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Histological effects of givinostat in boys with Duchenne muscular dystrophy

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Abstract

Duchenne Muscular Dystrophy (DMD) is caused by mutations in the dystrophin gene leading to dystrophin deficiency, muscle fiber degeneration and progressive fibrotic replacement of muscles. Givinostat, a histone deacetylase (HDAC) inhibitor, significantly reduced fibrosis and promoted compensatory muscle regeneration in *mdx* mice. This study was conducted to evaluate whether the beneficial histological effects of Givinostat could be extended to DMD boys. Twenty ambulant DMD boys aged 7 to <11 years on stable corticosteroid treatment were enrolled in the study and treated for ≥12 months with Givinostat. A muscle biopsy was collected at the beginning and at the end of treatment to evaluate the amount of muscle and fibrotic tissue. Histological effects were the primary objectives of the study. Treatment with Givinostat significantly increased the fraction of muscle tissue in the biopsies and reduced the amount of fibrotic tissue. It also substantially reduced tissue necrosis and fatty replacement. Overall the drug was safe and tolerated. Improvement in functional tests was not observed in this study, but the sample size of the study was not sufficient to draw definitive conclusions. This study showed that treatment with Givinostat for more than 1 year significantly counteracted histological disease progression in ambulant DMD boys aged 7 to 10 years.

Keywords: Duchenne muscular dystrophy; Givinostat; Histone deacetylase inhibitor; Histology; Clinical trial

1. Introduction

Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy in childhood [1]. The disease is caused by mutations in the dystrophin gene, leading to dystrophin deficiency and subsequent cell membrane instability. This instability determines uncontrolled calcium influx, inflammation, necrosis, and replacement of muscle with fibrotic tissue and fat, which leads to severe muscle wasting and weakness.

Pharmacological blockade of the histone deacetylase activity, which is constitutively active in DMD muscles [2] by

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HDAC inhibitors (HDACi), prevents fibrosis and promotes compensatory regeneration in the *mdx* mouse, a model of DMD [3,4].

Givinostat (aka ITF2357) is a potent HDACi currently being developed for the treatment of DMD. In *mdx* mice [3], Givinostat dose and concentration dependently increased the cross-sectional area of myofibers, decreased the cellular inflammatory infiltrate and prevented the formation of fibrotic scars. These findings strongly suggested that in this DMD animal model Givinostat was able to inhibit all the processes which determine muscle fibrotic substitution (inflammation, necrosis, fatty replacement and fibrosis) and to stimulate muscle regeneration with the formation of larger muscle fibers and overall more muscle tissue. Results also suggested that exposures of Givinostat of 300 ng*h/mL are required to exert the beneficial effect.

To evaluate the potential of Givinostat as a treatment for DMD, we conducted the study summarized in this manuscript. The primary objective of this study was to confirm also in humans that Givinostat can counteract the histological signs of the disease.

2. Methods

2.1. Patients

Twenty boys aged 7 to <11 years with an immunohistochemical and molecular diagnosis of DMD were enrolled in this study after an informed consent form was signed by a parent/guardian and child had assented to be in the study (if applicable). Boys were on a stable dose of systemic corticosteroids for at least six months and were able to complete the two screening 6 minute walk tests (6MWT) with a minimal distance of at least 250 meters each with the results of these tests within ± 30 meters of each other. Exclusion criteria were aimed at avoiding confounding factors from other potentially active treatments, and at recruiting boys without significant co-morbidities and without clinical alterations that could be worsened by Givinostat, e.g. low platelet counts. Detailed inclusion/exclusion criteria are summarized in the Supplementary Information.

2.2. Design

This was an open label two-part, phase 2 study. The primary study objective was the evaluation of the histological effects of Givinostat comparing baseline and end of treatment muscle biopsies (brachial biceps). Secondary objectives of the study were safety and tolerability, and functional assessments (6MWT, North Star Ambulatory Assessment (NSAA) and Performance of Upper Limb (PUL)).

Fig. 1 summarizes the study design. Part 1 was a dose escalation study. Boys were asked to return to sites every week for physical examination, vital signs, ECG, laboratory tests, AEs collection, drug dispensing and PK sampling (only at week 2).

All the boys who completed Part 1 entered Part 2. One boy entered the study directly in Part 2. Boys visited their study site at months 1, 2, 3, 4.5, 6, 7.5, 9, 10.5 and 12 of Part 2 for physical examination, vital signs, ECG, laboratory tests, AEs collection, drug dispensing and PK sampling (only at month 12). 6MWT, NSAA, and PUL were evaluated at screening, start of part 2, and at months 3, 6 and 12. Echocardiogram and spirometry were conducted at screening and at the end of part 2.

2.3. Standard protocol approvals, registrations and patient consents

The study was sponsored by Italfarmaco S.p.A. (Milan, Italy), performed in compliance with Good Clinical Practice and the Declaration of Helsinki and it was registered (Identifier NCT01761292) at www.Clinicaltrials.gov. The study was approved by the local Ethics Committees and authorized by the Competent Authority of Italy. A parent or guardian of the participants provided informed written consent and each subject provided written assent before participation.

2.4. Treatments

All boys were treated with Givinostat. During part 1 the dose was escalated from 25 mg BID to 50 mg BID and then reduced to 37.5 mg BID dose. During part 2 all boys were started on the 37.5 mg BID; seven completed the study on this dose and twelve reduced the dose to 25 mg BID (see below for details). All boys continued the steroid treatment regimen they were on at screening.

2.5. Endpoints

Histology: the primary endpoint was the change in histology comparing the brachial biceps biopsies before and after ≥12 months of treatment with Givinostat. The histological parameters assessed were: muscle fiber area fraction (MFAF), cross-sectional area (CSA), necrosis, hypercontracted (hyaline) fibers, fatty replacement and fibrosis (total, endomysial, perimysial). Details on muscle biopsy collection, preparation and histological assessments are provided in the Supplementary Information.

Muscle Function Tests: change in 6MWT, NSAA and PUL after treatment with Givinostat were secondary endpoints. Details on these function tests were reported previously [5–7].

Safety and tolerability were assessed by AEs collection, laboratory tests, physical examination, vital signs, ECG, echocardiogram and spirometry. The following laboratory tests were performed: hematology, total bilirubin, alkaline phosphatase, amylase, ALT, AST, LDH, C-reactive protein, creatine kinase, total protein, albumin, uric acid, sodium, potassium, chloride, calcium, glucose, creatinine, BUN, and CPK, creatinine clearance, and urinalysis. Laboratory tests were conducted at local laboratories.

2.6. Statistical analysis

Based on the results reported in the Desguerre publication [8], a sample size of 20 boys completing the study provided a 90% power (at a 2-sided alpha level of 5%) to detect at least a 25% relative increase in MFAF between pre- and post-treatment using a paired t-test and assuming a normal distribution.