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COMMENTARY & VIEW

Preventive study in subjects at risk of fatal familial insomnia: Innovative approach to rare diseases

Gianluigi Forloni[1,*], Mauro Tettamanti[1], Ugo Lucca[1], Yasmin Albanese[1], Elena Quaglio[1], Roberto Chiesa[1], Alessandra Erbetta[2], Flavio Villani[2], Veronica Redaelli[2], Fabrizio Tagliavini[2], Vladimiro Artuso[3], and Ignazio Roiter[3]

[1]Department of Neuroscience; IRCCS; Istituto di Ricerche Farmacologiche "Mario Negri" Milano; Milano, Italy;
[2]Fondazione IRCCS; Istituto Neurologico "Carlo Besta"; Milano, Italy
[3]ASL9 Ca' Foncello, Treviso, Italy

ABSTRACT. The text describes a preventive clinical trial with drug treatment in a very rare neurodegenerative disease (Fatal familial Insomnia, FFI) designed with the help of individuals at genetic risk of developing the disease, asymptomatic carriers, who have agreed to be exposed over a 10-year period to doxycycline, an antibiotic with anti-prion activity. At least 10 carriers of the FFI mutation over 42 y old will be treated with doxycycline (100 mg/die) and the incidence of the disease will be compared to that of an historical dataset. For ethical reasons a randomized, double-blind, placebo-controlled trial was not feasible, however the study design and the statistical analysis ensure the scientific value of the results. This approach might represent an important breakthrough in terms of potential therapy and knowledge of rare diseases that could give some hopes to these neglected patients.

KEYWORDS. doxycycline, neurodegeneration, preventive treatment in FFI, Prion's diseases, trial

Treating asymptomatic individuals at risk of developing neurodegenerative disorders is a hot topic on the agenda of a scientific community that has faced a long series of failures with putative disease-modifying drugs. Several initiatives have been organized to test

*Correspondence to: Gianluigi Forloni; Email: Forloni@marionegri.it
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preventive approaches in individuals at genetic risk to develop Alzheimer's disease and other neurodegenerative disorders.[1,2] Here we describe a preventive clinical trial based on the availability of a considerable number of subjects carrying a rare disease-associated mutation who accepted to be exposed for a long period of time to a drug potentially preventing development of disease.

Prion diseases, also known as transmissible spongiform encephalopathies (TSE), are a group of invariably fatal neurodegenerative disorders that can arise sporadically, be genetically inherited or acquired by infection.[3,4] Genetic prion diseases have a pattern of autosomal dominant inheritance and are linked to mutations in the *PRNP* gene encoding the cellular prion protein (PrP^{C}), on chromosome 20. The pathogenic mutations are believed to promote PrP^{C} misfolding and aggregation, favoring the formation of an infections isoform (PrP^{Sc}), which propagates by imprinting its abnormal conformation onto PrP^{C} molecules.

PRNP mutations are associated with different clinical and neuropathological phenotypes: Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia (FFI). FFI is associated with the *PRNP* D178N/M129 haplotype; it is characterized clinically by profound sleep alterations and autonomic dysfunction, neuropathologically by severe degeneration of the anterior ventral and mediodorsal nuclei of the thalamus.[5] The disease is devastating usually leading to death within 2 y from onset, and no treatment is available. We have taken advantage of the unique opportunity to collaborate with a group of subjects belonging to a large Italian FFI kindred[6] organized in association, to design a clinical trial to evaluate the efficacy of a preventive drug treatment. Eighty-five individuals belonging to the kindred were screened for the *PRNP* D178N/M129 mutation, and 22 of them were found to carry the genetic defect. Based on the analysis of previous cases (46 subjects), it was determined that the highest risk of developing FFI is within the range of 50−55 y of age since the disease penetrance is very high (94% in this particular kindred) and the possibility of cure after clinical onset is remote, we designed a study to test a preventive treatment with doxycycline (DOXY) in carriers who were born between 1960 and 1969 (i.e. over 42 y old at recruitment). The potential efficacy of DOXY in prion diseases was suggested by experimental investigations[7–12] and 2 observational clinical studies in Italy and Germany, which reported positive effects on survival of CJD patients with negligible side effects.[12] More recently a double blind trial did not support the use of DOXY in CJD subjects with overt clinical disease.[13] These disappointing results were discussed with the participants of the preventive study, a large part of the them were agree that they support the need to develop a preventive approach in a pathological condition that, when full-blown, leaves little possibility of cure with a drug treatment. Furthermore, a case report recently published of asymptomatic subject treated with DOXY for 4 y indicate the exposure to the drug as the potential cause of the long survival of the subject.[14,15]

The subjects gave their informed consent to participate in the study on the condition that they would not be made aware of their genotype. To comply with this request, both carriers and non-carriers belonging to the family were recruited based on the risk age (42–52 y old). All of them followed exactly the same procedures with the only difference that non-carriers received the placebo instead of the DOXY. However, to reduce the possibility that a participant could accidentally discovery to which treatment group she/he belonged (due to possible side effects of the drug), also non-carriers were administered DOXY for a limited period of time (1–2 months). Thus, 10 carriers receiving DOXY (100 mg/die orally) were matched with 15 non-carriers belonging to the same family group. Before starting the treatment and every second year afterward, all participants included in the study were clinically examined following identical procedures, the neuropsychological examination is performed every year and the blood sample analysis every 6 months. At the time of writing, the

recruited subjects had already undergone the initial medical evaluation at the including endocrinological exams and neuropsychological assessment, then neurological examinations according to the recent guidelines for diagnosis of FFI.[16] The neuropsychological assessment was performed by a trained psychologist blind to the condition of the recruited subjects, a wide range of cognitive functions are explored. The neurological examination and the instrumental analysis (MR/MRI analysis, polysomnography, cardiovascular autonomic reflex testing) are conducted at the Carlo Besta Neurological Institute in Milan by expert physicians blind to the conditions of the subjects. The clinical and anamnestic information is collected in an electronic database/CFR managed by personnel located at the Mario Negri Institute in Milan. DOXY hydrocloride (Bassado, 100 mg of pure base) and placebo tablets indistinguishable by shape or taste were prepared by a specialized company. Although the size of the sample is limited, according to the statistical analysis of historical data (44 out of 46 subjects died because of FFI, median of age at clinical onset calculated on 42 subjects was 49 years), we should be able to establish the efficacy of the treatment within 10 y. Based on data of the recorded cases with age at onset equal or higher than 42 y a survival curve was estimated, and probability of showing clinical signs in the following 10 y was calculated for each one of the subjects in study according to his/her age at the date of inclusion. Using these single probabilities we calculated the probability of observing no new FFI in the following 10 y. Then we calculated the probability of having exactly one, exactly 2 and exactly 3 new FFI cases out of 10 subjects in the same time period. The cumulative probability of observing 3 or less new cases in the 10 y following beginning of treatment for the 10 subjects studied is less than 0.05. Therefore if we will observe less than 4 cases we will declare the experiment had succeeded in demonstrating an effect of the drug (against a historical cohort). As a consequence the probability of 3 or fewer subjects getting ill in the course of 10 y is 0.0144, which is low enough to declare it statistically significant under the standard 0.05 cut-off value. Even though not having (or having a very low number of) FFI incident cases in the 10 y of the experiment will show the effect of doxycycline on the disease this does not rule out the possibility that the subjects may display the disease later (in this case the effect would be a 10 y postponement rather than a complete prevention). The participants have requested psychological support during the study, thus a specific psychological support plan has been elaborated by a specialized team that will follow the participants for the whole study. This help is needed also to face the consequences of the possible appearance of the FFI symptoms in a subject, to avoid the instinctive reaction to quit the study by the other participants. Since the occurrence of FFI cases does not mean automatically that the treatment is not active, it is extremely important to obtain conclusive results.

The protocol (DOXIFF) was reported in *The National Monitoring Centre on Clinical Trials* (OsSC) operating under the control of the Italian Medicines Agency (Eudra CT 2010–02223328). The protocol submitted on April 2011 was approved by the Ethics Committees on December 2011. We are now proceeding with the first follow up of the subjects that are going to finish the first 2 y of treatment. Hopefully the study will be closed within 2023.

This is the first preventive study in FFI, which takes advantage of the unique opportunity to treat in controlled conditions a large pedigree of individuals at risk of developing the disease. The absence of reliable biological marker compels us to a study of incidence and the limited number of carriers, as well as ethical considerations, make us rule out the possibility of having a control group. In this regard, since 3 carrier members of the family at the time of the recruitment withdrew their willingness to participate, they could be considered as an external control group. The clinical, neuropsychological, neurological and instrumental assessment performed every 2 y will enable us to keep numerous parameters potentially

affected by the disease under control. Thus, in the case of development of the FFI, it would be possible to collect complete information on the pre-symptomatic status of the subjects, useful to elaborate new strategies of investigation and identify biological markers. The present study could be a prototype for similar preventive investigations in other genetic neurodegenerative diseases.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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