

**A double-blind, randomized, placebo-controlled trial to test the efficacy, safety and tolerability of Dimethyl Fumarate in Friedreich Ataxia (DMF-FA-201).**

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## 1. Summary

Friedreich ataxia (FRDA), an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia among Caucasians. The disease is characterized by gait and limb ataxia, dysarthria, usually absent tendon reflexes, bilateral Babinski sign, impaired vibratory sense. Cardiomyopathy is the predominant cause of death.

The molecular defect is the trinucleotide GAA expansion in the first intron of the *FXN* gene that encodes frataxin, a 210 amino acid mitochondrial protein. Compared to controls, *FXN*/mRNA is reduced to 20% in patients, and to 53% in carriers, and shows an inverse correlation with the size of the GAA repeat.

A reduction in antioxidant defenses has been reported in fibroblasts from FRDA patients, highlighted by a deficiency in Nrf2 activation, suggesting that *FXN* deficiency may cause lower Nrf2 activation and decreased mitochondrial antioxidant protection.

Dimethyl fumarate (DMF) induces Nrf2 in vitro and in vivo modifying cysteine residues in Keap1. Nrf2-binding sites exist in the *FXN* gene, as demonstrated by Chromatin Immuno- Precipitation experiments, which represents one of DMF's modes of *FXN* induction.

A screening protocol for repurposed drugs that rescue frataxin-dependent defects in FRDA fibroblasts resulted in the identification of DMF, which dose-dependently increases frataxin in FRDA patient lymphoblasts. Also, dosed in the FRDA mouse models YG8 and KIKO in the dose range 3-10 mg/kg, DMF caused a dose dependent increase in frataxin protein in cerebellum, and in multiple other tissues. We recently found that DMF is able to increase *FXN*/mRNA by more than 70% in Multiple Sclerosis patients taking DMF as part of their standard of care treatment. DMF caused a 70% increase in mitochondrial copy number in PBMCs, >100% increase in 4/4 mitochondrial transcripts, indicating it can also stimulate mitochondrial biogenesis in-vivo.

Although several trials have been performed in FRDA patients, no effective treatment is available, and clinical practice consists in preventing the development of cardiomyopathy, and in the symptomatic management of the disease.

Based on the previous points, the aim of our study is to further investigate the role of DMF in FRDA and to demonstrate if DMF is able to correct the biological deficits of FRDA. We propose a double-blind, placebo-controlled, randomized, phase II clinical trial to test the effect of DMF on *FXN* transcription in FRDA patients. Secondary objectives of the study will be the effect of DMF on frataxin protein, the nrf2 pathway, on mitochondrial biogenesis, safety and tolerability, and clinical aspects of the disease.

The statistical hypothesis is that DMF is superior to placebo in its ability to increase *FXN*/mRNA.

The study is composed of a screening visit and two sequential phases of identical length of 12 weeks each: a core phase and an extension phase. During the core phase, patients will be randomly assigned to either DMF or placebo. During the extension phase, all patients will be treated with DMF. The study will enroll 40 patients with a molecular diagnosis of FRDA with a homozygous GAA expansion, age 12 years or higher, and with a body weight of at least 30 Kg. Onset of FRDA occurs usually when patients are in their teenage and causes patients to be wheelchair bound approximately 10 years after diagnosis, and to develop cardiomyopathy 10 years later. The result is that the social costs for such disability are both direct, for the costs of physiotherapy and symptomatic drug management, but also indirect for their loss in productivity. The availability of an effective treatment able to halt or slow disease progression may be of immense value as it may help cut both healthcare and social costs of the disease, and offer patients a therapeutic opportunity.

## 2. Background

Friedreich ataxia (FRDA), an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia among Caucasians.[1] The disease is characterized by gait and limb ataxia, dysarthria, usually absent tendon reflexes, bilateral Babinski sign, impaired vibratory sense. Cardiomyopathy is the predominant cause of death.[2]

The molecular defect is the trinucleotide GAA expansion in the first intron of the *FXN* gene[3] that encodes frataxin, a 210 amino acid mitochondrial protein. Compared to controls, *FXN*/mRNA is reduced to 20% in patients, and to 53% in carriers.[4] In patients, frataxin protein is 36% of the level seen in controls, and shows an inverse correlation with the size of the GAA1 repeat. Although the exact physiological function of frataxin is not known, it may have a role in iron–sulfur (Fe–S) cluster biogenesis, iron binding/storage, and scavenging against reactive oxygen species (ROS).[5]

All cells have an intrinsic protective mechanism from ROS that is controlled through the transcription factor Nrf2 that binds to the antioxidant response element (ARE) in the regulatory regions of target genes.[6] Nrf2 is normally sequestered in the cytoplasm through interaction with Keap1, which results in constitutive ubiquitination and proteosomal degradation. Excessive ROS results in the modification of Keap1 such that Nrf2 is no longer constitutively degraded.[7] A reduction in antioxidant defenses has been reported in fibroblasts from FRDA patients, highlighted by a deficiency in Nrf2 activation,[8] suggesting that *FXN* deficiency may cause lower Nrf2 activation and decreased mitochondrial antioxidant protection.[9]

Dimethyl fumarate (DMF) induces Nrf2 in vitro and in vivo modifying cysteine residues in Keap1[7]. DMF also regulates HDAC expression and increases acetylation of H3.3 and H2A. [10] Nrf2-binding

sites exist in the *FXN* gene, as demonstrated by Chromatin Immuno- Precipitation (ChIP) experiments,[11] which represents one of DMF's modes of *FXN* induction.

A screening protocol for repurposed drugs that rescue frataxin-dependent defects in FRDA fibroblasts resulted in the identification of DMF,[11] that dose-dependently increases frataxin in FRDA patient lymphoblasts. Also, dosed in the FRDA mouse models YG8 and KIKO in the dose range 3-10 mg/kg, DMF caused a dose dependent increase in frataxin protein in cerebellum, and in multiple other tissues. We recently found that DMF induces mitochondrial biogenesis and function in human fibroblasts, increases mitochondrial copy number, the mitochondrial biogenesis factor TFAM, 10 mitochondrial transcripts, and maximal mitochondrial O<sub>2</sub> consumption rate.[12] *NCF2* and *PDLIM1* were also recently validated in human FRDA lymphoblast lines as markers of DMF treatment.[13]

We performed an exploratory study to limit expenses and risks related to a straight phase II trial with DMF in FRDA patients. We enrolled multiple sclerosis (MS) patients, for which a decision to start a therapy with DMF (n=14) had already been taken as part of normal clinical practice, at the standard dose of 240 mg BID. We added to this study a parallel group of patients that started a treatment with fingolimod (n=12), a recently approved oral treatment for MS that served as a control group. We also enrolled twelve healthy controls. Baseline levels of *FXN*/mRNA in PBMCs were similar between controls and MS patients (p=0.818). After 3 months of treatment, *FXN*/mRNA expression increased by 85.1% in the DMF group, and 13.7% in the fingolimod group (relative increase in DMF +71.4%; p=0.011; Fig. 1). DMF also caused a 70% increase in mitochondrial copy number in PBMCs, >100% increase in 4/4 mitochondrial transcripts, indicating it can stimulate mitochondrial biogenesis in-vivo.[12] Since MS patients showed comparable levels of *FXN*/mRNA to healthy controls, it is possible that this conclusion may be extended to healthy individuals.

Therapeutic strategies in FRDA include increasing frataxin protein and/or *FXN*/mRNA levels and replacing frataxin function (i.e. antioxidation, iron chelation). Antioxidants, such as idebenone, a short chain quinone analog of CoQ10, are ineffective on the clinical course of the disease.[14] Erythropoietin (EPO) is a glycoprotein that acts as a main regulator for erythropoiesis. EPO increases frataxin levels in cultured human lymphocytes from FRDA patients. A phase IIb multicenter, randomized, placebo-controlled clinical trial showed no effect of Epoetin alfa on exercise capacity and on peak oxygen uptake (VO<sub>2</sub> max) at the cardiopulmonary exercise test in FRDA patients.[15] Deferiprone, an orally administrated lipophilic iron chelator, is able to scavenge iron from mitochondria and transfer chelated iron to biologic acceptors, but it showed several Adverse Events (AEs) limiting its use in FRDA patients [16].

Histone deacetylase inhibitors (HDACi) revert silent heterochromatin to active chromatin, increasing gene transcription, including the *FXN* gene. Treatment of lymphocytes of FRDA patients, with specific HDACi produces a considerable increase in *FXN*/mRNA and frataxin protein (up to 2-fold). Most promising HDACi are RG2833 (Repligen corporation) and Nicotinamide/Vitamin B3. Both have been tested in phase I clinical trials in FRDA patients [17]. RG2833 showed the ability to increase frataxin in 30% of patients after the administration of the highest planned dose, but produced cardiotoxic metabolites. Nicotinamide appears to be effective in increasing *FXN*/mRNA and frataxin protein levels when used at doses much higher than recommended, but several adverse events (AEs) occurred during the phase I trial of nicotinamide, and affected virtually all treated patients (nausea, lightheadness, vomiting, headache).[18] This will probably affect drug adherence and limit the long-term use of the drug. Considering that FRDA is a chronic condition, this may be a serious limitation to nicotinamide. Although several trials have been performed in FRDA patients, no effective treatment is available, and clinical practice consists in preventing the development of cardiomyopathy, and in the symptomatic management of the disease.

Based on the previous points, the aim of our study is to further investigate the role of DMF in FRDA and to demonstrate if DMF is able to correct the biological deficits of FRDA. The study will add important information on the ability in-vivo of DMF, at currently approved doses for MS and psoriasis, to increase the expression of the *FXN* gene and to increase frataxin protein.

## 2.1. Dimethylfumarate

DMF is a fumaric acid ester that has been used for treatment of psoriasis[19] due to its suppressive actions on pro-inflammatory T-cell activation and to shift T-cell polarity from Th1 to Th2.[20] Recently DMF received approval from EMA for the treatment of psoriasis based on the positive results of the BRIDGE trial.[21] DMF has also been considered for treatment of multiple sclerosis (MS)[22] and was approved for the treatment of relapsing remitting MS.

After oral intake, DMF is rapidly hydrolyzed by esterases to its active circulating metabolite MMF. DMF is currently administered due to its lower incidence of gastrointestinal side effects compared to MMF.[23] MMF's bioavailability is decreased by concurrent food ingestion, though it remains bioavailable. MMF is the most bioactive metabolite [23], and typically reaches peak serum concentrations around 20 µM. MMF is eliminated mainly through breathing, only small amounts of intact MMF are excreted through urine or feces. There is no evidence for a cytochrome P450-dependent metabolism in the liver, thus few drug interactions would be expected. MMF's half-life in vivo is around 12 hours.

The safety profile of DMF is well known and adverse events of special interest that have been considered during currently being considered for Skilarence are[24]: leukocytopenia and lymphopenia, flushing, gastrointestinal disorders, hepatic injury, malignancies, renal injuries and proteinuria, serious and opportunistic infections. The reported incidence of leukocytopenia is 13.3%, and lymphopenia is 10.0%, with very few patients (<1%) experiencing severe lymphopenia (<0.5/L) leading to discontinuation. Flushing has an incidence up to 20.8% of patients and is more frequent during the first weeks of treatment and tends to resolve during long term administration of DMF. Gastrointestinal disorders were reported in 62.7% of patients receiving DMF. They usually resolve after the first few months of treatment. The proportion of patients with increase in hepatic enzymes is 7.5% and all are usually mild/moderate intensity. There is no evidence that long-term treatment with DMF is associated with an increased risk of malignancies. The proportion of patients reporting renal injury/proteinuria is low (<3%). Several of these cases of renal toxicity occurred with doses of fumaric acid esters that are much higher than the dose recommended for DMF in psoriasis/MS. In all of these cases, the renal function returned to normal after stopping treatment. The number of infections during DMF treatment was very low (<5%). All infections resolved by the end of the studies or during the 2- month follow-up period. No relation was found between leukopenia/lymphopenia and infections.

### 3. Rationale

The present trial is of extreme interest for the Italian National Health Services and for the entire study population. First, no approved therapy for FRDA exists. This results in the use of symptomatic therapy, when depression and urinary disturbances occur, and in the chronic support through physiotherapy. Both cannot halt disease progression, which occurs in time. Onset occurs usually when patients are in their teenage and causes patients to be wheelchair bound approximately 10 years after diagnosis. The result is that the social costs for such disability are both direct, for the costs of physiotherapy and symptomatic drug management, but also indirect for their loss in productivity. The availability of an effective treatment able to halt or slow disease progression may be of immense value as it may help cut both healthcare and social costs of the disease. Rationale for the selected treatment dose is derived from: approved dose of DMF for Multiple Sclerosis and Psoriasis patients, previously reported effect of DMF 240 mg BID in Multiple Sclerosis patients [12].

## 4. Objectives

We propose a double-blind, placebo-controlled, randomized, phase II clinical trial to test the effect of DMF on *FXN* transcription in FRDA patients. Secondary objectives of the study will be the effect of DMF on frataxin protein, the nrf2 pathway, on mitochondrial biogenesis, safety and tolerability, and clinical aspects of the disease.

The statistical hypothesis is that DMF is superior to placebo in its ability to increase FXN/mRNA.

## 5. Study design

The study is composed of a screening visit and two sequential phases of identical length of 12 weeks each: a core phase and an extension phase. During the core phase, patients will be randomly assigned to either DMF or placebo. During the extension phase, all patients will be treated with DMF.

The use of placebo is based on several factors. On one side the need for a more accurate evaluation on the biochemical effect on FXN/mRNA and frataxin, that can show fluctuations during time. For this reason, an increase of FXN/mRNA in an open-label trial could be erroneously attributed to treatment. The second reason is that secondary endpoints (CPET, Clinical scales, etc.) can show a clear placebo effect once patients take part to a clinical trial. There is no known risk of ending in the placebo group as there is no treatment available for FRDA.

The study will begin with a screening visit where patients will sign the informed consent. We will then assess inclusion and exclusion criteria. Patients fulfilling all inclusion and none of the exclusion criteria will undergo endpoint measurement and will enter the core phase of the study.

Entering the core phase, patients will be randomized to receive either DMF or placebo in a 1:1 ratio. For the first week of the core phase, patients randomized to the treatment group, will receive one 120 mg tablet BID, for a total daily dose of 240 mg. Starting from the second week of treatment, the dose will be increased to two 120 mg tablet BID for a total daily dose of 480 mg. The placebo group will receive identical tablets of placebo. Patients will refer to the study center for endpoint re-assessment, and drug accountability procedures after 1, 4, 8, and 12 weeks. Patients completing the 12-week core phase will continue in a 12-week extension phase. During the extension phase, patients receiving DMF in the core phase will continue with DMF 480 mg/day. Patients receiving placebo in the core phase will undergo a one-week titration with one 120 mg tablet BID, followed by 11 weeks of two 120 mg



tablets BID for a total of 480 mg/day of DMF. Patients will again be visited after 1, 4, and 12 weeks after entering in the extension phase. A detailed description of the study activities is shown in Appendix 1.

## 6. Study population

The trial will enroll patients for our outpatient clinic of hereditary ataxias at the AOU Federico II University. Our outpatient currently follows 120 patients with molecular diagnosis of FRDA with regular bi-annual visits. An additional number of patients attend the center on a less regular basis. Patients' clinical and molecular data are stored in an electronic database that will be used for pre-screening purposes. We will enroll patients based on prescreening procedures, i.e. already known patients, at through enrollment calls via the Italian association for Ataxia (AISA).

During our last phase II trial with EPO, we managed to recruit 46 patients in a 24-week interval.[30] For that study, inclusion/exclusion criteria were more stringent as patients were required to be able to perform a cardiopulmonary exercise test with an arm ergometer. Based on the inclusion/exclusion criteria of this trial, we will be able to complete enrollment during a 24-week interval.

Based on inclusion criteria, and in a similar way to the previous trial[30], we will include patients with an age equal or higher than 12 years. The reason for dosing minors is that FRDA is considered a disease affecting teenager and young adults. This group is considered as a whole and exclusion of minors would lead to biased results and exclude those patients that would benefit more from an innovative treatment. In addition, the use of DMF in minors does not require any additional test, nor did it raise any additional side effects as compared to adults.

The study will have specific withdrawal criteria linked to the safety of DMF treatment. This will be an abnormal laboratory result showing abnormalities in either one of the following:

lymphocyte count ( $<0.5/L$ ), neutrophils ( $<0.5/L$ ), AST ( $> 5xUNL$ ), ALT ( $>5xUNL$ ). In addition to these specific criteria, the treating physician will be free of excluding patients with any adverse event or laboratory results that he/she considers life threatening or that could interfere with the study.

In the case of withdrawal, patients will be monitored with laboratory exams and clinical visits as needed until resolution of the abnormality.

### 6.1. Inclusion criteria

1. Molecular diagnosis of Friedreich Ataxia with a homozygous GAA expansion
2. Age  $\geq 12$  years and  $<70$  years
3. Body weight  $\geq 30$  Kg
4. Patients able to read and sign the informed consent

## 6.2. Exclusion criteria

1. Treatment with DMF in the previous 12 months
2. Treatment with Idebenone, coenzyme Q10, or any other vitamin supplements in the previous 30 days
3. Patients in treatment with any other not allowed drug
4. Any Cardiac and/or Renal and/or Hepatic disease judged as clinically significant by the investigator (any abnormal and clinically non significant cardiac disease associated with Friedreich Ataxia is not an exclusion criteria)
5. Any clinically significant ECG abnormalities that may interfere with the study
6. Any abnormal and clinically significant laboratory exams at screening visit that may interfere with the trial
7. Any acute disease that could interfere with the study, as judged by the investigator
8. Patient positive to the Human Immunodeficiency Virus (HIV) or Hepatitis B or C test
9. Patients with a positive history of neoplasia, with the only exception of a completely excised basal cell carcinoma
10. Positive history of alcohol or drug abuse in the past 2 years, except for medical use of cannabis
11. Hypersensitivity to DMF or any other component of the study drug
12. Patients not able to comply with the study
13. For female patients (Sexually not active, hysterectomized, sterilized, menopause patients are excluded from the following criteria):
  - Pregnancy, or
  - Breastfeeding, or
  - Inadequate contraception

## 7. Intervention

**Dose:** 120 mg gastro-resistant tablets of DMF or equivalent placebo.

**Packaging and labeling:** DMF or placebo will be supplied in blisters of 14 units packaged in kits of 2 or 8 blisters. Each kit will be labeled with a numeric code. Packaging for the core phase will be labelled “Core”, for the roll-over “Extension phase (Initiation)”, and for the Extension phase “Extension phase”.

**Duration of treatment:** During the core phase, patients will receive 1 tablet BID for 1 week, then 2 tablets BID for additional 11 weeks. During the extension phase, patients already treated with DMF will receive 2 tablets BID for 12 weeks. Patients treated with placebo during the core phase, will have a one-week titration with 1 tablet BID for one week, and 2 tablets BID for 11 weeks.

**Procedure for monitoring subjects compliance:** Compliance will be monitored at each visit by counting the number of used tablets and by checking the correct intake procedures. These will be annotated by each patient on a drug diary, that will indicate the time of assumption, side effects, meals taken before assumption.

**Stopping rules, discontinuation criteria:** The study will have specific withdrawal criteria linked to the safety of DMF treatment. This will be an abnormal laboratory result showing abnormalities in either one of the following: lymphocyte count ( $<0.5/L$ ), neutrophils ( $<0.5/L$ ), AST ( $> 5xUNL$ ), ALT ( $>5xUNL$ ). In addition to these specific criteria, the treating physician will be free of excluding patients with any adverse event or laboratory results that he/she considers life threatening or that could interfere with the study.

In the case of withdrawal, patients will be monitored with laboratory exams and clinical visits as needed until resolution of the abnormality. Patients can decide to withdraw the consent to the study at any moment. The patient will be followed and will receive the best treatments available.

**End of Study for each subject:** study will end if previous stopping rules apply or in case of patients' decision. In the case of withdrawal, patients will be monitored with laboratory exams and clinical visits as needed until resolution of the abnormality. Patients can decide to withdraw the consent to the study at any moment. The patient will be followed and will receive the best treatments available. In all previous cases, patients will undergo an end of study visit (EOS).

**End of the clinical trial:** the trial will end when the last visit of the last subject occurs, no other estimated end date can be foreseen or are planned for this study.

**Early termination of the trial:** due to the quick enrollment period (estimated in 6 months), treatment period for each patient (6 months), and to the time needed for FXN/mRNA measurement (3 months), there will not be an early efficacy/futility analysis. On the other hand, there will be an early termination of the trial if signals of DMF harm will be detected. In case of death and SAEs, a thorough investigation of each case will be conducted with PI, treating physician, and needed external advisors (based on the adverse event itself and narrative description). The sponsor will decide if these events are linked to DMF and if it has ongoing safety concerns for the study population it will decide to terminate the study.

**Accountability procedures:** each drug dispensation at the study center will be followed by proper accountability in the accountability logs, that will be under the responsibility of the trial coordinator.

### Prohibited medications

At all time of the trial, and starting from a pre-specified time before screening in parenthesis, patients are not allowed on the following drugs: DMF (180 days), Idebenone and other coenzyme Q10 analogues (30 days), Nicotinamide (90 days), Nicotinic acid (90 days), Valproic acid (30 days), Interferon-gamma (90 days), Erythropoietin (180 days), Etravirine (180 days), any experimental drugs (180 days). The rationale for prohibited medications is the demonstrated in-vitro or in-vivo ability to increase FXN/mRNA or frataxin levels.

## 8. Outcomes

### 8.1. Primary Endpoint

The effect of DMF compared to placebo on FXN gene expression level considering the change from baseline to 12 weeks (core phase of the DMF-FA-201 study).

### 8.2. Secondary Endpoints

- Effect of DMF on frataxin protein; change from baseline to week 12 and week 24
- Effect of DMF on FXN and frataxin protein at week 1 of the core phase, and at week 1 of the extension phase for those patients transitioning from placebo to DMF, compared to other time-points obtained after a DMF dose of 240 mg BID
- Effect of DMF on the cardiopulmonary exercise outputs (VO2max, anaerobic threshold, peak workload). change from baseline to week 12 and week 24.
- Effect of DMF on echocardiographic measures, change from baseline to week 12 and week 24
- Effect of DMF on FXN and frataxin protein at week 4 of the core phase
- Effect of DMF on FXN and frataxin protein from pooled data from the core and extension phase of the study
- Effect of DMF on FXN at all remaining visits compared to baseline (week 8, 16, 20)
- Effect of DMF on Nrf2 pathway genes: NFE2L2, NQO1, HMOX1, PDLIM1, NCF2; change from baseline to week 12 and week 24
- Effect of DMF on mitochondrial biogenesis genes (mt-ND6, mtCYB, mt-CO2, mt-ATP6) and on mtDNA/nDNA; change from baseline to week 12 and week 24
- Number and distribution of serious and non-serious adverse events between DMF and placebo
- Difference in the Scale for the Rating and assessment of Ataxia (SARA) and modified Friedreich Ataxia Rating Scale (mFARS) between DMF and placebo; change from baseline to week 12 and week 24

- Difference in the 9-hole pegboard test (9HPT) and Pata Rate Test (PRT) between DMF and placebo; change from baseline to week 12 and week 24
- Difference in the EQ-5D and ADL/IADL between DMF and placebo; change from baseline to week 12 and week 24

## 9. Methods

An independent rater, blinded to laboratory results and patients side effects, will measure clinical endpoints (SARA, 9HPT). This will be necessary in order to assure that clinical measures will not be influenced by the treating physician in the case he accidentally recognizes treatment allocation, based on side effects or laboratory results. EQ-5D and ADL/IADL will be self-rated. FXN/mRNA and frataxin protein are recognized endpoints for phase II clinical trials in FRDA patients.[18] Nrf2 pathway and mitochondrial biogenesis genes are the logic consequence of DMF's mechanism of action and should be closely monitored during a clinical trial.

### 9.1. The Scale for the Rating and Assessment of Ataxias (SARA) and the modified Friedreich Ataxia Rating Scale (m-FARS)

The SARA scale is a scale consisting of 8 items (gait, stance, sitting, speech disturbance, finger chase, nose-finger test, fast alternating hand movements, heel-shin slide). The scale can score from 0 to 40, with 40 being the worst condition. The scale was validated in FRDA and is the current European standard for the measurement of ataxic symptoms over time.[25] It has been the standard for previous clinical trials.[15]

The m-FARS includes a bulbar, upper and lower limb coordination, and upright stability tests. The scale ranges from 0 to 93. It is a modified version of the original FARS and has been approved by the FDA as a clinical outcome for registrative trials.

### 9.2. 9-Hole Pegboard test (9-HPT)

The 9-HPT is a brief, standardized, quantitative test of upper extremity function. Both the dominant and non-dominant hands are tested twice. On a start command when a stopwatch is started, the patient picks up the nine pegs one at a time as quickly as possible, puts them in the nine holes, and, once they are in the holes, removes them again as quickly as possible one at a time, replacing them into the shallow container. The total time to complete the task is recorded. Two consecutive trials with the dominant hand are immediately followed by two consecutive trials with the non-dominant hand.

### 9.3. Pata Rate Test (PRT)

The PRT allows for quick screening of dysarthria and was first used in a composite index for Spinocerebellar Ataxia patients (Schmitz-Hübsch T, Giunti P, Stephenson DA et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. *Neurology* 2008;71:486-492.). Proband is invited to repeat the syllables “PA-TA” as quickly as possible during a ten-second interval. The test is immediately repeated for a second time. During each attempt the voice is recorded and played at half of the original speed to calculate the number of PA-TA repetitions. Both trials are then averaged for final score determination. Normative values are available for the general population and allow for immediate comparison (Pane C, et al. Adult normative value for the PATA Rate Test. *J Neurol* 2018;265:1102-5.)

### 9.4. The Cardiopulmonary exercise test (CPET)

CPET will be performed using an upper limb cycle ergometer (Ergoselect 400, Ergoline GmbH, Blitz, Germany). A ramp protocol of 5 W/min will be used and continued until limiting symptoms, chest pain, signs of ischemia, or arrhythmias developed or other indications for exercise termination appear. Subjects will be instructed to keep pedaling at a constant rate (50 to 60 rpm) during the test. Subjects were advised that they were free to stop whenever they wished but were encouraged to continue for as long as possible. Respiratory gas exchange measurements will be obtained breath by breath by a commercially available system (Vmax 29C; SensorMedics, Yorba Linda, CA, USA). Peak oxygen uptake ( $\text{VO}_{2\text{max}}$ ), and respiratory exchange ratio (RER), will be recorded at the mean value of  $\text{VO}_2$  during the last 20 sec of the test. The ventilatory anaerobic threshold will be detected by the use of the V-slope method. The ventilation per minute (VE) vs carbon dioxide production ( $\text{VCO}_2$ ) relationship (ventilatory efficiency) will be measured by plotting ventilation against  $\text{VCO}_2$  obtained every 10 sec of exercise (VE/ $\text{VCO}_2$  slope). The VE/ $\text{VCO}_2$  slope will be calculated as a linear regression function, excluding the non-linear part of the relationship after onset of acidotic drive to ventilation.

Peak exercise oxygen pulse will be calculated by dividing derived  $\text{VO}_{2\text{max}}$  by the maximum heart rate (HR) during exercise and will be expressed in milliliters per beat. HR will be recorded by ECG at rest, at the AT and at peak exercise.

There will be a body weight cut-off at 30 Kg based on the need for an appropriate estimation of  $\text{VO}_2$  calculation in children that cannot be reliable for a lower body weight.



### 9.5. Echocardiography (EcoCG)

An ultrasound system equipped with a 2.5 MHz multifrequency transducer (Aplio, Toshiba, Japan) will be used for complete M-mode, two-dimensional, Doppler and Tissue Doppler Imaging (TDI) echocardiographic analyses. M-mode and two-dimensional recordings were made with the patients in the lateral recumbent position. Measures of LV end-diastolic volume (EDV) and endsystolic volume (ESV) will be measured by the modified Simpson's rule. Accordingly, ejection fraction (EF) will be calculated as follows:  $EF = (EDV - ESV) / ESV \times 100$ . LV mass will be calculated according to the American Society of Echocardiography-recommended formula:  $LV\ mass = 0.8 \times \{1.04[(LVIDd + PWTd + SWTd)^3 - (LVIDd)^3]\} + 0.6\ g$  where PWTd and SWTd are posterior wall thickness at end diastole and septal wall thickness at ed, respectively. The following parameters of diastolic function will be measured as the mean of three to five consecutive beats: diastolic transmitral peak velocities, E/A ratio, Isovolumic Relaxation Time (IRT), mitral deceleration time and pulmonary vein velocities. Quantitative diastolic data will be derived from TDI analysis as elsewhere described.

### 9.6. Quantitative real time PCR (q-PCR)

Total mRNA will be extracted from PBMCs using TRIzol® reagent (Thermofisher) following manufacturer's instructions. One µg mRNA will be reversely transcribed using the one-step High Capacity RNA-to-cDNA Master Mix (Thermofisher, Carlsbad, CA, USA) following manufacturer's instructions in a total volume of 20 µL. About 2 µL of cDNA were amplified using the TaqMan® Gene Expression Master Mix and TaqMan® Gene Expression Assay for frataxin (Applied biosystems, catalog n°Hs00175940\_m1) in a StepOne real-time PCR. We will test ten reference genes in order to choose the most stable in our experimental conditions using normfinder software. Relative expression will be calculated with the efficiency-calibrated model. The entire procedure will follow the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.

### 9.7. Frataxin measurement

Peripheral Blood Mononuclear Cells will be extracted from 15ml of EDTA anticoagulated whole blood, and stored until analysis. PBMCs were lysed with an extraction buffer (Abcam n.AB193970-50, Cambridge, UK), and total protein measured using the bicinchoninic acid assay. Each sample was assayed in duplicate using a commercially available ELISA kit (Frataxin profiling ELISA kit, n.AB110173, Abcam, Cambridge, UK), and normalized with a standard curve using full length human frataxin

(n.AB110353, Abcam, Cambridge, UK). This method will be used to measure frataxin at baseline, weeks 4, 12 and 24 of the study.

### 9.8. Study safety

Safety and tolerability will be guaranteed monitoring adverse events and laboratory parameters with the specified timing of the study appendix:

Biochemical routine: Sodium, Potassium, Calcium, Blood Urea Nitrogen, Glucose, Creatine, Total Protein and Albumin, Total Iron, Ferritin, Transferrin, Uric Acid, Total Bilirubin, Direct Bilirubin, Total Cholesterol, Triglyceride, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, Gamma-glutamyl transferase, Lactate Dehydrogenase, Creatine Phosphokinase, Amylase; Na, K, Ca, urea, glucose, creatin, total protein and albumin, total iron, ferritin, transferrin, uric acid, total bilirubin, total cholesterol, triglyceride, AST, ALT, ALP, GGT, LDH, CPK, AMS;

Haematology: Red blood cell count, Hematocrit, Hemoglobin, White blood cell count, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets, Reticulocytes; RBC, HCT, Hb, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, PLT, reticulocytes;

Urine examination: Glucose, pH, Proteins, Bilirubin, Hemoglobin, Red blood cells, White blood cells.  
Pregnancy test: serum beta human chorionic gonadotropin. Glucose, pH, proteins, bilirubin, Hb, RBC, WBC. Pregnancy test: serum beta HCG

### 9.9. Randomization and drug dispensation

Randomization will start with the retrieval of randomization lists that will be generated by the private CRO (FullCRO) involved in the study. Randomization will be in blocks of four and will be stratified based on GAA triplet repeat expansion (group I <635; group II ≥635). For safety reasons, the treating physician will be able to break the code of a single patient and to be informed of the allocation between active treatment and placebo. This will be followed by the immediate discontinuation of the patient. investigator will be able to open the randomization codes only in cases of extreme criticality and / or clinical emergency and only after having taken all possible actions to adequately manage the patient (e.g. a serious adverse event that the Investigator cannot adequately treat if not knowing which drug was used in the double-blind study

### 9.10. Masking

All personnel involved in the study will be masked to treatment allocation. This will be achieved through identical drug boxes and tablets for both active drug and placebo. Drug boxes will have a random code,

and the allocation of that code to either active drug or placebo will be blinded to the treating and evaluating physician, and to the trial coordinator. A study pharmacist will be in charge of uploading the drug box codes once they arrive at the study center with their correct allocation.

#### **9.11. Study Personnel**

The administrating physician will be in charge of: informed consent, inclusion/exclusion criteria, demographics and diagnosis, past/actual medical history, concomitant therapies, ECGs, drug accountability and administration, adverse events. The study rater will be blinded to adverse events, medical records, laboratory exams and other study related data. He will be in charge of neurological examination, SARA, mFARS, 9HPT, EQ-5D, ADL/IADL. Additional personnel: Study nurse (blood sampling), Study coordinator and Biologist (laboratory procedures, study coordination, data entry), Local pharmacist (IVRS entry).

#### **9.12. Information retrieval**

When the use of an electronic clinical reporting form (e-CRF) is envisaged, only validated systems that address traceability are acceptable (for instance, excel spreadsheets do not represent an adequate system for recording data. The measures/indicators used in the trial have been described in the outcomes section, including their validity/reliability. Potential sources of bias are the increase in variability of laboratory measures caused by performance issue of commercially available kits. Although this has never been published, commercially available kits to measure frataxin protein (Abcam) have been used in the past years with conflicting results and the worldwide opinion is that their results are not reliable. For this reason, we will use the Mayo clinic commercial test, which is validated in a clinical setting, and showed to be very reliable with a certified clinical/diagnostic use. For the same reason we will measure the nrf2 pathway and mitochondrial biogenesis using a commercial service of the UC Davis that is experienced in both measurements both in-vitro and in-vivo.

#### **9.13. Error mitigation**

Additional sources of bias are the clinical measures of the SARA and 9HPT. Both may have an inter-rater variability that may add noise to the final results of the study. To reduce this, the same blinded rater will perform all SARA, mFARS and 9HPT measures throughout the study. As for patient reported outcomes (EQ-5D and ADL/IADL), it is known that they might be influenced by the physician's

evaluation of the clinical status. Therefore, they will be measured before any clinical activity during each visit.

Follow-up after study termination is not included in this study. If possible, we will plan an open-label extension of additional 6 months to gather safety information. This will be secondary to funding availability and will be part of a separate study. We expect to lose up to 20% of patients at follow-up. This may be secondary to gastrointestinal side effects and has been accounted for in the sample size calculation.

#### 9.14. Electronic Case Report Form (eCRF)

All variables will be stored in the eCRF of the study that will be specifically designed for the present study using Filemaker 19.5 software. It will be accessible through the web with a common browser. It will be hosted on a server based at the coordinating center with RAID type data protection. The eCRF will follow all indications of the Italian drug agency (AIFA) for non-profit clinical trials, including audit-log trail, data protection and backup, sheet freezing, data unlock for deviations.

#### 9.15. Sample size calculation

Sample size calculation was performed based on our preliminary results of DMF treatment in Multiple Sclerosis patients and is based on the estimated increase in FXN expression with following assumptions derived from our preliminary data [12]. We previously found a significant +85% increase in the group treated with DMF compared to no increase in the control group (time\*treatment interaction  $p=0.02$ ), that resulted in a partial  $\eta^2$  of 0.198. Sample size in this study was 14 patients treated with DMF and 13 with reference control treatment. The partial  $\eta^2$  of 0.198 results in an effect size of 0.497 ~~which could be considered a very high effect size.~~

Estimating the effect size based on preliminary data on MS patients is an underestimation of the potential effect of DMF in FRDA patients. Indeed, DMF can overexpress FXN through two mechanisms. One is the stimulation of nrf2 and the increase in transcription of several genes, including antioxidant genes and frataxin. This mechanism is clearly involved in MS patients treated with DMF. The second mechanism is the ability of DMF to induce transcription initiation and reduce transcriptional pausing in mutant FXN gene. This mechanism is unique to FRDA patients as it requires a mutated gene to be effective. In support to this, experiments in patients' lymphoblasts treated with DMF have found up to a +260% increase relative to baseline[14], compared to our finding of +85% increase in MS patients treated with DMF.

~~This effect size is relative to Multiple Sclerosis patients treated with DMF and could be different in FRDA treated patients. For this reason, we decided to use a more conservative and standard approach with an effect size of 0.25.~~

Based on this data, we calculated the appropriate sample size for this study using G\*Power version 3.1.9.6. We used an ANOVA for repeated measures, within-between interaction, as the appropriate test. Effect size was set at ~~0.4970-25~~, alpha error at 0.05, power at 0.8, number of groups 2, number of measurements 2, ~~correlation among repeated measures 0.5~~, non-sphericity correction 1. This results in a total population of ~~346~~ patients (~~178~~ treated + ~~178~~ placebo). We will prudently include 40 patients total, allowing us to compensate for a lost at follow-up up to ~~180~~% of randomized patients.

#### 9.16. Statistical analysis

All data will be preliminary tested for normality using the Kolmogorov-Smirnov test. Since qPCR data are frequently not normal, a normalization attempt, using ln or log transformation, will be performed. qPCR data will be calculated as relative increases imputing a baseline expression level of 1.0. Main statistical analysis of both FXN expression and frataxin protein level will be conducted with a Generalized Linear Model (GLM) for repeated measures. GLM incorporates a 2-way ANOVA for repeated measures and can accommodate a multivariate analysis of factors and covariates. Treatment with DMF or placebo will be considered as a factor, and baseline levels of frataxin will be considered as a covariate. FXN baseline expression will not be used in the model as it is already integrated using relative expression with common baseline. All other available data (sex, age, disease duration, GAA1, and treatment specific findings, etc.) will be preliminarily tested one by one as covariates. In case of resulting p values <0.1 they will be integrated in the final multivariate model with treatment as a factor. In case of a significant Mauchly's sphericity test, we will use the Greenhouse-Geisser correction. We will use a similar analysis approach for remaining secondary endpoints. This will include: different time-points from FXN and frataxin, and other qPCR analysis, SARA, 9HPT, PRT, ADL/IADL, EQ-5DVAS. The main analysis will be conducted with results from the core phase of the trial (20 patients treated with DMF and 20 with placebo), and will be used to draw conclusions on the efficacy of DMF. A secondary analysis will be conducted adding to the model patients from the extension phase that switched from placebo to DMF (total of 40 DMF vs 20 placebo). This analysis will be considered as descriptive and not used to drive efficacy conclusions. For adverse events (AEs), we will perform a descriptive analysis of AEs including their distribution in DMF and placebo treated patients, AEs duration, severity, and connection to the study drug. To test if treatment with DMF increases the likelihood of having an AE, we will use a Generalized

Linear Model with Poisson distribution and treatment as a factor. To test if a single AE is more frequently linked to DMF treatment we will use the exact test of Goodness of fit, assuming an equal proportion of adverse events in the treatment and placebo group. This will be possible only for adverse events occurring at least one time in both groups. For baseline variables a descriptive analysis will be performed, and difference between DMF and placebo patients will be tested with an unpaired t-test and chi-square test, depending on the variable. In case on non normal distribution a Mann-Whitney test will be used in place of a t- test. A two-sided significance level of 5% will be required to reject the null-hypothesis that the outcome measures are the same before and after treatment, and for all other comparisons. The Bonferroni correction for multiple analyses will be used. Data will be analyzed using the SPSS 23 for MAC (IBM, Chicago, USA).

#### 9.17. Organizational characteristics

This will be a single center trial with only one center activated at the AOU Federico II University Hospital. Our center will provide all of the necessary figures. This will include the Principal Investigator, the treating physician, the trial coordinator and biologist, the site pharmacist, the study nurse. Our center is equipped with all the technology necessary for this trial. For the eCRF we are equipped with a server with RAID protection data with an additional local hard backup and external cloud backup. For laboratory procedures we are equipped with: two refrigerated centrifuges, two non-refrigerated centrifuges, one chemical hood, one biological hood, one PCR hood, two thermocyclers, one real time PCR (StepOne plus), two electrophoretic chambers, one gel photodocumentation system. We also have certified fridges (+4°C) and freezers (-20°C, -80°C). Samples for routine biochemistry, hematology, and urine will be processed at our local laboratory, which is ISO 9001 certified.

#### 9.18. Timing

- The study will require 3 months of study start-up procedures. These will include the ethics committee submission and approval, the contracts for the trial coordinator and biologist, and the start of the trial insurance.
- Enrollment will take 6 months at a rate of 2 new enrolled patients every week. First Patient First Visit (FPFV) will take place 3 months after the beginning of the project. Last Patient Last Visit (LPLV) will occur 15 months after project start.

- Laboratory analysis will proceed in parallel to the generation of samples and will take place between month 7 and 20. Database lock after monitoring procedures and data export will be completed by month 20.
- Data analysis and generation of the final report will take place from month 21 and 24.

## 10. Safety Issues

### 10.1. Adverse Events

An adverse event is any untoward medical occurrence in a patient administered a medicinal product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (including an abnormal finding or lack of expected pharmacological action), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition based on International Conference on Harmonization [ICH]).

This includes any occurrence that is new in onset or worsened in severity from baseline, or abnormal results of any diagnostic procedure carried out as per clinical practice.

### 10.2. Adverse Drug Reaction

An adverse drug reaction (ADR) is defined as a response to a medicinal product that is noxious and unintended. The phrase “response to a medicinal product” means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility.

An ADR, in contrast to an adverse event, is characterized by the fact that a causal relationship between the medicinal product and the occurrence is suspected. All adverse events judged by either the reporting physician or the sponsor as having a reasonable causal relationship to the medicinal product qualify as ADRs.

An unexpected adverse drug reaction (UADR) is an adverse drug reaction in which the nature or severity is not consistent with the available product information. ADRs that are more specific or more severe than described in the available product information should also be considered unexpected.

### 10.3. Serious Adverse Event

A serious adverse event (SAE) based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use, is any untoward medical occurrence that at any dose:

- Results in death

- Is life-threatening (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires patient hospitalization or prolongation of ongoing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important\*

\* Medical and scientific judgment should be exercised in order to establish whether situations should be considered serious, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed above.

For reports of hospitalization, it is the sign, symptom or diagnosis which led to hospitalization that represents the serious adverse event.

Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following reasons:

- Hospitalizations not intended to treat an acute illness or adverse event (eg, social reasons such as pending placement in long-term care facility)
- Surgery or procedure(s) planned before entry into the study (should be documented in the CRF). Note: Hospitalizations that were planned before the start of data collection, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.

All adverse events which do not meet any of the criteria for serious should be regarded as non-serious adverse events.

The AE reporting period for safety surveillance begins when the subject is included in the trial (date of signature of informed consent) and continues until 30 days after the last dosing.

Lymphocyte count  $<0.5/L$  will be considered as a serious adverse event and will lead to immediate drug discontinuation. AST, ALT, GGT levels higher than 3x the upper limit of normality (ULN) will require an additional evaluation of biochemical routine until normalization. Values higher than 5x ULN will require a study drug interruption. In case of adverse events the treating physician will be free to use drugs to mitigate the effect of DMF. These include, but are not limited to: proton pump inhibitors, prokinetics, anti diarrhea agents, acetylsalicylic acid.



#### **10.4. Causality assessment- definitions**

An adverse event is considered associated with the use of the product according to the definitions listed below:

Related: Suspected to be reasonably related to any study medication

Not related: Not suspected to be reasonably related to any study medication. A reasonable alternative explanation must be available.

#### **10.5. Severity Criteria**

An assessment of the severity grade will be made using descriptors outlined in NCI CTCAE v.5.0 toxicity grading.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the patient (e.g., laboratory abnormalities).

Where applicable, an assessment of severity grade will be made using the following categorical descriptors:

Grade 1 Mild

Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities.

Grade 2 Moderate

Sufficient discomfort is present to cause interference with normal activity.

Grade 3 Severe

Extreme distress, causing significant impairment or incapacitation. Prevents normal everyday activities.

Grade 4 Life threatening

Grade 5 Death

#### **10.6. Recording of Adverse Events**

At each trial visit, the subject will be queried on changes in his or her condition. During the reporting period, any unfavorable changes in the subject's condition will be recorded as AEs, whether reported by the subject or observed by the investigator.

Complete, accurate and consistent data on all AEs/SAE experienced for the duration of the AE reporting period (defined above) will be reported on an ongoing basis in the appropriate section of the CRF. All SAEs must be documented and reported using the SAE Reporting form.

The AE report should include a description of the event, its duration, onset and resolution dates, its severity, its causal relationship with the trial treatment, any other potential causal factors, any treatment given or other action taken, including dose modification or discontinuation of the investigational medicinal product, and its outcome. In addition, serious adverse events should be identified and the appropriate seriousness criteria documented.

AEs will be coded according to the latest version of MedDRA.

#### **10.7. Pregnancy Reporting**

All pregnancies with an estimated conception date occurring from the date of enrollment until 30 days after the last drug administration must be recorded in the CRF. This applies both to pregnancies in female participants in the study and to pregnancies in female partners of male participants. The investigator must notify the Sponsor of any pregnancy within 24 h using the appropriate form as per SAE reporting. The Sponsor must be notified on any pregnancy regardless of its association with an AE or not.

Investigators must actively follow up, document, and report on the outcome of any pregnancies even if the subject is withdrawn from the study. If an abnormal outcome occurs, the safety data collection form is to be completed and sent to the Sponsor.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered serious adverse events and must be reported using a Serious Adverse Event Form.

#### **10.8. SAE Reporting**

Serious adverse events will be recorded throughout the study period, defined as the period up to 30 days after the last dose of study treatment or the end of the study. In accordance with local procedures, statutes and the European Clinical Trial Directive, the Investigator must notify the sponsor on any serious adverse event occurring at the site within 24 hours since the acknowledge. The Sponsor will ensure medical review of all the SAEs. The Sponsor will ensure the notification of the appropriate Ethics Committees, Competent Authorities and participating Investigators of all serious adverse events occurring at the site in accordance with local legal requirements, statutes and the European Clinical Trial Directive. Any late SAE (occurring within 30 days after the last drug administration) possibly or probably related to the study treatment will follow the same reporting procedure.

Any death occurring between the study enrollment and 30 days following the treatment must be reported to the Sponsor within 24 hours, as a Serious Adverse Event, regardless of the relation to study treatment as serious adverse event.

Deaths occurring during the study follow-up period (i.e. later than 30 days after the last drug administration) need only to be reported as serious adverse events if it is believed that there is a possible relationship to the study treatment. All deaths should be reported in the death report form section of the CRF regardless of cause.

#### Procedure for Follow-up Information

Serious adverse events occurring during the study must be monitored and followed up by the Investigator until stabilization or until the outcome is known, unless the patient is lost to follow-up.

Reporting of any new information on a previously reported SAE or non-serious ADR (follow-up) will follow the procedures and timelines of the original report.

The sponsor contact for SAEs, Pregnancies and deaths reporting is the following:

[farmacovigilanza.DMF-FA-201@fullcro.org](mailto:farmacovigilanza.DMF-FA-201@fullcro.org)

### 10.9. Safety Investigator responsibility

The investigator is responsible for reporting all adverse events, assessing and explaining the severity of the adverse event and the causal link between it and the drugs in use.

### 10.10. Benefit/Risk Ratio

As to the risk/benefit ratio, it is important to note that DMF has already been on the market for the treatment of MS and has recently received approval for psoriasis. More than 200.000 patients worldwide have already been treated with DMF for MS, and a similar number received DMF as fumaric acid esters for the treatment of psoriasis. The safety profile of the drug is reassuring, and side effects can be minimized with clinical decisions, e.g. assumption of the drug with a full stomach to minimize gastrointestinal side effects, or with an adequate risk management plan for leuco-/lymphopenia and increase in liver enzymes. Study safety is assured through procedures explained in section 9.7 /Safety), 6.2 (Exclusion criteria), 7.0 (stopping rules, discontinuation criteria).

### 10.11. Adequate contraceptive methods

The following methods will be considered adequate during the trial:

- Estrogen and progestogene containing hormonal methods associated with inhibition of ovulation (oral, intravaginal, transdermal)
- Progesteron-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
- Intrauterine device
- Intrauterine hormone-releasing system
- Vasectomy partner
- Sexual abstinence

## 11. Ethical and Administrative Aspects

### 11.1. Good Clinical Practices

Physicians will perform the study in accordance with ICH Good Clinical Practice and Good Clinical Practice for Trials on Medicinal Products in the European Community (ISBN 92 - 825-9563-3).

The Investigator is required to ensure his/her compliance to procedures required by the protocol. The Investigator agrees to provide all information requested in Case Report Form in an accurate manner.

The Investigator is required by Italian Health Authorities to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms.

Specific hazards for this study are the respect of inclusion and exclusion criteria. It is critical that all patients are correctly diagnosed with a molecular test and that this is available as a documentation of the study. Not allowed medications are another critical point as it could interfere with the study drug. This will be accurately checked by the study monitor before patients receive their first treatment. It is also critical that patients comply with the drug dose

regimen and this will be checked through compliance pill counts, house diaries, and drug accountability logs. Monitors will be required to check all of these procedures at each visit. Data generated during the study, and that will be collected in the eCRF, will be laboratory results, clinical scales, self-reported scales, concomitant treatment, adverse events. All will be monitored at each visit for 100% coverage of all imputed data. A total of six monitoring visits will be performed during the study.

Compliance to the study protocol and risk minimization will be achieved through training of all site personnel at the beginning of the study and with periodical (every 3 months) meeting with update on enrollment, side effects, monitoring findings, DMF new SUSARs, and adherence to the programmed schedule. Meeting outcome will be shared with the study personnel and with the study monitor.

### 11.2. Ethical aspects

This protocol is in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and amendments laid down by the 29th (Tokyo 1975), the 35th (Venice, 1983), the 41st (Hong Kong, 1989), the 48th (Somerset West, 1996), the 52nd (Edinburgh, 2000), 53rd (Washington DC, 2002, Note of Clarification added), 55th (Tokyo, Note of Clarification added), 59th (Seoul, 2008) and 64th (Fortaleza 2013) World Medical Assemblies (see appendix).

The protocol will obtain approval from to the local Ethics Committee and competent Authority before starting the study. The EC approval must report trial identification data (title, protocol number and version), documents evaluated (protocol, informed consent,) and the date of their version.

Protocol amendments must not be implemented without prior EC and competent authority approval, or if the relevant competent authority has raised any reasons for non-acceptance..

### **Informed Consent**

The investigator must explain each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the physician. The informed consent will be released as written statement, written in non-technical language. The patient should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the document, oral presentation may be made or signature be given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign documents. No patient can enter the study before his/her informed consent has been obtained. The informed consent is part of the protocol and must be submitted by the investigator to the local ethical committee.

A copy of the patient's signed written consent will be kept by the center in the Investigator Site File. In case of patient consent withdrawal, no further data will be collected. However, any previously collected data will be used for study analyses.

We are not aware of specific ethical risks for subjects participating to the study. The study protocol is not predicted to interfere with subjects' privacy, except for the acquisition of demographical data. These will be treated in accordance to our hospital's procedures.

### **11.3. Privacy aspects**

The names of patients will not be recorded in the eCRF; a sequential identification number will be attributed to each patient enrolled in the trial. This number will identify the patient and must be included on all electronic Case Report Forms.

The Investigator is required to maintain adequate and accurate eCRFs for each patient enrolled in the study. All eCRFs should be completed to ensure accurate interpretation.

All eCRF entries, corrections, and alterations must be made by the participating physicians or authorized participating site personnel.

Participating physicians agree to complete a patient identification and enrollment log to allow easy identification of each patient during and after the study. The document will be reviewed by the sponsor. The patient identification and enrollment log will be treated as confidential and will be filed by the participating physicians in the site file. No copies will be made in order to ensure patient confidentiality.

All reports and communications relating to the study will identify participating patients by the anonymized patient identification number.

Investigators will guarantee that all persons involved in this study will respect the confidentiality of any information concerning the trial.

All parties involved in this clinical trial will maintain the strict confidentiality to assure that neither the personal nor the family privacy of the patient participating in the trial is violated; appropriate measures shall be taken to avoid the access of non-authorized persons to trial data. The processing of patient personal data, and in particular regarding consent, shall comply with Italian privacy laws (Legge delega 127/2001) and with the General Data Protection Regulation GDPR (2016/679).

#### **11.4. Insurance**

An insurance with the company HDI Gerling will be stipulated during start-up procedures, and will be in agreement to Italian law DM 14 July 2009.

## **12. Data Quality Assurance and Monitoring**

Investigators will be responsible for ensuring data quality. Reported information will be checked for consistency, completeness and accuracy during monitoring visit and audits.

### **12.1. Monitoring**

The sponsor or his designee will perform on-site monitoring visits as required.

The nature and location of all source documents will need to be identified to ensure that all original data sources required to complete the CRF are known to the sponsor and participating site personnel and are accessible for verification by the sponsor or designee.

Direct access to source documentation must be allowed for verification of consistency with data recorded in the CRF.

**12.2. On-Site Audits**

Representatives of the sponsor may visit a participating site at any time during or after completion of the study to conduct an audit. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected. The participating physician and participating site personnel are required to be available for consultation during the site visits conducted by the sponsor or designees.

Similar inspections may also be conducted by agents of any regulatory body. Participating physicians should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an inspection.

**13. Archiving**

The participating Investigators/sites will maintain all source documents that support the data collected for each patient, as well as all study documents specified by the applicable regulatory requirement(s). The participating physician/site will take measures to prevent accidental or premature destruction of these documents.

Should it be necessary for the sponsor or appropriate regulatory authority to review documentation relating to this study, the participating physician and/or site must allow access to the aforementioned documents.



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## 15. Study Synopsis

<b>Title</b> A double-blind, randomized, placebo-controlled trial to test the efficacy, safety and tolerability of Dimethylfumarate in Friedreich Ataxia (DMF-FA-201).	
<b>Objectives</b> We propose a double-blind, placebo-controlled, randomized, phase II clinical trial to test the effect of DMF on <i>FXN</i> transcription in FRDA patients. Secondary objectives of the study will be the effect of DMF on frataxin protein, the <i>nrf2</i> pathway, on mitochondrial biogenesis, safety and tolerability, and clinical aspects of the disease. The statistical hypothesis is that DMF is superior to placebo in its ability to increase <i>FXN</i> /mRNA	
<b>Primary Endpoint</b> • The effect of DMF compared to placebo on <i>FXN</i> gene expression level considering the change from baseline to 12 weeks (core phase of the DMF-FA-201 study).	
<b>Secondary Endpoints:</b> • Effect of DMF on frataxin protein; change from baseline to week 12 and week 24 • <u>Effect of DMF on <i>FXN</i> and frataxin protein at week 1 of the core phase, and at week 1 of the core phase for those patients transitioning from placebo to DMF, compared to other time-points obtained after a DMF dose of 240 mg BID</u> • Effect of DMF on the cardiopulmonary exercise outputs (VO2max, anaerobic threshold, peak workload); change from baseline to week 12 and week 24 • Effect of DMF on echocardiographic measures; change from baseline to week 12 and week 24 • Effect of DMF on <i>FXN</i> and frataxin protein at week 4 of the core phase • Effect of DMF on <i>FXN</i> and frataxin protein from pooled data from the core and extension phase of the study • Effect of DMF on <i>FXN</i> at all remaining visits compared to baseline (week 8, 16, 20) • Effect of DMF on <i>Nrf2</i> pathway genes: <i>NFE2L2</i> , <i>NQO1</i> , <i>HMOX1</i> , <i>PDLIM1</i> , <i>NCF2</i> ; change from baseline to week 12 and week 24 • Effect of DMF on mitochondrial biogenesis genes (mt-ND6, mt-CYB, mt-CO2, mt-ATP6) and on mtDNA/nDNA; change from baseline to week 12 and week 24 • Number and distribution of serious and non-serious adverse events between DMF and placebo • Difference in the Scale for the Rating and assessment of Ataxia (SARA) and modified Friedreich Ataxia Rating Scale (mFARS) between DMF and placebo; change from baseline to week 12 and week 24 • Difference in the 9-hole pegboard test (9HPT) between DMF and placebo; change from baseline to week 12 and week 24 • Difference in the EQ-5D and ADL/IADL between DMF and placebo; change from baseline to week 12 and week 24	
<b>Study Design</b> We will progressively enroll patients after signing the informed consent. We will assess inclusion and exclusion criteria during a screening visit. Patients fulfilling all inclusion and none of the exclusion criteria, will undergo endpoint measurement and will enter the core phase of the study. Patients will be randomized to receive either DMF 120 mg or placebo in a 1:1 ratio. Patients will refer to the study center for endpoint re-assessment, and drug accountability procedures after 1, 4, 8, and 12 weeks. Patients completing the 12-week core phase will continue in a 12-week extension phase. Patients will again be visited after 1, 4, and 12 weeks after entering in the extension phase.	
<b>Phase:</b> II	<b>Study Drug:</b> Dimethylfumarate or placebo

#### Drug Administration

Study drug will be Dimethylfumarate or placebo. Both will be administered in gastric-resistant tablets of 120 mg. For the first week of the core phase, patients randomized to the treatment group, will receive one 120 mg tablet BID, for a total daily dose of 240 mg. Starting from the second week of treatment, the dose will be increased to two 120 mg tablet BID for a total daily dose of 480 mg. The placebo group will receive identical tablets of placebo.

During the extension phase, patients receiving DMF in the core phase will continue with DMF 480 mg/day. Patients receiving placebo in the core phase will undergo a one week titration with one 120 mg tablet BID, followed by 11 weeks of two 120 mg tablets BID for a total of 480 mg/day of DMF.

#### Study Population

The study will enroll 40 patients

##### Inclusion Criteria:

- Molecular diagnosis of Friedreich Ataxia with a homozygous GAA expansion
- Age  $\geq 12$  years and  $< 70$  years
- Body weight  $\geq 30$  Kg
- Patients able to read and sign the informed consent

##### Exclusion Criteria:

- Treatment with DMF in the previous 12 months
- Treatment with Idebenone, coenzyme Q10, or any other vitamin supplements in the previous 30 days
- Patients in treatment with any other not allowed drug
- Any Cardiac and/or Renal and/or Hepatic disease judged as clinically significant by the investigator (any abnormal clinically non significant cardiac disease associated with Friedreich Ataxia is not an exclusion criteria)
- Any clinically significant ECG abnormalities that may interfere with the study
- Any abnormal and clinically significant laboratory exams at screening visit that may interfere with the trial
- Any acute disease that could interfere with the study, as judged by the investigator
- Patient positive to the Human Immunodeficiency Virus (HIV) or Hepatitis B or C test
- Patients with a positive history of neoplasia, with the only exception of a completely excised basal cell carcinoma
- Positive history of alcohol or drug abuse in the past 2 years, except for medical use of cannabis
- Hypersensitivity to DMF or any other component of the study drug
- Patients not able to comply with the study
- For female patients (Sexually not active, hysterectomized, sterilized, menopause patients are excluded from the following criteria):
  - Pregnancy, or
  - Breastfeeding, or
  - Inadequate contraception

#### Study Center

UOS Centro Sclerosi Multipla  
AOU "Federico II"  
Via Pansini, 5  
80131 Napoli  
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Codice campo modificato

Formattato: Inglese (Regno Unito)

## 16. Study Procedures Appendix

Phase		Core Phase					Extension phase				
Visit	1	2	3	4	5	6	7	8	9	10	EOS
Activities and Evaluations	Screening (-1 to -2 weeks)	Baseline	Week 1 – day 7 (±1 day)	Week 4 – day 28 (±4 Days)	Week 8 – day 56 (±1 week)	Week 12 – day 84 (±1 week)	Week 13 – day 91 (±1 day)	Week 16 – day 112 (±4 Days)	Week 20 – day 140 (±1 week)	Week 24 – day 168 (±1 week)	Study Interruption
Informed consent	X										
Inclusion/exclusion criteria	X	X									
Demographics	X										
Diagnosis	X										
Past and actual medical history	X										
Randomization		X									
Physical examination and vital signs	X	X	X	X	X	X	X	X	X	X	X
Concomitant therapies	X	X	X	X	X	X	X	X	X	X	X
ECG	X					X				X	X
Neurological examination	X	X				X				X	X
SARA	X	X				X				X	X
m-FARS	X	X				X				X	X
PATA rate Test		X				X				X	X
9HPT		X				X				X	X
CPET		X				X				X	X
ECOCG		X				X				X	X
EQ-5D		X				X				X	X
ADL/IADL		X				X				X	X
Drug administration/accountability		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X
Routine biochemistry	X	X	X	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X	X	X	X
Urine examination	X	X	X	X	X	X	X	X	X	X	X
Blood sampling for protein/RNA	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X			X	X	X		X	X	X	X

17. Study Overview

Study 201 Overview

