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To
**Ufficio Sperimentazione Clinica
AIFA
Via del Tritone, 181
00187 Roma
Roma, 17 April 2023**

Object: EU CT number 2022-503016-16-00, Responses to RFI considerations of 06 April 2023 and 14 April on PART I and RFI considerations of 12 April 2023 on PART II

| | |
|-------------------------|---|
| EudraCT number | 2021-006274-23 |
| EU CT number | 2022-503016-16-00 |
| Protocol Title | A double-blind, randomized, placebo-controlled trial to test the efficacy, safety and tolerability of Dimethyl Fumarate in Friedreich Ataxia. |
| Protocol Code | DMF-FA-201 |
| Phase | II |
| Sponsor | Dipartimento di Neuroscienze, Scienze Riproduttive e Odontostomatologiche Università degli Studi di Napoli Federico II Via Sergio Pansini, 5 - 80131 Napoli, Italia |
| Applicant | Fullcro srl, Via Ignazio Guidi 5. 00147 Roma, Italia |
| Coordinator Site | UOS Centro Sclerosi Multipla AOU "Federico II" Via Pansini, 5 80131 Napoli |

Fullcro srl, as representative of the Sponsor, replies to the RFI considerations in object with the following responses. As consequence, the application and the relevant documents sections of Form of Part I and Part II was updated.



RFI of 6 April 2023 part I

1) Consideration number RFI-CT-2022-503016-16-00-IN-005-01:

Consideration number RFI-CT-2022-503016-16-00-IN-003-01: issue not solved - the rationale supporting the dose chosen for the enrolled patients is not acceptable. The reference provided in the response document (Hayashi et al., 2017) can not be considered sufficient to support the chosen dose for the proposed trial. Moreover, the dose scheme proposed in the trial is the one authorized for Multiple Sclerosis, whose pathogenesis is different from that of Friedreich Ataxia. The Sponsor is requested to provide further data in order to justify the pediatric and adult dose scheme for the proposed trial.

Sponsor response

Thank you for bringing this issue to the discussion, we agree that there is no direct rationale for using the present dose in FRDA and that translating the approved dose for Multiple Sclerosis and Psoriasis may seem insufficient. We would like to support our choice by pointing out that no known drug metabolism issues have ever been observed in FRDA that could lead to a plasmatic and tissue concentration of MMF that differs compared to healthy volunteers and to Multiple Sclerosis and Psoriasis. Second, stimulation of nrf2 through an FDA recently approved treatment, Omaveloxolone, seems to benefit FRDA patients and no direct harm signals have been reported. Third, we observed a 85% increase in FXN/mRNA in our previous study in Multiple Sclerosis patients using the 240 mg BID dose of DMF. This increase could be able to restore FXN/mRNA levels similar to healthy carriers and healthy individuals.

We are aware of the criticism generated by our choice and in order to accommodate your point we now added a secondary endpoint where we will measure FXN/mRNA and frataxin protein after one week of 120 mg BID treatment in the core phase, and after one week of the extension phase for subjects transitioning from placebo to DMF, and compare it to the other measurements obtained after 240 mg BID, allowing for dose finding purposes. We also added safety laboratory measurements at visit 3 and 7.

2) Consideration number RFI-CT-2022-503016-16-00-IN-005-02:

Consideration number RFI-CT-2022-503016-16-00-IN-003-03: issue not solved - The list of laboratory tests in section 9.7 is acceptable. Please add a list of abbreviations of these tests and of the other acronyms

Sponsor response

We now updated section 9.8 Study Safety with all measures spelled out without abbreviations.



3) Consideration number RFI-CT-2022-503016-16-00-IN-005-03:

Consideration number RFI-CT-2022-503016-16-00-IN-003-28: Issue not solved. Furthermore to the choice of the statistical test (RE to Consideration number RFI-CT-2022-503016-16-00-IN-003-16), is necessary to explicit and justify how the effect size is determined also by referring values from literature or preliminary data.

Sponsor response

*The effect size is now determined exclusively through our previously reported effect of DMF in Multiple Sclerosis patients (Hayashi et al., 2017). Since we are the authors of this paper and have access to the raw data, we found a partial eta square of 0.198 for the significant increase of FXN/mRNA after DMF administration compared to fingolimod (pseudo-placebo). Analysis was performed using a general linear model for repeated measures in SPSS. For sample size calculation, we entered the partial eta square value directly into G*Power software using the option "Effect size specification as in SPSS". This results in an effect size of 0.497. Final sample size is different than previously reported because of the different option used (it was previously selected as in "G*Power 3.0"). See software output attached for reference.*

Estimating the effect size based on our 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 and also in FRDA. 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 (see reference 14), compared to our finding of +85% increase in MS patients treated with DMF. For this reason, we believe that an estimated effect size of 0.497 is appropriate for the use of DMF in FRDA and does not represent an overestimation of the potential effect of DMF in FRDA.

4) Consideration number RFI-CT-2022-503016-16-00-IN-005-04:

Consideration number RFI-CT-2022-503016-16-00-IN-003-16: Issue not solved. The output of the determination of the sample sizing was not attached and the statistical determination of the sample sizing still to be unclear. Although the primary end-point is aimed to evaluate difference pre-post among the two study group, the updated sample sizing was set to study the interaction term. By following this approach, since the interaction represent a squared variable, it is necessary a more appropriate evaluation and adjustment for the alpha error. Please note that an unreadable symbol must be changed in the sentence "that resulted in partial ?2" which does not to allow to clearly understand the sample sizing.



Sponsor response

*We are now attaching the output of the sample size calculation using G*Power. Unfortunately, “partial eta square” was unreadable, we are now spelling it out instead of using symbols. Using SPSS effect size specification, we should have solved the issue and have a more appropriate alpha error adjustment.*

5) Consideration number RFI-CT-2022-503016-16-00-IN-005-05:

Consideration number RFI-CT-2022-503016-16-00-IN-003-13: issue not solved - The current version of the protocol contains discontinuation criteria for enrolled subjects. Please add clinical trial termination criteria.

Sponsor response

Sorry for misunderstanding this point, we will not have early termination criteria for early evidence of efficacy. This is due to the quick enrollment that we foresee for patients, and to the time needed for FXN/mRNA measurement. We estimate 6 months for enrollment and 6 months treatment for each subject. Biochemical analysis will take 3 months leaving not enough time for an efficacy and futility analysis. On the other hand, we can add an early termination if signals of DMF harm will be detected during the trial. In case of death and SAEs, each case will determine an unblinding of those subjects and a thorough investigation 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. We now added a sub-section to Section 7.

6) Consideration number RFI-CT-2022-503016-16-00-IN-005-06:

Consideration number RFI-CT-2022-503016-16-00-IN-003-07: issue not solved - According to the Regulation 536/2014 Annex I D17 (o) the protocol should contain a clear and unambiguous definition of the end of the clinical trial and, if it is not the date of the last visit of the last subject, a specification of the estimated end date and a justification thereof.

Sponsor response

Thank you for citing Annex I D17 letter o, we now provide a sub-section called end of clinical trial (Section 7) that states that the trial will end at the date of the last visit last subject.



7) Consideration number RFI-CT-2022-503016-16-00-IN-005-07:

Consideration number RFI-CT-2022-503016-16-00-IN-003-05: issue not solved - The Sponsor is required to add the justification for the use of placebo in the protocol, including the appropriate ethical considerations;

Sponsor response

Thank you for making this point clear, we now added a comment in section 5 (Study design) explaining the reason for placebo and the risk of this for patients. Since no available treatments are available, we do not foresee any risks.

RFI 14 April 2023 Part I

Consideration number RFI-CT-2022-503016-16-00-IN-007-01

Considering that the cited effect size refers to increase of FXN/mRNA after DMF administration compared to fingolimod that are two different line of treatment for SM and moreover, as also defined by the researchers, the cited effect size is "very high effect size". A more conservative effect size would be preferable to reduce the probability of a negative or inconclusive study. Please justify the effect size chosen and the decision not to use a more conservative effect size

Sponsor response

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RFI 12 April 2023 Part II

Consideration number RFI-CT-2022-503016-16-00-IN-006-01

All references to incidental findings must be eliminated in all information sheets (mature minor, adults, parents and relative privacy consents).

Sponsor Response: all patient documents were corrected

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