Journal of Immunological Methods 440 (2017) 41–51

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Antibodies that neutralize cellular uptake of elosulfase alfa are not associated with reduced efficacy or pharmacodynamic effect in individuals with Morquio A syndrome

Andrew C. Melton *, Russell K. Soon Jr., Troy Tompkins, Brian Long, Becky Schweighardt, Yulan Qi, Catherine Vitelli, Anil Bagri, Celeste Decker, Charles A. O'Neill, Stephen J. Zoog, Lynne Jesaitis

BioMarin Pharmaceutical Inc., Novato, CA, United States

ARTICLE INFO

Article history:
Received 6 August 2016
Received in revised form 20 October 2016
Accepted 21 October 2016
Available online 24 October 2016

Keywords:
Morquio A
Neutralizing antibody titer
Lysosomal storage disease
Elosulfase alfa
Enzyme replacement therapy

ABSTRACT

Many enzyme replacement therapies (ERTs) for lysosomal storage disorders use the cell-surface cation-independent mannose-6 phosphate receptor (CI-M6PR) to deliver ERTs to the lysosome. However, neutralizing antibodies (NAb) may interfere with this process. We previously reported that most individuals with Morquio A who received elosulfase alfa in the phase 3 MOR-004 trial tested positive for NAbs capable of interfering with binding to CI-M6PR ectodomain in an ELISA-based assay. However, no correlation was detected between NAb occurrence and clinical efficacy or pharmacodynamics. To quantify and better characterize the impact of NAbs, we developed a functional cell-based flow cytometry assay with a titer step that detects antibodies capable of interfering with elosulfase alfa uptake. Serum samples collected during the MOR-004 trial were tested and titers were determined. Consistent with earlier findings on NAb positivity, no correlations were observed between NAb titers and the clinical outcomes of elosulfase alfa-treated individuals with Morquio A.

1. Introduction

The mucopolysaccharidosis (MPS) diseases are a group of lysosomal storage disease caused by a deficiency of enzymes catalyzing the stepwise degradation of glycosaminoglycans (GAGs). Individuals with the autosomal recessive MPS disorder Morquio A syndrome (mucopolysaccharidosis IVA, MPS IVA; OMIM 253000) have mutations in the gene encoding the enzyme *N*-acetylgalactosamine-6-sulfatase (GALNS; EC 3.1.6.4), resulting in deficient GALNS enzyme activity and accumulation of the GAGs keratan sulfate and chondroitin-6-sulfate in the lysosomes of individuals with Morquio A syndrome. Although symptoms can vary, Morquio A syndrome generally manifests as a progressive disorder with multiple organ and tissue involvement that is characterized by restricted growth, severe skeletal malformations, corneal opacity, restricted hearing, and premature death (Harmatz et al., 2013; Hendriksz et al., 2013). Elosulfase alfa (Vimizim®; rhGALNS; BMN 110) is a US Food and Drug Administration-approved enzyme replacement therapy (ERT) for the treatment of Morquio A syndrome. The previously reported results of a pivotal phase 3 clinical trial, MOR-004, which demonstrated that elosulfase alfa administered either weekly (QW) or every other week (QOW) for 24 weeks to individuals with Morquio A syndrome had an acceptable safety profile, and QW dosing significantly improved 6-min walk test (6MWT) distance, 3-min stair climb test (3MSCT), and respiratory function (maximum voluntary ventilation [MVV]). In addition, the high urine levels of the pharmacodynamic marker keratan sulfate (uKS) characteristic of individuals with Morquio A syndrome were significantly reduced with elosulfase alfa treatment (Hendriksz et al., 2014).

A number of ERTs for the treatment of various lysosomal storage disorders, including elosulfase alfa, contain mannose-6-phosphate (M6P) moieties and depend on the CI-M6PR transmembrane receptor for internalization and trafficking to the lysosome. Biological therapies can elicit an immune response, resulting in the generation of antidrug antibodies capable of binding to the drug product and, in some cases, interfering with receptor binding on the target cell. Furthermore, these antibodies may negatively impact efficacy (Brooks et al., 2003; Desnick and Schuchman, 2012; Banugaria et al., 2011). The MOR-004 study showed that all individuals treated with elosulfase alfa developed drug-specific total antibodies (TAb). A subset of drug-specific antibodies

Abbreviations: TAb, total antibody; NAb, neutralizing antibody; LSD, lysosomal storage diseases; GAGs, glycosaminoglycans; ERTs, enzyme replacement therapies; rhGALNS, recombinant human *N*-acetylgalactosamine-6-sulfatase; 6MWT, six minute walk test; 3MSC, three minute stair climb; MVV, maximum voluntary ventilation; uKS, urine keratan sulfate; M6P, mannose-6-phosphate; CI-M6PR, cation-independent mannose-6-phosphate receptor; TQC, titer quality control; LQC, low quality control; HQC, high quality control; NPS, normal (human) pooled serum; SI, signal inhibition; AbPC, antibody positive control; MRD, minimum required dilution; NQC, negative quality control; MFI, median fluorescence intensity; CCP, confirmation cut point; SCP, screening cut point; TCP, titer cut point.

* Corresponding author at: 791 Lincoln Ave, San Rafael, CA 94901, United States.
E-mail address: AMelton@bmrn.com (A.C. Melton).

http://dx.doi.org/10.1016/j.jim.2016.10.006

may be elosulfase alfa-specific neutralizing antibodies (NAbs) capable of interfering with CI-M6PR binding, positivity for which was revealed using an ELISA-based in vitro assay (CI-M6PR binding assay). Although elosulfase alfa-specific antibody development was universal among treated individuals in the MOR-004 study, no relationship was detected between TAb titers or NAb positivity and the magnitude of physical improvements or reductions in uKS levels (Schweighardt et al., 2015).

In this study, we describe a novel, cell-based flow cytometry method for identifying and titering NAbs capable of inhibiting cellular uptake (functional NAb assay) to determine if this measurement might be more clinically meaningful than measuring inhibition of receptor binding, as was measured in the original study. Serum samples were co-incubated with Alexa488-labeled rhGALNS, and flow cytometry was used to detect interference of drug internalization by human Jurkat cells, which express transmembrane CI-M6PR on the cell surface. Assay sensitivity, precision, specificity, selectivity, as well as drug tolerance were determined, and cut points were calculated to set thresholds for assessing positive samples. In support of our previous findings that NAb positivity had no correlation with clinical outcomes or pharmacodynamic effects (Schweighardt et al., 2015), testing of MOR-004 samples with the functional NAb assay demonstrated that NAb titer is not correlated with efficacy outcomes or pharmacodynamic effects of elosulfase alfa in individuals with Morquio A syndrome.

2. Materials and methods

2.1. Clinical study design

MOR-004 (NCT01275066) was a phase 3, randomized, double-blind, placebo-controlled study designed to assess the safety and efficacy of elosulfase alfa administered at 2.0 mg/kg QW or QOW in individuals with Morquio A syndrome. The study protocol was approved at each participating clinical site by an institutional review board, independent ethics committee, or research ethics board. Prior to entering the study, each participant or his/her legally authorized representative provided written informed consent. The assigned treatment was blinded to investigators, site personnel, and patients until the completion of the final analysis. Of the 176 study participants, 59 received placebo, 58 received elosulfase alfa 2.0 mg/kg QW, and 59 received elosulfase alfa 2.0 mg/kg QOW. The MOR-004 study design, inclusion criteria, and endpoints were reported previously (Hendriksz et al., 2014).

2.2. Immunogenicity testing

Serum samples were collected from all individuals for immunogenicity testing prior to dose administration at baseline, weeks 2 and 4, and every 4 weeks thereafter (or within 1 week of the early-termination visit). Detailed methods for sample preparation and the CI-M6PR binding assay were reported previously (Schweighardt et al., 2015). In brief, TAb titers were determined by a validated bridging electrochemiluminescence (ECL) assay, and positivity for NAb was determined by a validated in vitro assay that assessed interference of elosulfase alfa binding to CI-M6PR immobilized on an ELISA plate.

2.3. Functional NAb assay

Alexa488 was conjugated to rhGALNS (BioMarin Pharmaceutical Inc.) using an Alexa Fluor 488 Protein Labeling Kit (Molecular Probes) according to the manufacturer's protocol. Alexa488-conjugated rhGALNS had an average labeling ratio of 1.75 mol of Alexa488 per mole of rhGALNS as determined by absorbance at 494 nm. Quality controls, serum samples, immunodepleted samples for confirmation, or 3-fold serially diluted serum samples for titration were diluted 1:2.5 in serum-free RPMI-1640 with high glucose, L-glutamine, and HEPES (ATCC) and incubated with 0.4 μg/mL Alexa488-conjugated rhGALNS in duplicate wells overnight at 4 °C. Samples were then added to Jurkat cells (clone E6-1, ATCC) plated at 7.5×10^4 cells per well in 96-well round bottom plates and incubated for 3 h at 37 °C and 5% CO_2. Cells were washed 3 times in PBS after centrifugation at 310 ×*g*, stained with 1:1000 LIVE/DEAD Fixable Red Dead Cell Stain Kit (Molecular Probes) for 15 min, centrifuged, and fixed with 1% paraformaldehyde for 10 to 15 min at room temperature or up to 72 h at 2 °C to 8 °C. Fixed cells were analyzed for fluorescence on a BD LSRII flow cytometer using BD FACSDiva software (BD Biosciences). Dead cells were excluded from the analysis, and approximately 10,000 events were recorded. Uptake of Alexa488-conjugated rhGALNS was reported as mean of duplicate median fluorescence intensity (MFI) measurements from adjacent wells.

2.4. Confirmation assay sample preparation

Samples that met the threshold for NAb positivity in the screening assay were verified using a confirmation assay. Briefly, tosyl-activated magnetic beads (Dynabeads®; ThermoFisher Scientific) conjugated with rhGALNS were washed 3 times in coupling buffer (1× DPBS, 0.01% Tween) and added to wells, and the buffer was removed. Samples diluted 1:2.5 in serum-free RPMI-1640 were added to the wells with the beads and incubated with shaking at 800 rpm for 1 h. The beads and samples were separated, and the eluent was tested in the functional NAb assay (described in Section 2.3).

2.5. Calculations and statistics

Percentage signal inhibition (%SI) was calculated using the equation $\%SI = [1 - (\textit{mean MFI of sample} / \textit{mean MFI of cut-point control})] \times 100$. Confirmatory assay data were analyzed as recovery ratio, defined as *mean confirmatory assay MFI of sample / mean screening assay MFI of*

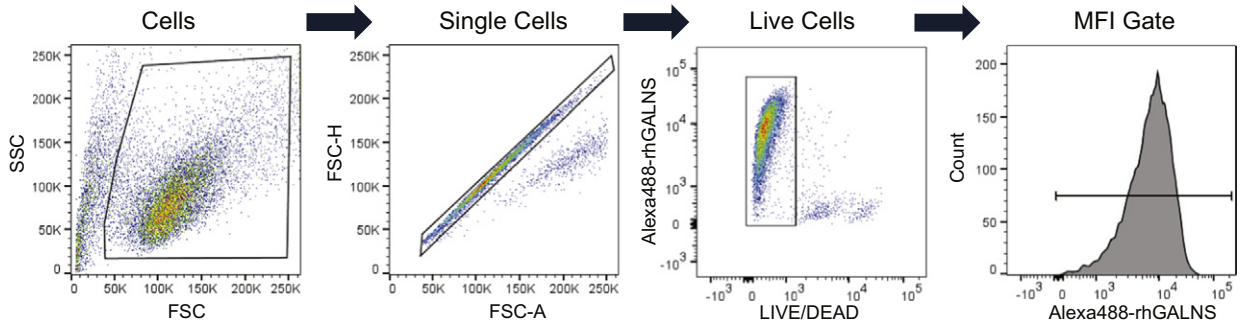

Fig. 1. Assay platform flow cytometry gating strategy. Jurkat cells were separated from all events captured by the instrument detector using the forward scatter (FSC) and side scatter (SSC) channels. Cell doublets were then removed by gating on single cells using the FSC area (FSC-A) and FSC height (FSC-H) parameters. Dead cells were removed from analysis by gating on cells not labeled with LIVE/DEAD cell stain. Median fluorescence intensity (MFI) of Alexa488-rhGALNS was calculated using live cells plotted as a histogram. Cells incubated with or without Alexa488-rhGALNS (red and blue histograms, respectively) are shown.