

**Date: July 2, 2018**

**RE: Statistical Analysis Plan for Protocol 15-NR-0072 Antioxidant therapy in RYR1-related congenital myopathy**

**Primary outcome measures**

**Where to find data (variables) for the below analyses in locked excel files:**

- 1) AE\_Spreadsheet\_Demographics\_LOCKED\_2018.3.29
- 2) Urinelsoprostane\_6MWT\_LOCKED\_2018.3.29
- 3) PillCount\_PlasmaRedox\_SalivaFBI\_SMOxidativeStressDCFH\_LOCKED\_2018.3.29

**Descriptive statistics**

- Assess data distribution (i.e. basic descriptive statistics) for demographics (sex, ethnicity, smoking\_status, alcohol\_consumption, age\_month0, height\_month0, weight\_month0, bmi\_month0)
  
- Assess data distribution for urine isoprostane (all variables)
  
- Assess data distribution for six-minute walk test (predicted\_distance\_walked\_month0, min\_6\_distance\_month0, predicted\_distance\_walked\_month6, min\_6\_distance\_month6, predicted\_distance\_walked\_month12, min\_6\_distance\_month12).
  
- Assess data distribution for skeletal muscle oxidative stress (all variables),
  
- If possible, also assess data distribution for plasma redox (GSH\_GSSG\_month0, GSH\_GSSG\_month6, GSH\_GSSG\_month12, CYS\_CYSS\_month0, CYS\_CYSS\_month6, CYS\_CYSS\_month12).
  
- Assess data distribution for pill count (percent\_compliance\_month12).

- Descriptive statistics (frequency, mean, median, SD, SEM, IQR, range) for all the above for entire cohort at baseline (month 0) for demographics.
- Repeat abovementioned descriptive statistics split by treatment group (NAC and placebo) at baseline.
- Assess the frequency of AEs by system organ class (System Organ Class (SOC) (AE only) and by Preferred Term (Preferred Term (AE only)).

### **Natural history phase analyses**

During the natural history phase of the study (month 0 to month 6), we will take one look at the data prior to the final analysis with the following two approaches:

1. After 30 participants complete their baseline visit, we will compare their baseline outcome measure values against healthy controls. The mean values for the primary outcome measures (corrected 15-F2t-isoprostane concentration and 6MWT distance) will be compared against existing data for otherwise healthy individuals using summary independent t-tests and/or standardized mean difference (i.e. Hedge's  $g$  which is a measure of effect size between two means, weighted by  $n$ ). This analysis will be repeated when all baseline visits are completed.

#### *Null Hypothesis ( $H_0$ )*

There will be no difference in mean corrected 15-F2t-isoprostane concentration or 6MWT distance, between the *RYR1*-RM and otherwise healthy individuals.

#### *Alternative Hypothesis ( $H_1$ )*

Corrected 15-F2t-isoprostane concentration will be significantly increased and 6MWT total distance will be significantly decreased in *RYR1*-RM myopathy patients when compared to otherwise healthy individuals.

2. After 30 participants complete their 2<sup>nd</sup> visit at 6 months (prior to starting study drug/placebo), disease progression will be assessed using paired t-tests to determine changes between 0 and 6-month visits for each outcome measure. For the primary outcome measures (corrected 15-F2t-isoprostane concentration and 6MWT) change between 0 and 6-month time points will be assessed. This analysis will be repeated when all 6-month visits are completed.

### *Null Hypothesis (H<sub>0</sub>)*

Corrected 15-F2t-isoprostane concentration and 6MWT total distance will not change significantly between 0 and 6 months in *RYR1*-RM myopathy patients.

### *Alternative Hypothesis (H<sub>1</sub>)*

There will be a statistically significant difference between 0 and 6-month values for corrected 15-F2t-isoprostane concentration and/or 6MWT total distance in *RYR1*-RM myopathy patients.

### **Intervention phase analyses (month 6 to month 12)**

- Use an independent t-test (or non-parametric equivalent) to compare pre-intervention (month 6) values for demographics, urine isoprostane and six-minute walk test between NAC versus placebo groups.
- Using multiple imputation (x40 datasets and minimum/maximum value constraints from *per protocol* data) impute missing month 12 data.
- The following GLM should be run both *per protocol* and with ITT datasets.
  1. Run a general linear model comparing month 12 month corrected 15-F2t-isoprostane concentration (corrected\_f2-isop\_month12, corrected\_f2-isop\_PGF2alpha\_ratio\_month12) between NAC and placebo groups controlling for pre-intervention value. Run this model again controlling for established *a priori* confounders including age, sex, height, weight, BMI, smoking status, alcohol status. Variables that do not contribute significantly to the model may be removed. (Exclude RYR023, RYR024, and all healthy volunteers).

### *Null Hypothesis (H<sub>0</sub>)*

In *RYR1*-RM myopathy patients, there will be no statistically significant difference in corrected 15-F2t-isoprostane concentration and/or corrected 15-F2t-Isop:PGF2 $\alpha$  ratio between NAC and placebo groups at month 12, after controlling for established *a priori* confounders.

### *Alternative Hypothesis (H<sub>1</sub>)*

At month 12, in *RYR1*-RM myopathy patients allocated to NAC treatment, there will be significantly decreased corrected 15-F2t-isoprostane concentration and/or 15-F2t-Isop:PGF2 $\alpha$  ratio compared to those allocated to placebo, after controlling for established *a priori* confounders.

2. Run a general linear model comparing month 12 6MWT total distance (min\_6\_distance\_month12) between NAC and placebo groups controlling for baseline value. Run this model again controlling for established *a priori* confounders including age, sex, height. Variables that do not contribute significantly to the model may be removed.

Run this model again and control for one additional hypothetical confounder (post hoc): FVC % predicted.

### *Null Hypothesis (H<sub>0</sub>)*

In RYR1-RM myopathy patients, there will be no statistically significant difference in 6MWT total distance between NAC and placebo groups at month 12, after controlling for established *a priori* confounders.

### *Alternative Hypothesis (H<sub>1</sub>)*

At month 12, in RYR1-RM myopathy patients allocated to NAC treatment, there will be significantly increased 6MWT total distance compared to those allocated to placebo, after controlling for established *a priori* confounders.

## **Adverse Event Analysis**

The 3 categories for AEs in the locked data are:

1. AE
2. UP/SAE
3. Non-UP/SAE

The other categories do not apply as they are not AEs.

Please use these categories in column F: "IRB determination of category".

Exclude HVs (healthy volunteers) and only use RYR numbers. Exclude RYR023 and RYR024.

For the remaining AE events (i.e. in the 3 categories delineated above) all have individual RYR IDs.

Assess:

1. Which AEs occurred in NAC group and which occurred in Placebo group (descriptive statistics) (see attached p.1 Mock Table 1)

2. Were there more specific AEs (by preferred term, Column H on excel) of any kind in NAC vs. Placebo groups? Or were AEs equally distributed between groups? Both Chi-square test and Fisher's exact test extended to general RxC tables, aka the Freeman-Halton test, will be used to compare AE frequencies between NAC and Placebo groups.
3. For those in NAC group, what was event causality (see AE excel column Q) and what was event severity (AE excel column R)? (for an example of this table, see p. 7 of attached, first table on p. 7). Capture the "body system" from our AE excel column G (AKA "system organ class").

## **Secondary Outcome Measures**

### **Natural History Phase (month 0 to month 6):**

**Aim 1:** plasma GSH:GSSG ratio; plasma CYS:CYSS ratio, (corrected DCF-fluorescence intensity (month 6 only))

1. We will compare baseline outcome measure values against healthy controls, when possible, using a summary independent t-test (or non-parametric equivalent). Hedge's  $g$  will be used to assess standardized mean differences between *RYR1*-RM and otherwise healthy population groups.

#### *Null Hypothesis ( $H_0$ )*

There will be no difference in mean plasma GSH:GSSG ratio; plasma CYS:CYSS ratio, or corrected DCF-fluorescence intensity, between *RYR1*-RM and otherwise healthy individuals.

#### *Alternative Hypothesis ( $H_1$ )*

Plasma GSH:GSSG, and CYS:CYSS ratios will be significantly decreased and corrected DCF-fluorescence intensity will be significantly increased in *RYR1*-RM myopathy patients when compared to otherwise healthy individuals.

2. We will compare baseline outcome measure values against values obtained at month 6 using paired t-tests (or non-parametric equivalent) to determine changes between 0 and 6-month visits for each outcome measure.

#### *Null Hypothesis ( $H_0$ )*

Plasma GSH:GSSG, and CYS:CYSS ratios and corrected DCF-fluorescence intensity will not change significantly between 0 and 6 months in *RYR1*-RM myopathy patients.

#### *Alternative Hypothesis ( $H_1$ )*

There will be a statistically significant difference between 0 and 6-month plasma GSH:GSSG, and/or CYS:CYSS ratios and/or corrected DCF-fluorescence intensity in *RYR1*-RM myopathy patients.

**Aim 2:** Timed function tests; Biodex; Myotools, MFM, MRI, PROMIS, FACIT, MFI, NeuroQoL scales.

1. We will compare baseline outcome measure values against healthy controls, when possible, using a summary independent t-test (or non-parametric

equivalent). When sufficient normative data is available, Hedge's  $g$  may be used to calculate the standardized mean difference between groups.

*Null Hypothesis ( $H_0$ )*

There will be no difference, in graded functional tests (time to completion); Biodex (fatigue indices); Myotools values (grip and pinch strength), MFM (D1, D2, D3 and total % of maximum score), MRI (intramuscular fatty infiltration), PROMIS (scale scores), FACIT (total score), MFI-20 (total score), NeuroQoL (scale scores), between *RYR1*-RM and otherwise healthy individuals.

*Alternative Hypothesis ( $H_1$ )*

In *RYR1*-RM patients, graded functional tests (time to completion); Biodex (fatigue indices); Myotools values (grip and pinch strength), MFM (D1, D2, D3 and total % of maximum score), PROMIS (scale scores), FACIT (total score), NeuroQoL (scale scores) will be significantly lower and MRI (intramuscular fatty infiltration), MFI-20 (total score) significantly higher when compared to otherwise healthy individuals.

2. We will compare baseline outcome measure values against values obtained at 6 months using paired t-tests (or non-parametric equivalent) to determine changes between 0 and 6-month visits for each outcome measure.

*Null Hypothesis ( $H_0$ )*

Graded functional tests (time to completion); Biodex (fatigue indices); Myotools values (grip and pinch strength), MFM (D1, D2, D3 and total % of maximum score), MRI (intramuscular fatty infiltration), PROMIS (scale scores), FACIT (total score), MFI-20 (total score), NeuroQoL (scale scores), will not change significantly or clinically between 0 and 6 months in *RYR1*-RM myopathy patients.

*Alternative Hypothesis ( $H_1$ )*

There will be a statistically significant and/or clinically meaningful difference between 0 and 6-month graded functional tests (time to completion); Biodex (fatigue indices); Myotools values (grip and pinch strength), MFM (D1, D2, D3 and total % of maximum score), PROMIS (scale scores), FACIT (total score), NeuroQoL (scale scores), MRI (intramuscular fatty infiltration), MFI-20 (total score) in *RYR1*-RM myopathy patients.

3. Additionally, the following will be analyzed:

Optimal measures for fatigability in *RYR1*-RM will be determined using paired t-tests for 6MWT distance and speed, time for graded functional tests, and fatigue index for biodex fatigue test.

Spearman rho correlation, Pearson correlation and/or Bland Altman analysis will also be used to examine the relationship between slow vital capacity and forced vital capacity (L and % predicted). Descriptive statistics and change between baseline and 6 months will be assessed for FVC and SVC (L and % predicted).

Reliability will be assessed for PROMIS, FACIT, MFI, MFM, and NeuroQoL by Cronbach's alpha for internal consistency and by Intraclass correlation coefficient for test-retest.

### **Intervention phase analyses (intent-to-treat)**

- Use an independent t-test (or non-parametric equivalent) to compare baseline values for all secondary outcomes above between NAC versus placebo groups. General linear modeling will be used to control for possible confounders such as such as but not limited to baseline values, gender, age, and baseline values. Log transformed values may be used if there is no bell curve. We will use an alpha of 0.05 to determine significance. Variables that do not contribute to the model may be removed.
- Using multiple imputation (x40 datasets and minimum/maximum value constraints from per protocol data) impute missing month 12 data.
- The following GLM should be run both *per protocol* and with ITT datasets.
- General linear model comparing month 12-month data between NAC and placebo groups (controlling for possible confounders such as but not limited to: baseline value, age, sex, height, weight, BMI, smoking status, alcohol status).

### **Exploratory Outcome Measures**

**Aim 1:** Anaerobic threshold, NIRS; secreted ER calcium monitoring proteins (SERCAMP) assay in blood and muscle

Anaerobic threshold using NIRS will be evaluated for patterns in individuals with *RYR1*-RM compared to healthy controls. From this evaluation, the tissue saturation index will be calculated as  $TOI = [O_2-Hb] / Tot-Hb$ . The rate of decline and rise in TOI will be compared to healthy controls. NIRS data will also be obtained during CPET and Biodex

testing and may be used to generate new hypotheses regarding oxidative metabolism in *RYR1*-RM.

SERcaMP levels will be tested in an exploratory fashion in patient-derived myotubes to assess whether  $Ca^{++}$  levels are normal in the sarcoplasm and/or cytosol. This will generate new hypotheses regarding  $Ca^{++}$  dysregulation in *RYR1*-RM.

**Aim 2:** CPET, EIM, salivary FBI, melatonin, cortisol and DHEA; QoL qualitative interview

Correlative analysis (Pearson correlation and/or Spearman rho) will be used to examine the linear relationship between subjective fatigue measures (FACIT and MFI) and the salivary biomarkers (FBI, melatonin, cortisol, DHEA) to the objective fatigability measures mentioned above, such as CPET. This data may be used to generate new hypotheses fatigue and fatigability in *RYR1*-RM.

The QoL interviews will be mined for common themes across participants. Interviews will also be mined for any comments about differences in QoL before and after treatment. This data may be used to generate new hypotheses regarding QoL in individuals with *RYR1*-RM.

All data may be used for additional exploratory analyses, particularly for the natural history phase of the study. These additional analyses which will not include or alter 12-month data/results of the intervention phase analysis. This is due to the rare nature of this disease and thus the substantial value of this data to contribute findings in this population.

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