECTIVE

1.2.1 Primary Objective

To evaluate the safety and tolerability of long-term dosing of losmapimod tablets in subjects with FSHD1

1.3 MAIN STUDY ENDPOINTS

1.3.1 Primary Endpoint(s)

The primary endpoint is the assessment of safety and tolerability based on adverse events (AEs), serious adverse events (SAEs), clinically significant laboratory test results, electrocardiograms (ECGs) and vital signs (safety endpoints)

1.3.2 Secondary Endpoint(s) / Pharmacodynamic

Secondary endpoints are as follows:

1. Changes from baseline in pHSP27, total HSP27, and in the ratio of pHSP27/total HSP27 as measured by sorbitol stimulated peripheral whole blood (pharmacodynamic [PD] endpoint).
2. Changes from baseline in pHSP27, total HSP27, and in the ratio of pHSP27/total HSP27 in muscle during the dosing period (pharmacodynamics [PD] endpoint).
3. Plasma and muscle concentrations of losmapimod (pharmacokinetic [PK] endpoint)

1.3.3 Exploratory Endpoint(s)

Changes from baseline during the dosing period in the following:

1. DUX4 activity and disease specific gene transcripts by quantitative polymerase chain reaction (qPCR) of skeletal muscle using a subset of DUX4-regulated and other disease specific gene transcripts
2. Skeletal muscle lean tissue volume by whole-body magnetic resonance imaging (MRI)
3. Skeletal muscle tissue replacement by fat using whole-body MRI
4. Correlations between MRI composite measures of classic TUG, FSHD TUG, and RWS and traditional measures of classic TUG, FSHD TUG, and RWS
5. Skeletal muscle echogenicity by ultrasound (selected muscles)
6. Reachable Work Space (RWS) with and without weights

7. Ambulatory function by classic and FSHD TUG
8. Physical function by Motor Function Measure (MFM) domain 1
9. Muscle strength by quantitative manual dynamometry
10. Disease impact by subject report using FSHD-Rasch-built Overall Disability Scale (FSHD-RODS)
11. Disease impact by subject report using FSHD-Health Index (FSHD-HI)
12. Disease impact by subject report using Patient Global Impression of Change (PGIC)
13. Upper and lower limb mobility in the outpatient setting using wearables
14. Change in ambulation as measured by the 6-minute walking test (6-MWT)
15. Change in lung ventilatory function as measured by Spirometry

1.4 EXTENSION ENDPOINT

1.4.1 Primary Endpoint

The primary endpoint is the safety and tolerability based on AEs, clinical laboratory test results, ECGs, and vital signs.

1.5 SUMMARY OF THE STUDY DESIGN

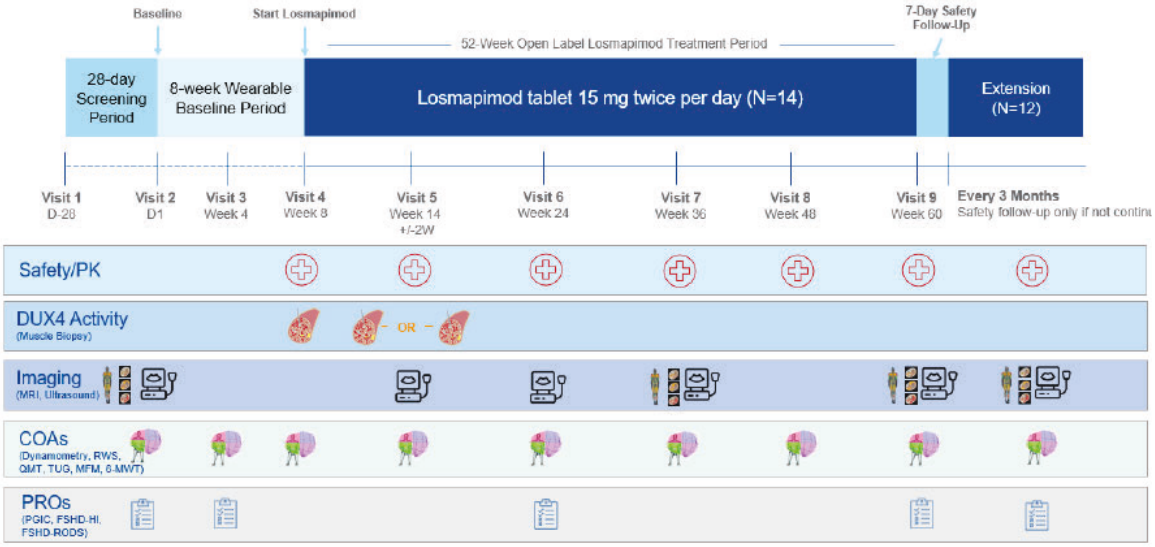
1.5.1 General Study Design and Plan

This is a single-center, open-label pilot study that will investigate the safety, tolerability, PK and target engagement during long-term dosing with losmapimod tablets in adult subjects with FSHD1. Subjects will be evaluated during an 8-week pre-treatment period (baseline) and will be treated with losmapimod for approximately 1 year and assessed at regular intervals for change from the baseline assessment.

During the extension, subjects will attend clinic visits approximately every 24 weeks and have a safety phone call 12 weeks between in-person clinic visits until study drug approval or until study termination. The extension of this study will enable continued investigation of the safety and tolerability during long-term dosing with losmapimod tablets in adult subjects with FSHD1.

The study design is depicted in Figure 1 below:

Figure 1 Study Design Schema



6-MWT=6-minute walking test; D=day; COAs=clinical outcome assessments; DUX4=double homeobox 4; FSHD-HI=facioscapulohumeral muscular dystrophy-Health Index; FSHD-RODS=FSHD-Rasch-built Overall Disability Scale; MFM=Motor Function Measure; MRI=magnetic resonance imaging; PGIC=Patients' Global Impression of Change; PK=pharmacokinetics; PRO=patient-reported outcome; QMT=quantitative myometry; RWS=Reachable Workspace; TUG=Timed Up and Go; W=week.
Only subjects terminating the study after Week 60 will have the Visit 10 End of Study visit. Subjects proceeding to the extension will continue to Week 72 visit.
Note: All visits during the extension will have a ± 4-week window; early termination visits can occur at any time.

1.5.2 Sample Size and Statistical Power Considerations

Sixteen subjects are planned to be treated with losmapimod. While this sample size is not based on statistical considerations, a sample size of 16 subjects receiving losmapimod is considered sufficient to obtain estimates of the safety, tolerability, PK, and target engagement properties of losmapimod tablets over long-term dosing and to inform initial assumptions on the potential impact of treatment on biomarkers and clinical outcome assessments compared to the pre-treatment period.

2 STATISTICAL CONSIDERATIONS

2.1 GENERAL CONSIDERATIONS

All descriptive statistics for continuous variables will be reported using mean, standard deviation (SD), median, minimum, and maximum. Standard error (SE) will be reported for all change from baseline tables. Categorical variables will be summarized as number of subjects and their percentages.

2.2 DEFINITIONS OF ANALYSIS SETS

There is only one analysis set for the study, i.e. safety analysis set, which is defined as subjects who received at least one dose of losmapimod.

2.3 DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

2.3.1 Baseline Definition

Baseline: Baseline value is defined as last observed measurement occurring prior to the study drug.

2.3.2 Study Day

Study Day will be calculated from the reference start date and will be used to show start/stop day of assessments and events. Reference start date (Day 1) is defined as the first day of treatment. Study Day = date of event – reference start date + 1. In the situation where the event date is partial or missing, Study Day, and any corresponding durations will appear partial or missing in the listings.

2.3.3 End of Treatment

End of treatment: The definition of end of treatment is the last non-missing visit post-baseline that the subject is on treatment.

2.3.4 End of Study

End of study: The definition of end of study is the subjects' last completed visit.

2.3.5 Analysis Visit Windows

The following non-overlapping visit windows will be used to assign data to visits for data analyses:

Table 1 Visit windows for measurements

Adjusted Defined Windows Visit	Scheduled Study Day	Maximum Windows
Baseline Visit (First dose/Day 1)	Day 1	<= Day 1
Week 4 post-treatment	Day 28	>Day 1 to <=Day 42
Week 8 post-treatment	Day 56	>Day 42 to <=Day 98
Week 20 post-treatment	Day 140	>Day 98 to <=Day 182
Week 32 post-treatment	Day 224	>Day 182 to <=Day 266
Week 44 post-treatment	Day 308	>Day 266 to <=Day 350
Week 56 post-treatment	Day 392	>Day 350 to <=Day 406
60 Weeks post-treatment (EOS, Protocol Visit 10)	Day 420	>Day 406
64 Weeks post-treatment (Protocol Week 72)	Day 448	>Day 406 to <=490

Adjusted Defined Windows Visit	Scheduled Study Day	Maximum Windows
76 Weeks post-treatment (Protocol Week 84)	Day 532	>Day 490 to <=Day 574
88 Weeks post-treatment (Protocol Week 96)	Day 616	>Day 574 to <=Day 658
100 Weeks post-treatment (Protocol Week 108)	Day 700	>Day 658 to <=Day 742
112 Weeks post-treatment (Protocol Week 120)	Day 784	>Day 742 to <=Day 826
124 Weeks post-treatment (Protocol Week 132)	Day 868	>Day 826 to <=Day 952
148 Weeks post-treatment (Protocol Week 156)	Day 1036	>Day 952 to <=Day 1120
172 Weeks post-treatment (Protocol Week 180)	Day 1204	>Day 1120 to <=Day 1288
196 Weeks post-treatment (Protocol Week 204)	Day 1372	>Day 1288 to <=Day 1456
220 Weeks post-treatment (Protocol Week 228)	Day 1540	>Day 1456 to <=Day 1624
244 Weeks post-treatment (Protocol Week 252)	Day 1708	>Day 1624 to <=Day 1792
268 Weeks post-treatment (Protocol Week 276)	Day 1876	>Day 1792 to <=Day 1960

Only subjects terminating the study after Week 56 post-treatment will have the Week 60 follow-up (EOS) visit. Subjects proceeding to the extension will continue to 64 Weeks post-treatment (protocol week 72) visit. Subjects will remain in the extension until 90 days after commercial drug is available post regulatory approval or until the study is discontinued by the sponsor. During the extension, subjects will attend clinic visits approximately every 24 weeks. All subjects who complete or discontinue from treatment will complete a safety follow-up visit 7 days (±3 days) after the last dose of study drug.

If multiple visits are recorded in the same analysis window, the later visit will be used in the analysis.

2.3.6 Missing Data Handling Rules

ADVERSE EVENTS

Partial AE onset dates will be imputed as follows:

1. If the onset date of an adverse event is missing day only, it will be set to:

a. First day of the month that the AE occurred, if month/year of the onset of AE is different from month/year of medication.

b. The day of treatment if the month/year of the onset of AE is the same as month/year of treatment and month/year of AE resolution date is different.

c. The day of treatment or day of AE resolution date, whichever is earlier, if month/year of the onset of AE and month/year of treatment and month/year of the AE resolution data are same.

2. If the onset data of an adverse event is missing both day and month, it will be set to the earlier of:

   a. January 1 of the year of onset, as long as this date is after the date of treatment.

   b. Month and day of treatment if the date is the same year that the AE occurred.

   c. The AE resolution date.

3. Completely missing AE onset dates will not be imputed.

Partial AE resolution dates not marked as ongoing will be imputed as follows:

1. If the resolution date of an AE is missing day only, it will be set to the earliest of the last day of the month of occurrence of resolution or the day of the date of death, if the death occurred in that month

2. If the resolution date of an AE is missing both day and month, it will be set to the earliest of December 31 of the year or the day and month of the date of death, if the death occurred in that year

3. Completely missing resolution dates will not be imputed.

Missing or partial time will not be imputed.

If the severity is missing for an AE, then it is considered as unknown. If relationship of AEs to the study medication could not be derived (i.e., missing or unknown), it will be considered as related, for analysis purpose.

DUX4 COMPOSITE SCORE

Missing composite score will be imputed by three methods as follows

1. Mean Imputation: For subjects missing “pre”, impute using the MEAN(pre) of non-missing subjects. For subjects missing “post”, impute using MEAN(post) from non-missing subjects.

2. Worst case imputation: For subjects missing “pre”, impute using MIN(pre) of non-missing subjects. For subjects missing “post”, impute using MAX(post) from non-missing subjects.

3. Median Imputation: For subjects missing "pre", impute using the MEDIAN(pre) of non-missing subjects. For subjects missing "post", impute using MEDIAN(post) from non-missing subjects.

2.3.7 Other Data Points Definition

NA

2.4 Multiple Comparisons/Multiplicity

No formal statistical tests will be employed in the study, therefore there is no multiplicity adjustment for type I error to be made.

2.5 Examination of Subgroups

Depending on the number of subjects in each sub-category, subgroup analyses may be conducted on [redacted]

3 STATISTICAL ANALYSES

3.1 Subject Information

Unless indicated otherwise, the results for subject information will be based on the safety analysis set.

3.1.1 Disposition of Subjects

The number of subjects screened and the number (percent) of subjects who failed screening will be summarized.

Study Completion: The number (percent) of treated subjects who completed the study (Week 60) and who discontinued from the study will be summarized according to the reasons for discontinuation.

Entered Study Extension: The number (percent) of treated subjects who entered the extension of the study.

3.1.2 Protocol Deviations

The number and percentage of subjects with significant protocol deviations will be presented in a summary table. Line listings of all collected protocol deviations will also be presented.

3.1.3 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized. The continuous variables will be summarized using descriptive statistics (n, mean, standard deviation [SD], median

and range [minimum and maximum]). The minimum and maximum observations will be presented to the same number of decimal places as the original data. The mean and median will be rounded to one more decimal place than the original data, while the SD will be to two more decimal places. The categorical variables will be summarized by absolute and relative frequencies. There are no formal hypotheses regarding baseline demographics and characteristics, hence no p-values will be provided.

Continuous demographic and baseline variables include age (years), baseline weight (kg), body mass index (BMI) in kg/m^2, etc. Categorical variables include sex (male, female, undifferentiated), race (White, Black or other), age group (18 to < 50 years, >=50 to <= 65 years, Ethnicity (Hispanic or Latino, not Hispanic or Latino), etc.

The status of the disease at screening will be summarized according to his/her age at diagnosis, number of FSHD repeats, Ricci score, and family history of FSHD. Subject's age at diagnosis will be summarized as a continuous variable and as a categorical variable, with categories of <18, ≥18 to < 50 years, and ≥ 50 to <= 65 years.

3.1.4 Medical History

Medical History will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 23.0. The number of subjects and their percentages reporting a history of any medical condition will be summarized by System Organ Class (SOC) and preferred term (PT). A subject data listing of medical and surgical history will be provided.

3.1.5 Prior and Concomitant Therapy

All investigator terms for medications will be coded using the World Health Organization (WHO) Drug Dictionary (Global B3, March 2019). The number (percent) of subjects who took prior and concomitant medications will be summarized using Anatomical Therapeutic Chemical (ATC) Classification, and WHO Drug preferred term.

Prior medications will be defined as medications that started before the first dose of study drug, regardless of when the medication ended. Concomitant medications will be defined as medications continued or newly received on or after the first dose date of study drug, up to 7 days following the last dose. A given medication can be prior, concomitant, or both.

3.1.6 Treatment Compliance

Descriptive summary statistics will be provided for time on treatment and compliance for all patients.

Mean, standard deviation, median, minimum, and maximum amount of study drug returned will be provided. The cell frequencies and percentage of patients in each category (<80%, >=80%) will be provided. Compliance for the main study will be calculated as (actual dose/planned dose)*100 where planned dose is 7.5 mg*2*2*total duration of treatment. Compliance for the study extension will be calculated as (actual dose/planned dose)*100 where planned dose is 7.5 mg*2*2*total duration of treatment when 2 tablets were given plus 15 mg*2*total duration of treatment when 1 tablet was given.

3.2 ANALYSIS FOR PRIMARY ENDPOINTS

Safety variables include treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), clinical laboratory parameters, vital signs, 12-lead electrocardiogram (ECG) results, and extent of exposure. Study Day 1 for all safety analyses is defined as the date of the first dose of study drug.

3.2.1 Extent of Exposure

Descriptive summary statistics will be provided for time on treatment.

Time on treatment in days will be derived using the following formula:

Time on treatment (days) = (date of last dose) – (date of first dose) + 1If the last dose date is missing, or if a subject is lost to follow-up, and the drug accountability log confirms that the subject has taken the study drug, the visit date following the last completed drug accountability log will be used.

3.2.2 Adverse Events (AEs)

The AE verbatim descriptions (investigator terms from the case report forms [CRF]) will be classified into medical terminology using the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be coded to primary System Organ Class (SOC) and preferred term (PT) using MedDRA, version 23.0.

A TEAE is defined as an AE that meets any of the following conditions:

- begins on or after the first dose of study drug and before the stop of study drug + 7 days;
- begins before the first dose of study drug and worsens on or after the first dose of study drug and before the stop of study drug + 7 days;
- is completely missing an onset date and an end date;

- is completely missing an onset date and the end date is on or after the first dose of study drug.

Only those AEs that are treatment emergent will be included in summary tables. All AEs, treatment emergent or otherwise, will be presented in subject data listings.

In summary tables, the incidence of TEAEs will be reported as the number of subjects and their percentages with TEAEs within SOC and PT. Subjects will be counted only once within a SOC and PT, even if the subject experienced more than one TEAE within a specific SOC and PT. The number of subjects and their percentages with TEAEs will also be summarized by maximum severity (mild, moderate, or severe) (or by highest common terminology criteria for AEs [CTCAE] grade).

3.2.2.1 Deaths, Serious and Other Significant Adverse Events

The number of subjects and their percentages with TEAEs leading to death will be summarized by MedDRA SOC and PT. A subject data listing of all AEs leading to death will be provided.

Note: A summary table will not be provided if there are no deaths in the study.

The number of subjects and their percentages with treatment-emergent SAEs will be summarized by MedDRA SOC and PT. A subject data listing of all SAEs will be provided.

The number of subjects and their percentages with TEAEs leading to discontinuation from study treatment will be summarized by MedDRA SOC and PT. A subject data listing of all AEs leading to discontinuation from study treatment will be provided.

The number of subjects and their percentages with TEAEs and the relation to study treatment will be summarized by MedDRA SOC and PT.

3.2.3 Clinical Laboratory Parameters

Laboratory values that are non-missing and reported as 'below the detectable limit' of an assay will be replaced by ½*LLOQ. Values that are reported as 'above the detectable limit' will be replaced by 1.5* ULOQ, to allow for summary tables to be generated. Laboratory results will be summarized using International System of Units (SI).

For all quantitative parameters listed in Section 8.1.1 of the protocol, the actual value and the change from baseline to each post-baseline visit and to the end of treatment will be summarized by visit, using descriptive summary statistics. Qualitative parameters will be summarized using frequencies (number and percent of subjects), and changes from baseline to each post-baseline visit and to end of treatment will be reported using shift tables. Percentages will be based on the number of subjects with both non-missing baseline and relevant post-baseline results.

Laboratory test results will be assigned an LNH classification according to whether the value is below (L), within (N), or above (H) the laboratory parameter's reference range. Within-treatment comparisons for each laboratory parameter will be based on 3-by-3 tables (shift tables) that compare the baseline LNH classification to the LNH classification at each post-baseline visit and at the end of treatment. Similar shift tables will also compare the baseline LNH classification to the LNH classification for the highest and lowest value during the treatment period.

3.2.4 Vital Signs, Physical Examination Findings, and ECG

3.2.4.1 Vital Signs

Descriptive statistics for vital signs parameters (diastolic and systolic blood pressure, pulse rate, respiration rate, temperature, weight (if collected) and changes from baseline will be presented by visit.

Vital sign results will also be assigned an LNH classification with respect to the normal ranges. Comparisons will be based on shift tables that compare the baseline LNH classification to the LNH classification at each visit. Shift tables will be used to compare the baseline LNH classification to the LNH classification for the highest and lowest value during the treatment phase.

3.2.4.2 Physical Examinations

Physical examination (PE) results are collected at screening (baseline). Each component of the baseline PE will be recorded as normal or abnormal and summarized using frequencies and percentages. Symptom-directed PE may also be performed at the discretion of the investigator. All PE data will be available in data listings.

3.2.4.3 Electrocardiogram (ECG)

ECG assessments are performed at visit 1 and visits 4-10. During the extension, ECGs are not mandatory assessments and will be carried out at the discretion of the investigator. Descriptive statistics for ECG parameters and changes from baseline will be presented.

Shift tables will present changes from baseline in ECG interpretation (categorized as normal; abnormal, not clinically significant; and abnormal, clinically significant) to end of treatment, by visit.

In addition, the number of subjects and their percentages with at least 1 post-baseline abnormal ECG result in QTc Bazett and QTc Fridericia during the treatment period will be summarized. Clinically abnormal ECG results in QTc Bazett and QTc Fridericia will be categorized as follows:

- Increase in QTc of > 60 msec from baseline
- QTc value of > 450 msec and ≤ 500 msec
- QTc value > 500 msec

3.3 ANALYSES FOR SECONDARY ENDPOINTS

Phosphorylated HSP27 (PD endpoint)

Phosphorylated HSP27 (pHSP27) and total HSP27 (tHSP27) in peripheral whole blood with ex-vivo sorbitol stimulation and in skeletal muscle (without sorbitol stimulation) will be measured at the time points specified in the schedule of assessments (Tables 2 and 3 of the protocol).

The Visit 4 (pre-treatment) value will be defined as the baseline value for change from baseline calculations.

All pHSP27 and tHSP27 values will be transformed using the natural logarithm function and will be summarized at each visit. If there is any value reported as zero (0) in either of the two parameters, a factor of 0.01 will be added to all observations prior to the log transformation.

The change from baseline in pHSP27, tHSP27 and pHSP27/tHSP27 - in sorbitol stimulated whole blood and in muscle - will be summarized at each post-treatment timepoint. The geometric mean for fold change from baseline and percent change from baseline (and 95% confidence interval) in pHSP27/total HSP27 will be calculated for each post-treatment timepoint. The percent change from baseline will be calculated as:

Percent change from baseline = 100*(exp(mean of change from baseline on the log-transformed scale) – 1).

Post-treatment will be also obtained at the estimated C_{max} time (e.g. 4h+-1h).

Summaries presented for subjects with second muscle biopsy at 4 weeks post-treatment (Biopsy Cohort Week 4), 8 weeks post-treatment (Biopsy Cohort Week 8), and for all subjects combined.

Plasma and muscle PK parameters of losmapimod (PK endpoint)

There will be no PK parameters calculated. Plasma and muscle concentrations of losmapimod will be summarized by time point. Individual concentrations will be plotted versus time using both linear and log scales for the y-axis. Additionally, concentration versus time curves will be plotted as a spaghetti plot. Individual subject listings will also be provided.

Summaries presented for subjects with second muscle biopsy at 4 weeks post-treatment (Biopsy Cohort Week 4), 8 weeks post-treatment (Biopsy Cohort Week 8), and for all subjects combined.

3.4 Analyses for Exploratory Endpoints

For all exploratory endpoints, in addition to descriptive statistics, graphical approaches - such as box plots, forest plots, scatter plots and spaghetti plots - may be used to describe the data.

3.4.1 Assessment of DUX4 Activity

A subset of DUX4-gene transcripts will be measured in skeletal muscle biopsies, using validated QRT-PCR assays, to measure the treatment effect of losmapimod on aberrant DUX4 activity. A composite DUX4 score will be derived for each patient, at each time point based on normalized gene expression values for the panel of pre-selected genes.

The quantification of gene expression using qPCR, normalization of qPCR and derivation of the composite DUX4 score are conducted at a central laboratory (Q2 Lab Solutions). For details on assay validation, gene subset selection criteria/process, and derivation of the composite DUX4 Score, see Appendices 1 – 5.

All subjects will undergo up to two muscle biopsies; one at baseline, pre-treatment (Visit 4, Week 8 ± 1 week), the second on-treatment muscle biopsy approximately 4 or 8 weeks later (Visit 5, Week 14 ± 2 weeks). Up to 8 subjects will have the second on-treatment biopsy at 4 weeks (Biopsy Cohort Week 4) and up to 8 subjects will have the second on-treatment biopsy at 8 weeks (Biopsy Cohort Week 8).

The DUX4 composite score will be summarized as change from pre-treatment (Week 8 biopsy) to on treatment (Week 14 ± 2 weeks). If the pre-treatment or on-treatment DUX-4 results are unavailable, then the assessment is considered missing. Missing results will be imputed by mean, worst case, and median methods (Section 2.3.6).

The individual components of the composite DUX4 score will also be summarized at each timepoint, along with change from baseline for post-treatment visits.

An additional DUX4 composite score based on a simple average, across all 6 genes, per subject, per timepoint, will be summarized as change from pre-treatment (Week 8 biopsy) to on treatment (Week 14 ± 2 weeks), to assess if a simpler approach to creating a composite score leads to equivalent interpretations of study results.

Similarly, another additional DUX4 composite score based on a simple average, across the top 2 genes, per subject, per timepoint, will be summarized as change from pre-treatment (Week 8 biopsy) to on treatment (Week 14 ± 2 weeks).

Composite scores based on simple averages will not be imputed. Summaries presented for Biopsy Cohort Week 4, Biopsy Cohort Week 8, and overall.

3.4.2 Assessment of Musculoskeletal MRI Muscle Fat Fraction, Lean Muscle Volume and Muscle Fat Infiltration

Measurement of the extent of skeletal muscle tissue replacement by fat in FSHD patients will be done through automatic skeletal muscle segmentation for the 3D muscle volumes and fat fraction analysis via robust algorithms using Dixon imaging. This will be assessed at Screening and Week 36.

Composite measures:
Composite variables, incorporating pre-selected muscles, will be derived for longitudinal analysis of muscle fat fraction, lean muscle volume and muscle fat infiltration (MFFtot, LMVtot, and MFItot respectively). The longitudinal composite observations of relative change in LMV are sensitive to low overall LMV, therefore an exclusion criterium is applied for such observations. See Appendix 6 for derivations and exclusion criteria. The scores will be derived and exclusions applied by scientists at the MRI Service Provider (AMRA Medical Inc.).

Descriptive statistics of MFFtot , LMVtot, and MFItot per visit as well as changes from baseline will be presented for the whole body, as well as muscle regions as applicable. Missing visit observations will be back calculated from provided screening and change from screening values.

Individual Muscles:
For each muscle location, a similar method will be used to analyze muscle fat fraction (%), lean muscle volume (cL), and muscle fat infiltration (%) per visit as well as changes from baseline.

Correlation:
Spearman correlations between MRI composite measures of classic TUG, FSHD TUG, and RWS and traditional measures of classic TUG, FSHD TUG, and RWS will be calculated. Correlations and p-values will be presented by visit. RWS correlations will be presented by dominant and non-dominant hand.

3.4.3 Skeletal Muscle Echogenicity by Ultrasound

Ultrasound for skeletal muscle will be measured at Baseline (Day 1), Week 14, Week 24, Week 36, and End of Treatment (Week 60). Ultrasound analysis will be performed by an outside vendor.

3.4.4 Reachable Work Space With and Without Weights

The reachable work space (RWS) is a 3-dimensional sensor-based system (using a single depth-ranging sensor) that can unobtrusively detect an individual's RWS and reflects an individual global upper extremity function, including shoulder and proximal arm. The evaluation will be performed with and without weights and on both the right and left arms at Baseline (Day 1), Week 4, Week 8, Week 14, Week 24, Week 36, Week 48, and End of Treatment (Week 60).

The absolute total RWS surface envelope area (m2) as well as areas for each of the quadrants will be calculated and provided by a vendor in a blinded fashion. The reachable workspace relative surface area (RSA) represents the portion of the unit hemisphere that is covered by an individual's hand movement.

Descriptive statistics of RSA results per visit as well as the change from baseline will be presented by quadrant, for total upper quadrants (Q1+Q3), total area (Q1+Q2+Q3+Q4), and total area (Q1+Q2+Q3+Q4+Q5) for the assessment with weights and without weights, and by dominant and non-dominant arm. Summary tables will be generated, showing percentage of subjects with RSA decline of >=5%, >=2% at each post-baseline timepoint. The functional work space (FWS) will also be summarized using number and percentages of subjects belonging to the categories of normal or low speed. The detected time within each target will be presented in a listing.

3.4.5 Classic and FSHD TUG

The TUG test is used to assess a person's mobility and requires both static and dynamic balance. It measures the time that a person takes to rise from a chair, walk 3 meters, turn around, walk back to the chair, and sit down. The FSHD TUG test is the classic TUG but adds the component of getting up from a laying position on a bed-like table in the clinic at the start of the test and laying back down on his or her back at the end of the test. The FSHD TUG will capture total completion time and completion times for each segment (supine to sit, stand-walk-sit, and sit to supine). Each test will be done twice per visit and will be assessed at Screening, Week 4, Week 8, Week 14, Week 24, Week 36, Week 48, and End of Treatment (Week 60).

The average of Trial 1 and Trial 2 assessments at each visit will be derived for both the classic TUG completion time and each of the FSHD TUG completion times (ie, supine to sit, stand-walk-sit, sit to supine, and total) will be used for the analysis.

Descriptive statistics of classic TUG average completion time and FSHD TUG average completion times in seconds per visit as well as the change from baseline will be provided in tables. The average completion time of the FSHD TUG is a key study endpoint. Descriptive statistics of FSHD TUG average completion times will be presented for each segment (supine to sit, stand-walk-sit, sit to supine, and total). Similar analysis will also be conducted for each of the individual components of the FSHD TUG.

3.4.6 FSHD-HI

The FSHD-HI is an FSHD-specific patient-reported measure of disease burden on activities of daily living, quality of life, and symptom prevalence and severity. It consists of a questionnaire with 116 items developed from qualitative interviews of patients. The measure consists of 14 subscales that measure a patient's perception of their ambulation and mobility, hand function, shoulder and arm function, emotional health, back/chest/abdomen strength, fatigue, pain, eating function, ability to do activities,

communication ability, satisfaction in social situations, performance situations, body image and cognition. The 116 items are combined into a total score, the score is then transformed onto a percentage scale, with 100 representing maximal disability and lower scores representing decreasing disability. This FSHD-HI total score will be calculated by a separate vendor. This score, along with the 14 subscale scores will be assessed at Screening, Week 4, Week 24, and End of Treatment (Week 60).

Descriptive of the FSHD-HI total and subscale scores per visit as well as the change from baseline will be presented.

3.4.7 FSHD-RODS

The FSHD-RODS is a patient-reported, linearly weighted scale that precisely measures activities of daily living and participation in subjects with FSHD using 50 items based on the Rasch model. This will be assessed at Screening, Week 4, Week 24, and End of Treatment (Week 60).

Descriptive statistics of the FSHD-RODS total and subscale scores per visit as well as the change from baseline will be presented.

3.4.8 Dynamometry

Dynamometry measures the static muscle strength without any movement. Manual dynamometry with hand-held devices and quantitative myometry testing (QMT) with bedframe equipment will be used to assess the skeletal muscle strength of study subjects in both the upper and lower limbs bilaterally. Manual dynamometry be assessed at Screening, Week 4, Week 14, Week 24, Week 48, and End of Treatment (Week 60). QMT will be assessed at Screening, Week 4, and End of Treatment (Week 60).

Descriptive statistics for percent predicted QMT as well as change from baseline will be presented for: (a.) all muscles combined; (b.) upper extremity strength, which includes the hands, shoulders and elbows combined; and (c.) lower extremity strength, which includes knee extension, knee flexion, and ankle dorsiflexors combined. Percent predicted QMT scores will be calculated using SAS® code provided in Appendix 9 (with additional extremity details in Appendix 10).

Descriptive statistics for total manual dynamometry as well as change from baseline will be presented for: (a.) all muscles combined; (b.) upper extremity strength, which includes the hands, shoulders and elbows combined; and (c.) lower extremity strength, which includes right and left ankle dorsiflexors combined. Raw scores will be presented.

Descriptive statistics of manual dynamometry average and maximum weight in kilogram per visit as well as the change from baseline and percent change from baseline will be presented according to assessment (dominant shoulder abductors, non-dominant shoulder abductors, dominant elbow flexors, non-dominant elbow flexors, dominant elbow

extensors, non-dominant elbow extensors, dominant hand grip, non-dominant hand grip, right ankle dorsiflexors, and left ankle dorsiflexors). Raw scores will be presented.

Descriptive statistics of normalized manual dynamometry average and maximum weight in kilogram per visit as well as the change from baseline will be presented by overall upper extremity and according to assessment (dominant shoulder abductors, non-dominant shoulder abductors, dominant elbow flexors, non-dominant elbow flexors, dominant elbow extensors, non-dominant elbow extensors, dominant hand grip, and non-dominant hand grip).

Manual dynamometry data will be normalized as (subject measure – reference mean)/reference standard deviation. For elbow flexion, elbow extension, and shoulder abduction the reference mean and standard deviation (converted to kg from Newton) from Douma et al. (see Appendix 7) will be used. For hand grip the reference mean and standard deviation from the Jamar® Smart Hand Dynamometer (see Appendix 8) will be used.

3.4.9 MMT

Manual muscle testing is one of the most commonly used forms of muscle testing. Subjects hold the corresponding limb or appropriate body part to be tested at the end of its available range while the evaluator provided opposing manual resistance.

Descriptive statistics for MMT as well as change from first observation will be presented for: (a.) all muscles combined; (b.) upper extremity strength; (c.) lower extremity strength; and (d.) individual muscles. See Appendix 11 for MMT raw score mapping.

3.4.10 PGIC

The PGIC is self-reported and reflects a subject's belief about efficacy of treatment. PGIC is a 7-point scale depicting a subject's rating of overall improvement. This will be assessed at Week 14, Week 24, Week 36, Week 48, and End of Treatment (Week 60).

Descriptive statistics of the PGIC responses per visit will be presented.

3.4.11 Outpatient Mobility Assessment

Upper and lower limb mobility in the outpatient setting using wearables will be measured at Baseline (visit 2) and intermittently to end of study. Mobility assessment will be analyzed by an outside vendor.

3.4.12 MFM Domain 1

The MFM scale measures the severity of the motor deficit in the main neuromuscular diseases as determined by a physical therapist. The total score provides a good measure of the overall functional severity across various domains. Domain 1 is used in this trial and focuses on ambulation and transfers ability. This will be assessed at Screening, Week 8, Week 24, Week 48, and End of Treatment (Week 60).

Descriptive statistics of scores per visit as well as the change from baseline will be presented by overall and according to assessment (lift pelvis in supine, lying to sitting, long sitting to standing up, sitting down on a chair, standing up from a chair, stand, standing on one foot, touching the floor while standing, walk on heels, walk on line, run, hop, squat).

3.4.13 Spirometry

Spirometry will be measured to assess the change from the pre-treatment period in lung ventilatory function. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) will be assessed at Baseline (visit 2), Week 24, Week 48, and End of Treatment (Week 60).

Descriptive statistics of FVC and FEV1 per visit as well as the change from baseline will be presented.

3.4.14 6-MWT

The 6-MWT will be performed to evaluate the change from the pre-treatment period in the distance a subject is able to walk over a total of 6 minutes. A course length of 20 meters will be marked with 2 traffic cones and subjects will be instructed to walk back and forth around the cones for 6 minutes. The distance walked during the first 2 minutes and the full 6 minutes will be recorded. This will be assessed at Screening, Week 4, Week 8, Week 14, Week 24, Week 36, Week 48, and End of Treatment (Week 60).

Descriptive statistics of the total distance walked per visit as well as the change from baseline will be presented.

3.5 Other Exploratory Analysis

Other exploratory analyses may be conducted as appropriate. Any exploratory analyses that are performed will be appropriately titled/labeled as ‘exploratory’ and will be clearly distinguished from planned analyses when results are reported in the Clinical Study Report (CSR).

Graphic methods - like box plots and spaghetti plots - may also be used to describe exploratory endpoints.

Descriptive statistics of Ricci scores per visit as well as the change from baseline will be presented.

3.6 Sensitivity Analysis

Sensitivity analyses will be conducted using an alternative baseline defined as the mean of all available pre-treatment measures. Selected exploratory endpoints will be repeated using the alternative baseline definition.

The sensitivity endpoints are as follows:

- Reachable Work Space (RWS) with and without weights
- Disease impact by subject report using FSHD-Rasch-built Overall Disability Scale (FSHD-RODS)
- Disease impact by subject report using FSHD-HI
- Physical function by Motor Function Measure (MFM) domain 1
- Manual Dynamometry
- Classic TUG and FSHD TUG
- Change in ambulation as measured by the 6-minute walking test (6-MWT)

4 INTERIM ANALYSIS

4.1 INTERIM ANALYSIS (IA)

There are two formal IAs planned:

- the first analysis will occur after approximately 6-8 subjects have completed the visit 5 biopsy (Week 4 or Week 8, post-treatment visit). For some subjects, the visit 5 biopsy occurs at 4-weeks post-treatment, and for others, it occurs at 8-weeks post-treatment. Analysis of DUX4 will be provided at baseline, Week 4 post-treatment and Week 8 post-treatment, with the corresponding change measures.
- the second IA will occur after approximately 6-8 subjects have completed Visit 7 (Week 36).

Further details of data to be analyzed and presented for each IA are described below:

IA #1 (n = 6 to 8 subjects):

- Summary table of Baseline demographics and disease characteristics (including baseline Ricci Scores)
- Medical History Summary
- PK/PD Summaries
- Summary tables of DUX4 activity for each of 11 gene transcripts: CCNA1; KHDC1L; LEUTX; MBD3L2; PRAMEF6; PRAMEF9; SLC2A3; SLC34A2; TRIM43; ZSCAN4; DUX4fl
    - Baseline, Week 4 post-baseline, Week 8 post-baseline and Change from Baseline summaries by gene transcript
- Summary tables expression levels for each of 18 transcripts: PNP; RBP7; MYH3; ACTA2; TNNT2; COMP; POSTN; SAA1; CCL13; FST; PLA2G2A; S100A6; C3; ADIPOQ; LEP; COL1A1; THBS4; TAGLN2
    - Baseline, Week 4 post-baseline, Week 8 post-baseline and Change from Baseline summaries by gene transcript
- Summary tables of DUX4 disease activity based on:
    - DUX4 Composite Score: Based on 6 gene transcripts. See Section 3.4 and Appendices 1-5.

- Box plots of DUX4 summary data, over time.

IA #2 (n = minimum 6 to 8 subjects and up to 14 subjects)

- Summary table of Baseline demographics and disease characteristics.
- Summary tables of MRI parameters, by different muscle location and whole body. MRI parameters include: Musculoskeletal MRI Fat Fraction; Musculoskeletal MRI Fat Infiltration; Musculoskeletal MRI Lean Muscle Volume; and composite scores of MRI measurements for whole body and regions (see Appendix 6).
    - Baseline, Visit 7/Visit 8 and Change from Baseline summaries by MRI parameter and location.
- Summary tables of classic TUG and FSHD TUG.
- PK Summaries
- Summary table of muscle strength by quantitative dynamometry
- Summary table of muscle strength by hand-held dynamometry
- Summary table of distance of walking measured by 6-MWT
- Summary table of reachable work space (RWS)
- Summary table lung function as measured by Spirometry
- Summary table of physical function by MFM domain 1
- Summary table of FSHD-HI total score
- Summary table patient global impression of change
- Summary table of FSHD-RODS total score

4.2 DATA MONITORING COMMITTEE

NA

5 SUMMARY OF MAJOR CHANGES IN THE PLANNED ANALYSES

Due to missed visits related to COVID-19, the FSHD-RODS and FSHD-HI results are presented by protocol visit as opposed to analysis weeks. Visits 6 and 7 are combined as individual subject visits were split between these two visits.

Due to missed visits related to COVID-19, the MRI results are presented by protocol visit as opposed to analysis weeks. Visits 7 and 8 are combined as individual subject visits were split between these two visits.

6 REFERENCES

1. ICH guidance for industry E9 Statistical Principles for Clinical Trials, 1998

2. ICH guidance for industry E3 Structure and Content of Clinical Study Reports, 1996

3. FDA Guidance for Industry, Adaptive Design Clinical Trials for Drugs and Biologics, Draft, 2010

4. FDA Reviewer Guidance. Conducting a Clinical Safety Review of a New Product Application and Preparing a Report on the Review. February 2005.

5. Douma *et al*.: **Reference values for isometric muscle force among workers for the Netherlands: a comparison of reference values**. *BMC Sports Science, Medicine, and Rehabilitation* 2014 6:10.

6. *Jamar® Smart Hand Dynamometer*. Patterson Medical

7 APPENDICES

APPENDIX 1: THE STEPS FROM RAW QPCR CT TO STATISTICAL ANALYSIS

1. Raw Cts from qPCR are determined for DUX4 regulated genes using a validated assay (Fluidigm), described in Appendix 2.
2. The raw Cts from the genes are normalized to reference (housekeeping) genes using the process described in Appendix 3. The document also describes the method for selecting the reference genes.
3. A composite DUX4 score is created for each subject using the normalized data, at baseline and post-baseline, using the derivation described in Appendix 4.
    a. Six DUX4-regulated gene transcripts were selected, a priori, to be in a composite DUX4 score. The process of selection of these 6 genes is detailed in Appendix 5.
4. Summary data is generated for the DUX4 composite score at baseline and post-baseline, to assess "change" over time.

APPENDIX 2: VALIDATION OF THE QPCR ASSAY

Method Validation Report VAL_M_0269

Gene Expression Assay Panel for EA18054 using Fluidigm Dynamic Arrays

Test Facility [redacted]

Date Issued TBD

APPROVALS

	Name and Title	Signature	Date
Authored By:	[redacted] Senior Scientist, Assay Development, Translational Genomics	See Attached	See Attached
Approved By:	[redacted] Ph.D. Senior Director and Global Head, Translational Genomics	See Attached	See Attached
QA Reviewed By:	[redacted] Associate Director, Quality Assurance	See Attached	See Attached

APPENDIX 3: QPCR NORMALIZATION

Fulcrum Therapeutics

Title

Normalization of qPCR data for Fulcrum Studies FIS-001-2019 and FIS-002-2019

REPORT: Addenda to FIS-001-2019 and FIS-002-2019 SAPs

INVESTIGATORS: [redacted]

COMPOUND NAME: Losmapimod

LOCATION: 26 Landsdowne St., Cambridge, MA 02139

AUTHORS: [redacted]

STUDY NUMBER(S): FIS-001-2019 and FIS-002-2019

REPORT DATE: January 22, 2020

APPENDIX 4: DERIVATION OF COMPOSITE DUX4 SCORE FROM DELTA CT

Select only rows pertaining to the following 6 gene transcripts: CCNA1; KHDC1L; MBD3L2; PRAMEF6; SLC34A2; ZSCAN4

Step 1 (Pre-Treatment data only): (By gene transcript)

I. Calculate Median (measure of average) of delta CT
   a. **Rationale:** median is a robust measure of average, less sensitive to extreme values

II. Calculate Median absolute deviation of delta CT (MAD: measure of variability)
   a. **Rationale:** MAD is a robust measure of variability

Step 2 (Pre-treatment and post-treatment: By gene transcript; by subject)

I. Use MAD obtained in Step 1 to calculate standardized score per gene transcript, per patient:

$$Standardized\ Score = (Normalized\ Delta\ Ct\ Value - Median) \div MAD$$

   a. **Rationale:** Standardize all gene measures to the same scale; reduce the impact of highly variable genes in the final composite measure

II. Select median of standardized scores for each patient, across all genes, by visit.
   a. Each patient's final score is the median standardized score across multiple transcripts.
   b. This "composite score" is then used in the analysis for change from baseline.
   c. Steps 1 and 2 may help resolve issue with censored (undetectable) data.

NOTE:

1. A composite score based on a simple average, across all 6 genes, per subject, per timepoint, may also be explored, to assess if a simpler approach to creating a composite score leads to equivalent interpretations of study results.
2. We will also explore composite scores based on the 6 genes: CCNA1; KHDC1L; PRAMEF6; SLC34A2; ZSCAN4; MBD3L2. The top two DUX4-driven transcripts will be identified as follow:
   a. At baseline, select the two highest magnitude transcripts per subject.
   b. For each subject, select only observations pertaining to only these two transcripts at both baseline and post-baseline. The two selected transcripts may vary across subjects.
   c. Calculate the composite score (DUX4 score) using only these two transcripts, per subject, per timepoint.

Composite DUX4 score = arithmetic mean (transcript 1, transcript 2)

APPENDIX 5: SELECTION OF 6 GENE TRANSCRIPTS

Fulcrum Therapeutics

TITLE: AN ANALYTICAL METHOD TO DEFINE A SUBSET OF DUX4-REGULATED TRANSCRIPTS TO SERVE AS THE SURROGATE FOR REDUCTION OF DUX4 EXPRESSION AND THE PRIMARY OBJECTIVE IN AN FSHD PROOF OF CONCEPT CLINICAL TRIAL OF LOSMAPIMOD

Version 1.0

REPORT:	**Addenda to Statistical Analysis Plan** **An Open-Label Pilot Study of Losmapimod to Evaluate the Safety, Tolerability, and Changes in Biomarker and Clinical Outcome Assessments in Subjects with Facioscapulohumeral Muscular Dystrophy 1 (FSHD1)**
INVESTIGATORS:	[redacted]
COMPOUND NAME:	Losmapimod
LOCATION:	26 Landsdowne St., Cambridge, MA 02139
AUTHORS:	[redacted]
STUDY NUMBER(S):	FIS-001-2019
REPORT DATE:	February 20, 2020

APPENDIX 6: DERIVATION OF COMPOSITE MRI ENDPOINTS (FOR EACH SUBJECT, AT EACH TIMEPOINT)

Muscle categorization

Each muscle is categorized into one of three categories[1]:

A. 'Normal' muscles are normal appearing (low MFI and MFF, muscle likely unaffected by disease)
B. 'Affected' muscles have a likely disease involvement (intermediate MFI and MFF)
C. 'End-stage' corresponds to that more than 50% of the muscle tissue has been replaced by fat, and most of the functional capacity is likely lost

Muscle category	MFF criteria		MFI criteria
A. Normal	MFF < 10%	AND	MFI < 10%
B. Affected	MFF < 50%	AND	MFI ≥ 10%
C. End-stage	MFF ≥ 50%	-	-

Quality control of measurements

Trained anatomical imaging experts evaluate the signal quality in each muscle. If a muscle is not analyzable, that muscle is marked as not analyzable and no measurements will be extracted from it. For cross-sectional analysis minor quality issues are accepted, but not for longitudinal analysis. Hence, as seen below, we'll use different statistical inclusion parameters depending on if we are doing a cross-sectional or longitudinal analysis.

Statistical inclusion parameters

The muscle categorization and quality control results are summarized in 3 statistical inclusion parameters which will be provided for each muscle:

- Statistical Inclusion Category: Can take the values **A**, **B** or **C**, which correspond to 'Normal', 'Affected' and 'End-stage'. Abbreviated SI_{cat} in equations.
- Statistical Inclusion Cross-sectional: Can take the values **0** or **1** (0 if there are major signal quality issues indicating unreliable measurement, 1 otherwise). Abbreviated SI_{cross} in equations.
- Statistical Inclusion Longitudinal: Can take the values **0** or **1** (0 if there are any signal quality issues, including minor, 1 otherwise). Abbreviated SI_{long} in equations.

Note: In general, if a SI parameter is 'Unable to Assess', 'NA' or '0', that muscle should be excluded from the corresponding data analysis. . As for other muscle measurements, the SI parameters will have a corresponding 'Quality Issues' column – these columns may be ignored.

Whole-body combined measurements

Cross-sectional analysis

MRI-based measurements from multiple muscles is combined to obtain global measurements of disease affection for correlation to disease severity or functional test scores in cross-sectional data:

$$LMV_{tot} = \sum_{i \in muscles} SI_{cross,i} \cdot LMV_i$$

[1] Note that AMRA® Researcher is for research purposes only, and the names of these categories do not correspond to any form of diagnosis.

$$MFI_{tot} = \frac{\sum_{i \in muscles} SI_{cross,i} \cdot MFI_i \cdot LMV_i}{\sum_{i \in muscles} SI_{cross,i} \cdot LMV_i}$$

$$MFF_{tot} = 1 - \frac{\sum_{i \in muscles} SI_{cross,i} \cdot LMV_i}{\sum_{i \in muscles} SI_{cross,i} \cdot TMV_i}$$

MFF_{tot} is likely to correlate best with functional tests as it reflects global muscle-to-fat replacement, while interpretation of total LMV at only one timepoint requires reference data for normal total LMV that is not available at the moment. Note that muscles are included regardless of category (A, B and C). A subject with no analyzable muscles should not be included in the cross-sectional analysis. The included muscles depend on the function of interest. Some examples are given below.

Functional test	Included muscles
Global tests: • **Disease severity / Whole-body function** • **FSHD TUG**	All muscles
Focused walk tests: • **Timed Up and Go (TUG) test**	Leg muscles: • Quadriceps • Hamstrings • Adductors • Tibialis Anterior • Gastrocnemius Medialis
Reach tests: • **Reachable workspace (RWS) test**	Arm, rotator cuff, and torso muscles: • Biceps Brachii • Triceps Brachii • Deltoideus • Pectoral Muscles • Rhomboideus Major • Latissimus Dorsi/Teres Major • Supraspinatus • Infraspinatus • Teres Minor • Subscapularis • Serratus Anterior

Longitudinal analysis

For longitudinal analysis of disease progression, we only want to include muscles that have category 'B' at baseline – the rationale being that inclusion of Normal appearing muscles (likely not affected by disease) or End-stage appearing muscles (likely not much function left) would only add noise and uncertainty to the analysis of longitudinal changes. Hence:

- If the muscle category is **A**, **C** or **NA** at baseline, the muscle should not be included in longitudinal analysis.
- If the muscle category is **B** at baseline, the muscle should be included in longitudinal analysis.

We include quality control SI parameters from both timepoints to ensure that we remove a muscle from the calculation if it's unanalyzable in any or both timepoints. For cleaner equations, we combine the statistical

inclusion parameters for the two timepoints here:

$$SI_{long}^{comb} = SI_{long,tp1} \cdot SI_{long,tp2}$$

The absolute change in total LMV, MFI and MFF between timepoint 1 (tp1) and timepoint 2 (tp2) becomes:

$$\Delta LMV_{tot} = \sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot \left(LMV_{i,tp2} - LMV_{i,tp1}\right)$$

$$\Delta MFI_{tot} = \frac{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot MFI_{i,tp2} \cdot LMV_{i,tp2}}{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot LMV_{i,tp2}} - \frac{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot MFI_{i,tp1} \cdot LMV_{i,tp1}}{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot LMV_{i,tp1}}$$

$$\Delta MFF_{tot} = \left(1 - \frac{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot LMV_{i,tp2}}{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot TMV_{i,tp2}}\right) - \left(1 - \frac{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot LMV_{i,tp1}}{\sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot TMV_{i,tp1}}\right)$$

The reason we only include the muscle category parameter from timepoint 1 is that we want to make sure to follow a muscle that is 'Affected' at baseline/tp1, even if it progressed into 'End-stage' at follow-up/tp2. A subject with no analyzable muscles should not be included in longitudinal analysis (this can be ensured by removing any subjects with total LMV = 0).

Since different number of muscles may be included in each individual patient, and each patient may have different muscle sizes, a relative change in total LMV may be a better endpoint than absolute change in total LMV (for MFI and MFF, absolute change makes most sense since they are fractions). This corresponds to:

$$\Delta LMV_{tot}^{relative} = \sum_{i \in SI_{cat,tp1=B}} SI_{long,i}^{comb} \cdot \left(\frac{LMV_{i,tp2} - LMV_{i,tp1}}{LMV_{i,tp1}}\right)$$

As a start, it makes sense to include all analyzable muscles in the longitudinal analysis, although muscles can also be grouped similarly to the cross-sectional analysis for assessment of longitudinal change of function.

Exclusion criteria for longitudinal composite scores

The longitudinal composite observations of relative change in LMV are sensitive to low overall LMV. Therefore, criteria are required for exclusion of such observations. To ensure that the relative change in LMV is reliable LMV_{ref} needs to fulfill one of the below:

- Be at least 25 cl and consist of measurements from at least 2 muscles.
- Be at least 50 cl with measurements from at least one muscle.

These criteria apply for all longitudinal composite measurements.

(1) If a subject's individual muscles couldn't fulfil both bullets above, the subject's change from reference time point in longitudinal composite scores for LMV (L), LMV (%), MFF and MFI should be set to missing. Here, LMV_{ref} refers to LMV at reference time point, such as reference screening, and cl means 0.01 liter.

(2) If a subject's individual muscles could fulfil at least one of the two bullets above, the subject's change from reference time point in longitudinal composite scores for LMV (L), LMV (%), MFF and MFI should not be set to missing.

(3) The first bullet above refers to at least 2 muscles with a combined LMV_{ref} (L) of at least 0.25 liter.

APPENDIX 7: NORMALIZATION FOR ELBOW FLEXION, ELBOW EXTENSION, AND SHOULDER ABDUCTION

Douma et al. BMC Sports Science, Medicine, and Rehabilitation 2014, 6:10
http://biomedcentral.com/2052-1847/6/10

BMC
Sports Science, Medicine & Rehabilitation

RESEARCH ARTICLE Open Access

Reference values for isometric muscle force among workers for the Netherlands: a comparison of reference values

Rob KW Douma[1*], Remko Soer[3,4], Wim P Krijnen[1], Michiel Reneman[2] and Cees P van der Schans[1,2]

Table 4 Dominant and non-dominant muscle strength means (sd) per age group for males

Male		Dominant		Non dominant	
Muscle force	Age group	N	Mean(Sd.)	N	Mean (Sd.)
Elbow flexion	20-29	48	281(48)	48	261(49)
	30-39	51	273(50)	51	266(51)
	40-49	70	271(59)	70	261(51)
	50-59	59	259(52)	59	245(47)
Elbow extension	20-29	48	186(38)	48	182(37)
	30-39	51	183(40)	51	179(45)
	40-49	70	185(46)	70	179(44)
	50-59	59	181(37)	59	173(36)
Knee flexion	20-29	48	267(57)	48	252(52)
	30-39	51	262(60)	51	250(55)
	40-49	68	274(77)	69	263(77)
	50-59	59	242(57)	59	234(55)
Knee extension	20-29	47	379(105)	47	371(112)
	30-39	51	351(99)	51	341(101)
	40-49	69	368(114)	70	341(107)
	50-59	59	337(103)	57	335(102)
Schoulder abduction	20-29	14	172(48)	14	173(35)
	30-39	26	181(38)	26	176(40)
	40-49	35	173(43)	35	177(40)
	50-59	37	178(39)	39	177(43)

Table 5 Dominant and non-dominant muscle strength means (sd) per age group for females

Female		Dominant		Non dominant	
Muscle force	Age group	N	Mean(Sd.)	N	Mean(Sd.)
Elbow flexion	20-29	51	191(30)	51	183(30)
	30-39	39	195(34)	39	186(35)
	40-49	66	191(37)	66	186(37)
	50-59	34	181(29)	34	166(22)
Elbow extension	20-29	51	132(28)	51	131(28)
	30-39	39	128(24)	39	125(26)
	40-49	66	131(28)	66	125(29)
	50-59	34	120(20)	34	118(27)
Knee flexion	20-29	51	198(38)	51	191(37)
	30-39	39	190(41)	39	188(35)
	40-49	66	190(51)	67	183(52)
	50-59	34	174(42)	34	169(45)
Knee extension	20-29	51	261(80)	51	260(75)
	30-39	38	273(87)	39	264(88)
	40-49	66	262(127)	67	245(79)
	50-59	34	244(66)	34	228(51)
Schoulder abduction	20-29	14	115(19)	14	124(23)
	30-39	22	116(26)	22	118(30)
	40-49	41	119(28)	41	118(26)
	50-59	15	114(22)	15	116(21)

APPENDIX 8: NORMALIZATION FOR HAND GRIP STRENGTH

CE

Jamar® Smart Hand Dynamometer

FROM PATTERSON MEDICAL

NORMATIVE GRIP STRENGTH DATA:

		Males		Females		Males		Females	
Age	Hand	Mean (lbs)	SD	Mean (lbs)	SD	Mean (kg)	SD	Mean (kg)	SD
6-7	R	32.5	4.8	28.6	4.4	14.7	2.2	13.0	2.0
	L	30.7	5.4	27.1	4.4	13.9	2.4	12.3	2.0
8-9	R	41.9	7.4	35.3	8.3	19.0	3.4	16.0	3.8
	L	39	9.3	33	6.9	17.7	4.2	15.0	3.1
10-11	R	53.9	9.7	49.7	8.1	24.4	4.4	22.5	3.7
	L	48.4	10.8	45.2	6.8	22.0	4.9	20.5	3.1
12-13	R	58.7	15.5	56.8	10.6	26.6	7.0	25.8	4.8
	L	55.4	16.9	50.9	11.9	25.1	7.7	23.1	5.4
14-15	R	77.3	15.4	58.1	12.3	35.1	7.0	26.4	5.6
	L	64.4	14.9	49.3	11.9	29.2	6.8	22.4	5.4
16-17	R	94	19.4	67.3	16.5	42.6	8.8	30.5	7.5
	L	78.5	19.1	56.9	14	35.6	8.7	25.8	6.4
18-19	R	108	24.6	71.6	12.3	49.0	11.2	32.5	5.6
	L	93	27.8	61.7	12.5	42.2	12.6	28.0	5.7
20-24	R	121	20.6	70.4	14.5	54.9	9.3	31.9	6.6
	L	104.5	21.8	61	13.1	47.4	9.9	27.7	5.9
25-29	R	120.8	23	74.5	13.9	54.8	10.4	33.8	6.3
	L	110.5	16.2	63.5	12.2	50.1	7.3	28.8	5.5
30-34	R	121.8	22.4	78.7	19.2	55.2	10.2	35.7	8.7
	L	110.4	21.7	68	17.7	50.1	9.8	30.8	8.0
35-39	R	119.7	24	74.1	10.8	54.3	10.9	33.6	4.9
	L	112.9	21.7	66.3	11.7	51.2	9.8	30.1	5.3
40-44	R	116.8	20.7	70.4	13.5	53.0	9.4	31.9	6.1
	L	112.8	18.7	62.3	13.8	51.2	8.5	28.3	6.3
45-49	R	109.9	23	62.2	15.1	49.8	10.4	28.2	6.8
	L	100.8	22.8	56	12.7	45.7	10.3	25.4	5.8
50-54	R	113.6	18.1	65.8	11.6	51.5	8.2	29.8	5.3
	L	101.9	17	57.3	10.7	46.2	7.7	26.0	4.9
55-59	R	101.1	26.7	57.3	12.5	45.9	12.1	26.0	5.7
	L	83.2	23.4	47.3	11.9	37.7	10.6	21.5	5.4
60-64	R	89.7	20.4	55.1	10.1	40.7	9.3	25.0	4.6
	L	76.8	20.3	45.7	10.1	34.8	9.2	20.7	4.6
65-69	R	91.1	20.6	49.6	9.7	41.3	9.3	22.5	4.4
	L	76.8	19.8	41	8.2	34.8	9.0	18.6	3.7
70-74	R	75.3	21.5	49.6	11.7	34.2	9.8	22.5	5.3
	L	64.8	18.1	41.5	10.2	29.4	8.2	18.8	4.6
75+	R	65.7	21.	42.6	11	29.8	9.5	19.3	5.0
	L	55	17	37.6	8.9	24.9	7.7	17.1	4.0

APPENDIX 9: SAS CODE FOR QMT PREDICTED PERCENT SCORES

SAS code below provided by [redacted] (Fulcrum) and [redacted]:

```
Below codes creates QMT predicted standardized scores for each QMT muscles:

*if medmax^=.5 then mm=(medmax+1); * Per Mike 6/26/95;
  mm=medmax+1;

  mdflp=4.6332-(0.00241*age)+(0.00607*height);
  fdflp=4.3991-(0.00241*age)+(0.00607*height);
  mdfrp=4.9847-(0.00277*age)+(0.00435*height);
  fdfrp=4.6962-(0.00277*age)+(0.00435*height);
  mdtlp=4.5529-(0.00522*age)+(0.00569*height);
  fdtlp=1.7941-(0.00522*age)+(0.01910*height);
  mdtrp=4.6812-(0.00650*age)+(0.00518*height);
  fdtrp=2.2375-(0.00650*age)+(0.01663*height);
  meelp=3.7628-(0.00151*age)+(0.00864*height);
  feelp=3.2676-(0.00151*age)+(0.00864*height);
  meerp=4.0617-(0.00232*age)+(0.00737*height);
  feerp=3.5597-(0.00232*age)+(0.00737*height);
  meflp=4.1605-(0.00266*age)+(0.00863*height);
  feflp=2.8467-(0.00266*age)+(0.01442*height);
  mefrp=5.0254-(0.00335*age)+(0.00430*height);
  fefrp=3.1534-(0.00335*age)+(0.01298*height);
  merlp=3.8313-(0.00365*age)+(0.00714*height);
  ferlp=3.3893-(0.00365*age)+(0.00714*height);
  merrp=3.9446-(0.00245*age)+(0.00625*height);
  ferrp=3.5009-(0.00245*age)+(0.00625*height);
  mhblp=3.6885-(0.00188*age)+(0.00960*height);
  fhblp=1.7266-(0.00188*age)+(0.01884*height);
  mhbrp=4.6122-(0.00258*age)+(0.00499*height);
  fhbrp=1.9143-(0.00258*age)+(0.01834*height);
  mhdlp=4.3537-(0.00331*age)+(0.00930*height);
  fhdlp=3.8317-(0.00331*age)+(0.00930*height);
  mhdrp=5.2077-(0.00387*age)+(0.00477*height);
  fhdrp=4.5916-(0.00387*age)+(0.00477*height);
```

```
mkelp=5.1832-(0.00837*age)+(0.00789*height);
fkelp=4.7644-(0.00837*age)+(0.00789*height);
mkerp=6.4124-(0.00701*age)+(0.00104*height);
fkerp=3.4479-(0.00701*age)+(0.01557*height);
mkflp=4.9876-(0.00591*age)+(0.00499*height);
fkflp=2.1190-(0.00591*age)+(0.01994*height);
mkfrp=4.9481-(0.00641*age)+(0.00524*height);
fkfrp=2.0670-(0.00641*age)+(0.02020*height);
mlgrip = -26.9062 - (0.1329 * age) + (0.4374 * height);
flgrip = -39.5766 - (0.1329 * age) + (0.4374 * height);
mrgrip = -9.7022 - (0.1732 * age) + (0.3696 * height);
frgrip = -24.8027 - (0.1732 * age) + (0.3696 * height);


if (test='DFL' and sex='M') then stanqmt=(log(mm)-mdflp)/0.20454;
if (test='DFL' and sex='F') then stanqmt=(log(mm)-fdflp)/0.20454;
if (test='DFR' and sex='M') then stanqmt=(log(mm)-mdfrp)/0.20380;
if (test='DFR' and sex='F') then stanqmt=(log(mm)-fdfrp)/0.20380;
if (test='SBL' and sex='M') then stanqmt=(log(mm)-mdtlp)/0.24541;*;
if (test='SBL' and sex='F') then stanqmt=(log(mm)-fdtlp)/0.24541;*;
if (test='SBR' and sex='M') then stanqmt=(log(mm)-mdtrp)/0.27474;*;
if (test='SBR' and sex='F') then stanqmt=(log(mm)-fdtrp)/0.27474;*;
if (test='EEL' and sex='M') then stanqmt=(log(mm)-meelp)/0.19315;
if (test='EEL' and sex='F') then stanqmt=(log(mm)-feelp)/0.19315;
if (test='EER' and sex='M') then stanqmt=(log(mm)-meerp)/0.17903;
if (test='EER' and sex='F') then stanqmt=(log(mm)-feerp)/0.17903;
if (test='EFL' and sex='M') then stanqmt=(log(mm)-meflp)/0.14704;
if (test='EFL' and sex='F') then stanqmt=(log(mm)-feflp)/0.14704;
if (test='EFR' and sex='M') then stanqmt=(log(mm)-mefrp)/0.14746;
if (test='EFR' and sex='F') then stanqmt=(log(mm)-fefrp)/0.14746;
if (test='ERL' and sex='M') then stanqmt=(log(mm)-merlp)/0.20397;
if (test='ERL' and sex='F') then stanqmt=(log(mm)-ferlp)/0.20397;
if (test='ERR' and sex='M') then stanqmt=(log(mm)-merrp)/0.19397;
if (test='ERR' and sex='F') then stanqmt=(log(mm)-ferrp)/0.19397;
if (test='HBL' and sex='M') then stanqmt=(log(mm)-mhblp)/0.20715;
if (test='HBL' and sex='F') then stanqmt=(log(mm)-fhblp)/0.20715;
if (test='HBR' and sex='M') then stanqmt=(log(mm)-mhbrp)/0.23012;
```

```
  if (test='HBR' and sex='F') then stanqmt=(log(mm)-fhbrp)/0.23012;
  if (test='HDL' and sex='M') then stanqmt=(log(mm)-mhdlp)/0.21990;
  if (test='HDL' and sex='F') then stanqmt=(log(mm)-fhdlp)/0.21990;
  if (test='HDR' and sex='M') then stanqmt=(log(mm)-mhdrp)/0.23142;
  if (test='HDR' and sex='F') then stanqmt=(log(mm)-fhdrp)/0.23142;
  if (test='KEL' and sex='M') then stanqmt=(log(mm)-mkelp)/0.21727;
  if (test='KEL' and sex='F') then stanqmt=(log(mm)-fkelp)/0.21727;
  if (test='KER' and sex='M') then stanqmt=(log(mm)-mkerp)/0.23823;
  if (test='KER' and sex='F') then stanqmt=(log(mm)-fkerp)/0.23823;
  if (test='KFL' and sex='M') then stanqmt=(log(mm)-mkflp)/0.19632;
  if (test='KFL' and sex='F') then stanqmt=(log(mm)-fkflp)/0.19632;
  if (test='KFR' and sex='M') then stanqmt=(log(mm)-mkfrp)/0.21062;
  if (test='KFR' and sex='F') then stanqmt=(log(mm)-fkfrp)/0.21062;

  if (test = 'HGL' and sex = 'M') then stanqmt = (medmax - mlgrip) / 5.25987;
  if (test = 'HGL' and sex = 'F') then stanqmt = (medmax - flgrip) / 5.25987;
  if (test = 'HGR' and sex = 'M') then stanqmt = (medmax - mrgrip) / 5.73428;
  if (test = 'HGR' and sex = 'F') then stanqmt = (medmax - frgrip) / 5.73428;

  drop mm mdflp--frgrip;
```

Below codes created predicted scores

```
predict2=2.71828**predict;
  *Percent=(medmax/predict2)*100;
  logmm=log(medmax+1);
if sex='M' then do;


    if test='DFL' then predict=mdflp;
     else if test='DFR' then predict=mdfrp;
     else if test='SBL' then predict=mdtlp;
     else if test='SBR' then predict=mdtrp;
     else if test='EEL' then predict=meelp;
     else if test='EER' then predict=meerp;
     else if test='EFL' then predict=meflp;
```

```
   else if test='EFR' then predict=mefrp;
   else if test='ERL' then predict=merlp;
   else if test='ERR' then predict=merrp;
   else if test='HBL' then predict=mhblp;
   else if test='HBR' then predict=mhbrp;
   else if test='HDL' then predict=mhdlp;
   else if test='HDR' then predict=mhdrp;
   else if test='KEL' then predict=mkelp;
   else if test='KER' then predict=mkerp;
   else if test='KFL' then predict=mkflp;
   else if test='KFR' then predict=mkfrp;
     else if test='HGR' then predict=mrgrip;
     else if test='HGL' then predict=mlgrip;
end;

else if sex='F' then do;
  if test='DFL' then predict=fdflp;
   else if test='DFR' then predict=fdfrp;
   else if test='SBL' then predict=fdtlp;
   else if test='SBR' then predict=fdtrp;
   else if test='EEL' then predict=feelp;
   else if test='EER' then predict=feerp;
   else if test='EFL' then predict=feflp;
   else if test='EFR' then predict=fefrp;
   else if test='ERL' then predict=ferlp;
   else if test='ERR' then predict=ferrp;
   else if test='HBL' then predict=fhblp;
   else if test='HBR' then predict=fhbrp;
   else if test='HDL' then predict=fhdlp;
   else if test='HDR' then predict=fhdrp;
   else if test='KEL' then predict=fkelp;
   else if test='KER' then predict=fkerp;
   else if test='KFL' then predict=fkflp;
   else if test='KFR' then predict=fkfrp;
     else if test='HGR' then predict=frgrip;
     else if test='HGL' then predict=flgrip;
 end;
```

```
  *predict2=2.71828**predict;
  *Percent=(medmax/predict2)*100;
  *logmm=log(medmax+1);



Below codes creates overall percent predicted and standardized scores for each muscles:

*Multiply each muscle to 9.8 and convert to newton;

data qma2; set qma;length test $5;
 Test='SBL'; MedMax=9.8*SAL; output;
  Test='SBR'; MedMax=9.8*SAR; output;

 Test='EFL'; MedMax=9.8*EFL; output;
  Test='EFR'; MedMax=9.8*EFR; output;

  Test='EEL'; MedMax=9.8*EEL; output;
  Test='EER'; MedMax=9.8*EER; output;

 Test='DFL'; MedMax=9.8*ADL; output;
  Test='DFR'; MedMax=9.8*ADR; output;

  Test='KFL'; MedMax=9.8*KFL; output;
  Test='KFR'; MedMax=9.8*KFR; output;

 Test='KEL'; MedMax=9.8*KEL; output;
  Test='KER'; MedMax=9.8*KER; output;

  Test='HGL'; MedMax=HGL; output;*hand grip stays in kg;
 Test='HGR'; MedMax=HGR; output;

  keep record_id redcap_event_name  sex age height  test medmax  ;
run;
*proc print; run;
```

```
*creates percent and standardized scores;


data qma3; set qma2;
  %include 'n:\research\FSH\qmt_stan_updated.sas';
  %include 'n:\research\ibm\predict.sas';
  predict2=2.71828**predict;
   if test='SBL' OR TEST='SBR' OR TEST='EFL' OR TEST='EFR' OR  TEST='EEL' OR TEST='EER' or
test='DFL' OR TEST='DFR'
OR TEST='KFL' OR TEST='KFR' OR TEST='KER' OR TEST='KEL' or test='DFL' or test='DFR'
THEN Percent=(medmax/predict2)*100;
  if test='HGR' or test='HGL' then percent=(medmax/predict)*100;
  logmm=log(medmax+1);run;



proc sort data=qma3;by record_id redcap_event_name   ;run;
proc means data=qma3 noprint; by  record_id redcap_event_name ;
  var stanqmt percent age;
  output out=qmameans mean=;
  label stanqmt='Standartized Overall QMT Score(SA, EF, EE, AD,KF,KE,HG)';
  label percent='QMT:Overall Percent Predicted(SA, EF, EE, AD,KF,KE,HG) ';
run;



*Below codes calculates Lower Extremity QMA standardized and percent scores;

data qma2u; set qma;
 Test='SBL'; MedMax=9.8*SAL; output; ********;
  Test='SBR'; MedMax=9.8*SAR; output; ********;

 Test='EFL'; MedMax=9.8*EFL; output;
  Test='EFR'; MedMax=9.8*EFR; output;

  Test='EEL'; MedMax=9.8*EEL; output;
  Test='EER'; MedMax=9.8*EER; output;
```

```
  Test='HGL'; MedMax=HGL; output;
 Test='HGR'; MedMax=HGR; output;

  keep record_id redcap_event_name  sex age height  test
medmax  ;
run;

data qma3u; set qma2u;
   %include 'n:\research\FSH\qmt_stan_updated.sas';
  %include 'n:\research\ibm\predict.sas';
  predict2=2.71828**predict;
  if test='SBL' OR TEST='SBR' OR TEST='EFL' OR TEST='EFR' OR  TEST='EEL' OR TEST='EER' THEN ;
Percent_upper=(medmax/predict2)*100;
  if test='HGR' or test='HGL' then percent_upper=(medmax/predict)*100;
  logmm=log(medmax+1);
run;

 data qma3u;set qma3u(rename=(stanqmt=stanqmt_upper ));
run;

proc sort data=qma3u;by record_id redcap_event_name ;  ;run;
proc means data=qma3u noprint; by  record_id redcap_event_name ;
  var stanqmt_upper percent_upper age;
  output out=qmameans_upper mean=;
run;
data qmameans_upper;set qmameans_upper;
label stanqmt_upper='Standardized Upper Extremity QMT Score(SA,EF,EE,HG)';
label percent_upper=' Upper Extremity Percent Predicted QMT Score(SA,EF,EE,HG)';run;


 *Below codes calculates Lower Extremity QMA standardized and percent scores;
data qma2l; set qma;


 Test='DFL'; MedMax=9.8*ADL; output; *******;
  Test='DFR'; MedMax=9.8*ADR; output; *******;

  Test='KFL'; MedMax=9.8*KFL; output;
```

```
  Test='KFR'; MedMax=9.8*KFR; output;

 Test='KEL'; MedMax=9.8*KEL; output;
  Test='KER'; MedMax=9.8*KER; output;


  keep record_id redcap_event_name  sex age height  test
medmax  ;
run;

data qma3l; set qma2l;
   %include 'n:\research\FSH\qmt_stan_updated.sas';
  %include 'n:\research\ibm\predict.sas';
  predict2=2.71828**predict;
  Percent_leg=(medmax/predict2)*100;
  logmm=log(medmax+1);run;

  data qma3l;set qma3l(rename=(stanqmt=stanqmt_leg ));
run;


proc sort data=qma3l;by record_id redcap_event_name date40; ;run;
proc means data=qma3l noprint; by  record_id redcap_event_name ;
  var stanqmt_leg percent_leg age;
  output out=qmameans_leg mean=;
run;
data qmameans_leg;set qmameans_leg;
label stanqmt_leg='Standardized Lower Extremity QMT Score(AD, KF, KE)';
label percent_leg='Lower Extremity Percent Predicted QMT Score(AD, KF, KE)';run;



*Merge the original qmt enties , overall qma , lower and upper qma means;

data
merge qmt qmameans qmameans_upper qmameans_leg;by record_id redcap_event_name ;run;
```

APPENDIX 10: QMT EXTREMITIES

For Upper _Extremity estimated from (SA, EF, EE, HG) :

SA (Shoulder Abduction)
69 salmvict Num 8 BEST12. BEST32. SAR (Shoulder Abduction-Left) QMT (kg)
70 sarmvict Num 8 BEST32. SAR (Shoulder Abduction-Right) QMT (kg)

EF(Elbow Flexion)
58 eflmvict Num 8 BEST12. BEST32. EFL((Elbow Flexion-Left) QMT (kg)
68 efrmvict Num 8 BEST12. BEST32. EFR (Elbow Flexion-Right) QMT (kg)

EE (Elbow Extension)
59 eermvict Num 8 BEST12. BEST32. EER (Elbow Extension-Right) QMT (kg)
60 eelmvict Num 8 BEST12. BEST32. EEL (Elbow Extension-Left) QMT (kg)

HG(Hand Grip):
55 hglmvict Num 8 BEST12. BEST32. HGL (Hand Grip-Left) QMT (kg)
56 hgrmvict Num 8 BEST12. BEST32. HGR (Hand Grip-Right) QMT (kg)

For lower Extremity: estimated from (AD, KF, KE):
AD(Ankle Dorsiflexors)
61 adrmvict Num 8 BEST12. BEST32. ADR (Ankle Dorsiflexors-Right) QMT (kg)
62 adlmvict Num 8 BEST12. BEST32. ADL (Ankle Dorsiflexors-Left) QMT (kg)

KF (Knee Flexion)
65 kfrmvict Num 8 BEST12. BEST32. KFR (Knee Flexion-Right) QMT (kg)
66 kflmvict Num 8 BEST12. BEST32. KFL (Knee Flexion-Left) QMT (kg)

KE (Knee Extension)
57 kelmvict Num 8 BEST12. BEST32. KEL (Knee Extension-Left) QMT (kg)
67 kermvict Num 8 BEST12. BEST32. KER (Knee Extension-Right) QMT (kg)

Over all QMT is estimated from (SA, EF, EE, AD,KF,KE,HG):
HG(Hand Grip):
55 hglmvict Num 8 BEST12. BEST32. HGL (Hand Grip-Left) QMT (kg)
56 hgrmvict Num 8 BEST12. BEST32. HGR (Hand Grip-Right) QMT (kg)

KE (Knee Extension)

57 kelmvict Num 8 BEST12. BEST32. KEL (Knee Extension-Left) QMT (kg)
67 kermvict Num 8 BEST12. BEST32. KER (Knee Extension-Right) QMT (kg)

EF(Elbow Flexion)
58 eflmvict Num 8 BEST12. BEST32. EFL((Elbow Flexion-Left) QMT (kg)
68 efrmvict Num 8 BEST12. BEST32. EFR (Elbow Flexion-Right) QMT (kg)

EE (Elbow Extension)
59 eermvict Num 8 BEST12. BEST32. EER (Elbow Extension-Right) QMT (kg)
60 eelmvict Num 8 BEST12. BEST32. EEL (Elbow Extension-Left) QMT (kg)

AD(Ankle Dorsiflexors)
61 adrmvict Num 8 BEST12. BEST32. ADR (Ankle Dorsiflexors-Right) QMT (kg)
62 adlmvict Num 8 BEST12. BEST32. ADL (Ankle Dorsiflexors-Left) QMT (kg)

KF (Knee Flexion)
65 kfrmvict Num 8 BEST12. BEST32. KFR (Knee Flexion-Right) QMT (kg)
66 kflmvict Num 8 BEST12. BEST32. KFL (Knee Flexion-Left) QMT (kg)

SA (Shoulder Abduction)
69 salmvict Num 8 BEST12. BEST32. SAR (Shoulder Abduction-Left) QMT (kg)
70 sarmvict Num 8 BEST32. SAR (Shoulder Abduction-Right) QMT (kg)

APPENDIX 11: MMT SCORE MAPPING

Raw MMT scores will be mapped as follows:

‘0’ = 0

‘1’ = 1

‘2-‘ = 1.67

‘2’ = 2

‘2+’ = 2.33

‘3-‘ = 2.67

‘3’ = 3

‘3+’ = 3.33

‘4-‘ = 3.67

‘4’ = 4

‘4+’ = 4.33

‘5-‘ = 4.67

‘5’ = 5