A randomised placebo-controlled crossover trial of micronised resveratrol as a treatment for Friedreich ataxia

Statistical Analysis Plan

Version 1
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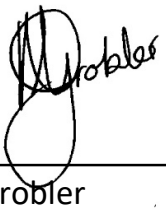

22 August 2022

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List of Abbreviations

AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
DSMC	Data Safety Monitoring Committee
GCP	Good Clinical Practice
ITT	Intent-To-Treat
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
SSI	Significant Safety Issue
SUSAR	Suspected Unexpected Serious Adverse Reaction
FRDA	Friedreich's Ataxia
FXN	Frataxin
mFARS	modified Friedreich's Ataxia Rating Scale
FARS	Friedreich's Ataxia Rating Scale
9HPT	9 Hole Peg Test
BBS	BERG Balance Scale
AIM-S	The Ataxia Instrumented Measure – Spoon
LISN-S	Listening in Spatialized Noise test
FAIS	The Friedreich Ataxia Impact Scale
MFIS	The Modified Fatigue Impact Scale

1. ADMINISTRATIVE INFORMATION

Protocol: Version 8 dated 8 April 2021
ClinicalTrials.gov register Identifier: **NCT03933163**

1.1 Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
	1.0			Initial release.	Not applicable.

1.2 Approvals

The undersigned have reviewed this plan and approve it as final. They find it to be consistent with the requirements of the protocol as it applies to their respective areas. They also find it to be compliant with ICH-E9 principles and confirm that this analysis plan was developed in a completely blinded manner (i.e. without knowledge of the effect of the intervention being assessed)

Name	Role on Study	Affiliation	Signature	Date

2. STUDY SYNOPSIS

TITLE	A randomised placebo-controlled crossover trial of micronised resveratrol as a treatment for Friedreich ataxia
OBJECTIVES	To evaluate the efficacy of micronised resveratrol on clinical and biological outcome measures compared to placebo in individuals with FRDA aged 16 years and over.
DESIGN	Double blind, randomised, placebo-controlled 2-period crossover trial of 2g/day of micronised resveratrol versus placebo
OUTCOMES	Primary outcome measure: Change in modified Friedreich Ataxia Rating Scale (mFARS) score from baseline to 24 weeks. Secondary outcome measures: Change in the following secondary outcome measures from baseline to 24 weeks: 1. The Nine-Hole Peg Test 2. The BERG Balance Scale 3. The Ataxia Instrumented Measure – Spoon (AIM-S) 4. Friedreich Ataxia Impact Scale (FAIS) and the Modified Fatigue Impact Scale (MFIS) 5. The full Friedreich Ataxia Rating Scale (FARS) 6. Measures of dysarthria (reading a paragraph, produce a prolonged vowel sound for 5 seconds, count from one to 20, and produce a 1-minute monologue on a pre-specified topic) and hearing (Listening in Spatialized Noise (LiSN-S) test 7. Cardiac parameters measured by echocardiography (m=mode, B-mode, Doppler, and tissue Doppler assessment) and ECG 8. Biomarkers at 24 weeks compared to baseline: o Frataxin levels in lymphocytes o Plasma F2-isoprostane levels o PGC-1α mRNA levels in lymphocytes o Nrf2 mRNA levels in lymphocytes
STUDY DURATION	52 weeks
INTERVENTIONS	2g/day of micronised resveratrol versus placebo
NUMBER OF PARTICIPANTS	40 participants
POPULATION	Subjects with Friedreich ataxia aged 16 years and over and homozygous for a GAA repeat expansion in intron 1 of the FXN gene; functional stage of the ataxia subscale of the FARS (Friedreich ataxia rating scale) of 1 of higher; adequate end organ function and written informed consent.

2.1. Primary Objective

The primary objective of this study is to compare the change in modified Friedreich Ataxia Rating Scale (mFARS) score from baseline to 24 weeks following treatment with 2g/day of micronised resveratrol, to treatment with placebo.

2.2. Secondary Objectives

The secondary objectives of this study are to:

1. Evaluate the effect of micronised resveratrol on functional measures (the nine-hole peg test and the Berg balance scale, and the Ataxia Instrumented Measure - Spoon) at 24 weeks compared to baseline
2. Evaluate the effect of micronised resveratrol on patient reported outcome measures, the Friedreich Ataxia Impact Scale (FAIS) and the Modified Fatigue Impact Scale (MFIS) at 24 weeks compared to baseline
3. Evaluate the effect of micronised resveratrol on full Friedreich Ataxia Rating Scale (FARS) score at 24 weeks compared to baseline
4. Evaluate the effect of micronised resveratrol on measures of dysarthria and hearing at 24 weeks compared to baseline
5. Evaluate the effect of micronised resveratrol on cardiac parameters measured by echocardiography and ECG at 24 weeks compared to baseline
6. Evaluate the effect of micronised resveratrol on relevant biomarkers at 24 weeks compared to baseline:
    a. Frataxin levels in lymphocytes
    b. Plasma F2-isoprostane levels
    c. PGC-1α mRNA levels in lymphocytes
    d. Nrf2 mRNA levels in lymphocytes

2.3. Study Population

This study will be an outpatient study that will take place at the Murdoch Children's Research Institute, The Royal Perth Hospital, The Royal Brisbane and Women's Hospital, and The Royal North Shore Hospital. Potential participants will be recruited from the Friedreich Ataxia research database at MCRI, the Friedreich Ataxia Research Association of Australasia (FARA) the Friedreich Ataxia Research Alliance (FARA USA), the Australasian patient support group for FRDA as well as patient databases from the Royal Perth, the Royal Brisbane and Women's, and the Royal North Shore Hospitals.

Eligibility Criteria:
Potential participants will be enrolled in this trial only if they meet all of the inclusion criteria and none of the exclusion criteria.

Inclusion criteria:
Each patient must meet all of the following criteria to be enrolled in this study:

1. Age ≥16 years.
2. Diagnosis of FRDA, genetically documented to be due to homozygosity for a GAA repeat expansion in intron 1 of FXN.
3. Functional stage on the Ataxia subscale of the full FARS of 1 or higher (a score of 1 is assigned if the subject has "Minimal signs detected by the physician during screening. Can run or jump without loss of balance. No disability."), and total mFARS score of ≤ 65.
4. Adequate end organ function defined as follows: (i) total bilirubin <2x upper limit of normal unless attributable to Gilbert disease, (ii) ALT and AST <1.5x upper limit of normal, (iii) Creatinine <2x upper limit of normal, (iv) neutrophils >$1.5x10^9$/L, (v) platelets >10^6/µL.
5. Written informed consent provided.

Exclusion criteria:

Patients meeting any of the following criteria will be excluded from the study:

1. Non-elective hospitalisation within the past 60 days that could be of concern in the investigator's judgment. Any hospitalisation in the previous 60 days will be assessed and if in the investigator's judgement it could compromise the individual or the study, that person will not be recruited. Examples include if the individual is hospitalised for management of cardiac morbidity such as uncontrolled arrhythmia or angina or for orthopaedic surgery for a lower limb fracture.
2. Women who are pregnant or lactating or men and women of childbearing potential who are unwilling to use contraception for the duration of the study.
3. FRDA due to compound heterozygosity for an expanded GAA repeat and a point mutation/ deletion in the FXN gene.
4. Current or recent (in last 12 months) arrhythmias including: atrial fibrillation, atrial flutter, sinus tachycardia >120/min, sinus bradycardia <50/min. Symptomatic paroxysmal arrhythmia which is recurring frequently. Cardiac insufficiency (by New York Heart Association >2). Reduced LV ejection fraction (<50%) in the last six months.
5. Medical illness that in the judgment of the investigator would jeopardise the safe completion of the study. Examples include cancer, chronic inflammatory disease, severe diabetes (type I or II, HbA1c >8%), chronic liver insufficiency, epilepsy, thrombocytosis.
6. Evidence of end organ dysfunction through failure to meet one or more parameters in inclusion criterion number 4.
7. Prior invasive cancer (excluding localised basal cell or squamous cell skin cancer).
8. Known hypersensitivity to resveratrol.
9. Use of any investigational agent within 30 days of enrolment.
10. Use of antioxidants such as vitamin E, coenzyme Q10 or idebenone within 30 days prior to enrolment.
11. Concomitant use of medications with potential for clinically relevant drug interactions. This includes medications with a narrow therapeutic range that are metabolised by the cytochrome P450 3A4, 2D6 or 2C9 systems e.g. warfarin, amiodarone.

2.4. Design and Intervention

The study will be a blinded, placebo-controlled randomised 2-period crossover trial of 2g/day of micronised resveratrol in Friedreich ataxia over 24 weeks.

There will be a total of 40 participants with Friedreich ataxia recruited into this study, each of whom will complete two 24-week periods of treatment during the trial. All participants will receive both resveratrol and placebo; 20 will receive resveratrol in the first period and placebo in the second and the other 20 will receive placebo in the first period and resveratrol in the second.

2.5. Randomisation and Blinding

Participants will be randomised to receive either 1g twice daily of micronised resveratrol in period 1 and twice daily placebo in period 2, or placebo in period 1 and micronised resveratrol in period 2, in a 1:1 ratio. Randomisation will be computer generated by an independent statistician using block randomisation, stratified by study centre (4 strata) and will be given to the study pharmacist. Patients and investigators will be blinded to treatment assignment throughout the study. After the first period

of 24 weeks, there will be a four week wash out period. Following the washout period, baseline data will be collected to provide a benchmark for period 2 and then participants will receive their allocated treatment for period 2 for a further 24 weeks.

2.6. Sample Size

The sample size calculation for this study was based on feasibility. We aim to recruit a total of 40 participants over 15 months across the 3 sites.

A sample size of 40 in a crossover study would enable us to identify differences of 0.45 standard deviation [SD] in our primary outcome, the change in mFARS score from baseline to 24 weeks, between the intervention and placebo periods with 80% power (based on a two-sided paired t-test with alpha=0.05). Based on observations from the Friedreich Ataxia Clinic at Monash Medical Centre in Melbourne, people with FRDA who are not treated with resveratrol have an average increase of 4.95 points in the FARS score per year (SD 5.89). This is in line with an annual increase of 5.5 points in the FARS score seen by Friedman and colleagues in untreated patients. Using these data, we would expect an increase of approximately 2.5 points on the FARS over a 24-week period whilst on placebo. We do not have data to estimate the SD of the difference in change from baseline to 24 weeks between the two treatment groups, but assuming a SD of 6 based on our previous data (assuming a lower SD given the reduced time period but allowing for the uncertainty in the outcome in each of the periods) a difference of 0.45 SD would equate to a difference of 2.7 in the change in the FARS score between the groups, or equivalently a reduction of 0.2 in the intervention period compared with an increase of 2.5 in the placebo period. Such a finding would be considered clinically meaningful.

By assuming a change in the mFARS of 1.27 at 6 months in the control group, a crossover study of 40 participants with 6-month follow-up per group will enable us to find a difference in the change from baseline in the mFARS of 2.67 and greater between the two groups (i.e. equivalent to a change of 3.437 in the intervention group) with 80% power based on a SD of 4.77.

2.7. Study Procedures

Study Visit	1	2	3	4	5	6	7	8	9
Week[1]	-1	0	4	12	24	28	32	40	52
Day relative to first dose	-30 to -1	1	28	84	168	196	224	280	364
Informed consent	x								
Eligibility (Inclusion/exclusion criteria)	x	x							
Randomisation		x							
Concomitant medication review	x	x	x	x	x	x	x	x	x
Drug dispensing		x	x	x		x	x	x	
Medical history	x								
Complete physical exam	x				x	x			x
Vital signs	x	x	x	x	x	x	x	x	x
Brief physical exam		x	x	x			x	x	
Urine pregnancy test	x			x		x		x	
Study drug accountability			x	x	x		x	x	x
Safety Bloods[2]	x		x	x	x	x	x	x	x
Full blood examination[3]	x			x	x	x		x	x
Urine dipstick (urinalysis)[4]	x		x	x	x	x	x	x	x
Urine microscopy[4]		x	x	x	x	x	x	x	x
Albumin:creatinine ratio[4]	x		x	x	x	x	x	x	x
Adverse event log		x	x	x	x	x	x	x	x
Biomarkers[5]		x			x	x			x
mFARS and FARS	x	x			x	x			x
9HPT, BBS, AIM-S, speech and hearing endpoints	x[6]	x			x	x			x
FAIS, MFIS		x			x	x			x
Echocardiogram		x			x				x
Electrocardiogram		x			x	x			x

Full details of the background to the trial and its design are presented in the protocol.

3. GENERAL STATISTICAL METHODOLOGY

3.1. Objectives of Analysis Plan

This analysis plan covers the analysis of all objectives.

3.2. Analysis Software

Data with be analysed using Stata v16.

3.3. Definition of Baseline

The baseline for each trail period within the crossover design is taken as the measures and assessments recorded at the pre-treatment visit for each time period (visit 2 and visit 6).

3.4. Definition of analysis populations

Data from this study will be analysed as an intention to treat analysis. Data from all participants who are randomised will be included in the intention to treat (ITT) analysis. The only exclusion criterion is if a participant has not participated in the entirety of one treatment period given the crossover

design. Such participants would be described in the demographics section and included in the CONSORT flow chart of the final manuscript, but would be excluded from the ITT analysis.

3.5. Definitions related to Adverse Events (AEs)

Other than standard adverse effects, the following specific adverse events will be reported on the CRF:

- Gastrointestinal symptoms: diarrhoea, nausea, vomiting, abdominal pain, cramping, flatulence, discomfort on passing faeces
- Neurologic symptoms: headache, dizziness, fatigue, seizure
- Dermatologic: rash, skin discolouration, pruritis, dry mouth, acne, eczema
- Cardiac: chest pain, palpitations, new ECG abnormalities, hypertension, postural hypotension
- Respiratory: shortness of breath, wheeze, chest infections
- Renal: haematuria, urine colour change, cystitis, urinary tract infection, proteinuria, cast nephropathy
- Hematologic: abnormalities in full blood count parameters
- Hepatic: jaundice, hepatomegaly, abnormal liver function tests
- Musculoskeletal: myalgia, cramps, raised creatine kinase, back pain
- Ophthalmologic: blurred vision, double vision, red eyes, itchy eyes
- Genitourinary: epididymitis

3.6. Adjustment for Multiplicity

Given that there is only a single primary outcome measure, there are no plans to adjust for multiplicity.

3.7. Interim Analyses

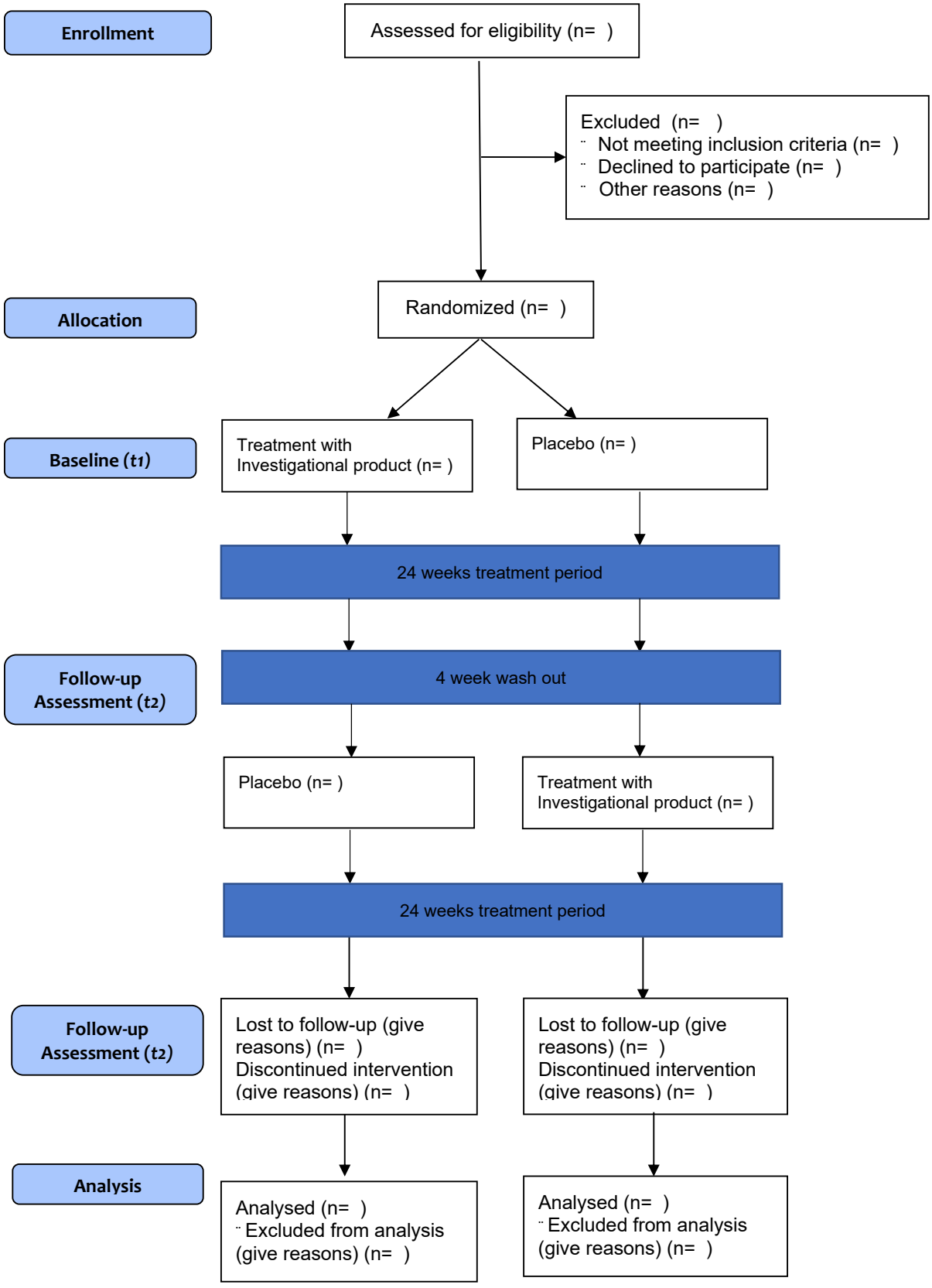
Interim analysis was not performed.

3.8. Handling of Missing Data

It is inevitable that some data will be missing. If less than ten percent of the total data is missing then no change to the ITT analysis will be made.

4. DESCRIPTIVE STATISTICS

4.1. Recruitment and Follow-up



4.2. Baseline Characteristics

Baseline population will be summarised using mean and standard deviations. Due to the crossover design, treatment and control groups will be identical and so will not be compared at baseline. The data summarised will include mean age, proportion of each sex and mean severity as measured by mFARS at screening. Genetic results will be described using mean expansion length and the range. Presence of scoliosis and pes cavus will be described using percentage incidence. Baseline scores for other secondary outcome measures including cardiac function measures, functional outcome measures, and patient reported outcome measured (see below section 6 for full details) will also be summarised using mean and standard deviations.

Demographic	
Age at screening	Mean (range)
Age at onset of FRDA symptoms	Mean (range)
Female Sex, n (%)	
mFARS score	Mean (st d)
FXN Gene Expansion	Mean (range)
Independently Ambulant	
Wheelchair dependent	
Comorbidities	
Cardiomyopathy, n (%)	
Cardiac Arrhythmia, n (%)	
Diabetes, n (%)	
Optic Atrophy, (%)	
Hearing Loss, n (%)	
Urinary urgency or incontinence, n (%)	
Scoliosis, n (%)	
Pes cavus, n (%)	
Functional Outcomes Baseline Data	
9HPT	
FARS	
BBS	
AIM-S	
FAIS	
MFIS	
Cardiac:	
Clinical diagnosis of cardiomyopathy	
LV posterior wall thickness from mmode	
LV fractional shortening	
GLS	
LV biplane ejection fraction	

4.3. Protocol Deviations

Participants who do not complete both treatment periods will be seen as a major protocol deviation.

4.4. Compliance

Compliance and participant adherence to study investigational product will be analysed based upon number of pills returned to pharmacy compared to expected and will be described in the manuscript or supplementary information using a table. The table will comprise headings number of pills

dispensed, actual number of pills returned, expected number of pills returned, difference in expected and actual number returned. The difference in expected and actual number of pills returned provides an assessment of number of pills and therefore doses missed and indicates compliance.

5. ANALYSIS OF THE PRIMARY OUTCOME(S)

5.1. Main Analysis

The primary outcome is the change in modified mFARS score from baseline to 24 weeks. The mFARS is derived from the full FARS. The full FARS consists of three subscales comprising a general score for ataxia, activities of daily living and neurologic examination whereas the mFARS consists of only the neurologic examination that excludes the peripheral nervous system subscale. The mFARS has a total score ranging from 0 to 99 with higher scores indicating greater disability. The FARS has been shown to have good inter-rater reliability and has been shown to have good face and concurrent criterion validity, as well as good responsiveness to change relative to other ataxia rating scales tested. The mFARS has recently been used as the primary endpoint for several ongoing clinical trials and has been approved by the US Food and Drug Administration as an acceptable endpoint for approval of a medication for marketing as a treatment for FRDA.

This analysis makes two assumptions, namely: no period effect and no treatment-period interaction.

Changes from baseline to 24 weeks (from baseline to week 24 in period 1 and from week 28 to 52 in period 2) in the mFARS score will be summarised descriptively as mean changes and their SDs, presented separately for resveratrol and placebo periods. Comparisons between the treatment groups will be made using mixed effects models applied to the change in the mFARS score from baseline during the two periods, with a fixed effect for treatment and period and using a random effect to allow for the multiple observations within an individual. Results will be presented as a mean difference in change and its 95% confidence interval (CI).

5.2. Sensitivity Analyses

Due to the global Covid pandemic in 2020 and 2021 and governmental restrictions in place over that time period, access to face-to-face research visits with participants was severely restricted. The decision was made to perform some assessments by video-call telehealth assessment. Although mFARS assessments by video are comparable to face-to-face assessments as found in a recent publication[1] a sensitivity analysis will be performed and these participants excluded from the analysis.

5.3. Subgroup Analyses

None

6. SECONDARY OUTCOMES

6.1 The Nine-Hole Peg Test (9HPT) which is a measure of upper limb function.

For each participant the difference between baseline and endpoint scores for the 9HPT for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.2 The BERG Balance Scale (BBS) is used to measure clinical balance.

The BBS evaluates performance in 14 balance activities, including sitting and standing. It has a maximum score of 56 and a minimum score of zero. A higher score indicates better balance.
For each participant the difference between baseline and endpoint scores for the BBS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.3 The Ataxia Instrumented Measure - Spoon (AIM-S)

The AIM-S is a device comprising a modified spoon equipped with a BioKin™ wireless motion capture device designed to capture complex movements of the hand and upper limb during the everyday task of eating. Equipment required is a non-slip mat, standard cereal bowl, dry oats and AIM-S. The participant is asked to pick up the spoon with their dominant hand from a standardized stationary position next to the bowl to retrieve a small quantity of dry oats from the bowl, transport to the mouth then return. This sequence of movements is repeated for five trials. The combination of kinematic parameters from three separate directions recorded by the AIMS during each phase of the movement contributes to a final composite score which reflects the upper limb impairment typical of Friedreich ataxia. The AIM-S is currently undergoing longitudinal validation in Friedreich ataxia.

For each participant the difference between baseline and endpoint scores for the AIM-S for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.4 The Friedreich Ataxia Rating Scale (FARS)

The FARS consists of three subscales comprising a general score for ataxia, activities of daily living and neurologic examination. The FARS has been shown to have good inter-rater reliability and has been shown to have good face and concurrent criterion validity, as well as good responsiveness to change relative to other ataxia rating scales tested.

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.5 Quality of life assessment

6.5.1 The Friedreich Ataxia Impact Scale (FAIS) - designed specifically for individuals with FRDA

For each participant the difference between baseline and endpoint scores for the FAIS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.5.2 The Modified Fatigue Impact Scale (MFIS) - which measures the impact fatigue takes on a person's daily life.

Quality of life which will be assessed using the Friedreich Ataxia Impact Scale (FAIS)(1), designed specifically for individuals with FRDA, and the Modified Fatigue Impact Scale (MFIS) which measures the impact fatigue takes on a person's daily life.

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.6 Audiological assessment.

Binaural speech perception assessment will be undertaken using the Listening in Spatialized Noise (LISN-S) test which measures the listener's capacity to segregate a target speech signal from a competing speech noise.
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.7 Speech assessment.

Subjects will be asked to read a paragraph, produce a prolonged vowel sound for 5 seconds, count from one to 20, and produce a 1-minute monologue on a pre-specified topic.
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.8 Change in echocardiogram measures from baseline to 24 weeks.

Subjects will undergo an echocardiogram to assess cardiac parameters prior to commencing resveratrol/placebo (week 0 and week 28) and again at 24 and 52 weeks. Standard transthoracic echocardiographic techniques will be used to assess cardiac function with simultaneous ECG monitoring in accordance with American Society of Echo guidelines.

6.8.1 Left Ventricular posterior wall thickness from mmode

For each participant the difference between baseline and endpoint scores for the LV posterior wall thickness from mmode for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.8.2 Left Ventricular fractional shortening

For each participant the difference between baseline and endpoint scores for the LV fractional shortening for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.8.3 Global longitudinal strain (GLS)

For each participant the difference between baseline and endpoint scores for the GLS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.8.4 Left Ventricular biplane ejection fraction

For each participant the difference between baseline and endpoint scores for the LV biplane ejection fraction for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.8.5 Left Ventricular pulse wave Tissue Doppler velocities

Septal E' and S wave
For each participant the difference between baseline and endpoint scores for the Septal E and S wave for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

Left Ventricular free wall E' and S wave
For each participant the difference between baseline and endpoint scores for the LV free wall E and S wave for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.9 Changes in relevant biomarkers from baseline to 24 weeks of treatment

6.9.1 Frataxin levels in lymphocytes.

For each participant the difference between baseline and endpoint scores for the mFARS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.9.2 Plasma F_2-isoprostane levels.

For each participant the difference between baseline and endpoint scores for the mFARS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.9.3 PGC-1α mRNA levels in lymphocytes.

For each participant the difference between baseline and endpoint scores for the mFARS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).
This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.9.4 Nrf2 mRNA levels in lymphocytes

For each participant the difference between baseline and endpoint scores for the mFARS for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

6.10 Subgroup Analyses

None

7. SAFETY OUTCOMES

Safety laboratory data, including blood haemoglobin, total white cell count, neutrophil count, platelet count, sodium, potassium, urea and creatinine levels and liver function test levels will be assessed to ensure there is no treatment emergent adverse effect on blood safety markers.

For each participant the difference between baseline and endpoint scores for each safety parameter listed above for each treatment period within the crossover trial design will be calculated (from baseline to week 24 in period 1, and from week 28 to 52 in period 2).

This will be analysed in the same way as described for the primary outcome in Section 5.1.

8. REFERENCES

1. Tai G, Corben LA, Woodcock IR, Yiu EM, Delatycki MB. Determining the Validity of Conducting Rating Scales in Friedreich Ataxia through Video. Mov Disord Clin Pract. 2021 Apr 6;8(5):688-693. doi: 10.1002/mdc3.13204. PMID: 34307740; PMCID: PMC8287168.1. Cano SJ, Riazi A, Schapira AH, Cooper JM, Hobart JC. Friedreich's ataxia impact scale: A new measure striving to provide the flexibility required by today's studies. Mov Disord. 2009;24:984-92.