A phase 1/2 study on intracerebroventricular tralesinidase alfa in patients with Sanfilippo syndrome type B

Nicole Muschol, … , Joseph Kovalchin, Eric H. Zanelli


BACKGROUND. Sanfilippo type B is a mucopolysaccharidosis (MPS) with a major neuronopathic component characterized by heparan sulfate (HS) accumulation due to mutations in the NAGLU gene encoding for alfa-N-acetyl-glucosaminidase. Enzyme replacement therapy for neuronopathic MPS requires efficient enzyme delivery throughout the brain in order to normalize HS, prevent brain atrophy and potentially delay cognitive decline.

METHODS. In this phase 1/2, open-label study, subjects (n=22) affected with MPS IIIB were treated with tralesinidase alfa administered intracerebroventricularly (ICV). Subjects were monitored for drug exposure, total HS and HS non-reducing end (HS-NRE) levels in both cerebrospinal fluid (CSF) and plasma, anti-drug antibody response, brain, spleen and liver volumes as measured by magnetic resonance imaging and cognitive development as measured by age-equivalent (AEq) scores.

RESULTS. In the Part 1 dose escalation (30, 100, and 300 mg) phase, tralesinidase alfa 300 mg was necessary to achieve normalization of HS and HS-NRE in CSF and plasma. In Part 2, tralesinidase alfa 300 mg sustained HS and HS-NRE normalization in the CSF and stabilized cortical grey matter volume (CGMV) over 48 weeks of treatment. Resolution of hepatomegaly and reduction in spleen volume were observed in most subjects. […]

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A Phase 1/2 study on intracerebroventricular tralesinidase alfa in patients with Sanfilippo syndrome type B

Nicole Muschol,1 Anja Koehn,1 Katharina von Cossel,1 Ilyas Okur,2 Fatih Ezgu,2 Paul Harmatz,3 Maria J de Castro Lopez,4 Maria Luz Couce,4 Shuan-Pei Lin,5 Spyros Batzios,6 Maureen Cleary,6 Martha Solano,7 Igor Nestrasil,8 Brian Kaufman,9 Adam J. Shaywitz,10,12 Stephen M. Maricich,11,13 Bernice Kuca,11 Joseph Kovalchin,11 and Eric Zanelli,11

Affiliations
1 University Medical Center Hamburg-Eppendorf, International Center for Lysosomal Disorders (ICLD), Hamburg, Germany
2 Gazi University Faculty of Medicine, Depts of Pediatric Metabolism and Genetics, Ankara, Turkey
3 UCSF Benioff Children’s Hospital Oakland, Oakland, USA
4 Hospital Clínico Universitario de Santiago, University of Santiago de Compostela, IDIS, CIBERER, MetabERN, A Coruña, Spain
5 Mackay Memorial Hospital, Taipei, Taiwan
6 Great Ormond Street Hospital, London, UK
7 Fundacion Cardio Infantil, Bogota, Colombia
8 Division of Clinical Behavioral Neuroscience, Department of Pediatrics, and Masonic Institute for the Developing Brain, University of Minnesota, Minneapolis, MN, USA
9 CLB Consulting, Falls of Neuse, Raleigh NC, USA
10 BioMarin Pharmaceutical, Novato CA, USA
11 Allievex Corporation, Marblehead MA, USA
12 Present address: BridgeBio Gene Therapy, Palo Alto, USA
13 Present address: Ashvattha Therapeutics, Inc. Redwood City, CA, USA

Conflicts of Interest
B.K., J.K. and E.Z are employees of Allievex Corporation. S.M.M. is a former employee of Allievex Corporation.

Corresponding Author
Eric Zanelli, Ph.D., Allievex Corporation, P.O. Box 1056, Marblehead, MA 01945, tel. 978-322-4554, eric@allievex.com

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Abstract

**Background.** Sanfilippo type B is a mucopolysaccharidosis (MPS) with a major neuronopathic component characterized by heparan sulfate (HS) accumulation due to mutations in the NAGLU gene encoding for alfa-N-acetyl-glucosaminidase. Enzyme replacement therapy for neuronopathic MPS requires efficient enzyme delivery throughout the brain in order to normalize HS, prevent brain atrophy and potentially delay cognitive decline.

**Methods.** In this phase 1/2, open-label study, subjects (n=22) affected with MPS IIIB were treated with tralesinidase alfa administered intracerebroventricularly (ICV). Subjects were monitored for drug exposure, total HS and HS non-reducing end (HS-NRE) levels in both cerebrospinal fluid (CSF) and plasma, anti-drug antibody response, brain, spleen and liver volumes as measured by magnetic resonance imaging and cognitive development as measured by age-equivalent (AEq) scores.

**Results.** In the Part 1 dose escalation (30, 100, and 300 mg) phase, tralesinidase alfa 300 mg was necessary to achieve normalization of HS and HS-NRE in CSF and plasma. In Part 2, tralesinidase alfa 300 mg sustained HS and HS-NRE normalization in the CSF and stabilized cortical grey matter volume (CGMV) over 48 weeks of treatment. Resolution of hepatomegaly and reduction in spleen volume were observed in most subjects. Significant correlations were also established between change in cognitive AEq and plasma drug exposure, plasma HS-NRE level and change in CGMV.

**Conclusion.** ICV administration of tralesinidase alfa effectively normalized HS and HS-NRE as a prerequisite for clinical efficacy. Peripheral drug exposure data suggests a role for the glymphatic system in altering tralesinidase alfa efficacy.

**Trial registration** Clinicaltrials.gov: NCT02754076
Introduction

Lysosomal storage diseases (LSDs) are a family of genetic disorders affecting the breakdown and recycling of complex molecules within the lysosomes (1). The lysosomal compartment contains more than 60 hydrolases including enzymes involved in the degradation of glycosaminoglycans (GAGs). LSDs associated with genetic deficiencies in GAG catabolism are regrouped under the term of mucopolysaccharidoses (MPS) (2, 3). MPS type III or Sanfilippo syndrome is a hereditary disorder characterized by the accumulation of heparan sulfate (HS) which leads to brain atrophy, developmental delay, behavioral disturbances, dementia and a life expectancy below twenty years of age in most cases (4).

Sanfilippo syndrome, or MPS type III, is divided into four subtypes, A to D, due to defects in genes coding for four different enzymes; Sanfilippo syndrome type B (MPS IIIB; OMIM #252920) is specifically due to mutations in a gene encoding for alfa-N-acetyl-glucosaminidase (NAGLU; EC 3.2.1.50)(5). In a recently published paper that characterizes the natural history of MPS IIIB subjects (6), it was established that the spectrum of disease severity in MPS IIIB subjects based on cognitive and adaptive behavior decline and cortical grey matter atrophy represents a single continuum with predicted trajectories (6). Although a few subjects have a genetically defined attenuated phenotype, the majority of MPS IIIB patients achieve an apex on both cognitive and adaptive behavior scales between 3 and 6 years of age as demonstrated by age-equivalent (AEq) scores. Development quotients (DQ) for both cognition and adaptive behavior follow a linear trajectory by which subjects reach a nadir by 8 years of age on average and by 13½ years at the latest. At baseline, all tested subjects had HS and HS non-reducing ends (HS-NRE) levels in cerebrospinal fluid (CSF) and plasma above normal range and signs of hepatomegaly (6).

Endogenous NAGLU contains mannose 6-phosphate (M6P)-residues, which enable receptor-mediated endocytosis and targeting to the lysosome where HS is degraded (7). Unfortunately, recombinant NAGLU expressed in Chinese hamster ovary (CHO) cells is not effectively taken up
by lysosomes because it contains little or no M6-P. To improve on its uptake by human cells, NAGLU was fused to insulin-like growth factor-2 (IGF-II) so that the resulting fusion protein, tralesinidase alfa, can be efficiently captured through the IGF-II binding site of cation-independent mannose-6-phosphate receptor (CI-MPR)(8). Animal studies in mice, dogs, and nonhuman primates have shown that intracerebroventricular (ICV) delivery of tralesinidase alfa can bypass the blood-brain barrier (BBB), result in normalization of total HS and disease-specific HS-NRE, and reduce disease-associated markers in brain tissues (9, 10). In both mouse and dog models, lysosomal size decreased with tralesinidase alfa treatment, demonstrating that it is effectively clearing HS from the targeted lysosome. Additional work in a dog model of MPS IIIB has shown that tralesinidase alfa-mediated reduction in HS and HS-NRE in both CSF and brain tissue leads to improvement in a T-maze learning task (11).

The objectives of this study were to evaluate the safety, tolerability and efficacy of tralesinidase alfa administered by an implanted ICV device to subjects with MPS IIIB. Hereafter, we report the pharmacokinetic and pharmacodynamic results of our clinical investigation of tralesinidase alfa in the treatment of children affected with MPS IIIB over a 48-week period. Overall, the current study highlights the ability of tralesinidase alfa to alter the natural course of MPS IIIB and suggests that this positive effect may lead to meaningful clinical benefits.
Results

Patients’ characteristics and adverse events. Interventional study 250-201 was divided into Part 1 and Part 2 (Figure 1); Part 1 was a dose escalation study in which 3 subjects, i.e., 9001, 9002 and 9003, received weekly doses of 30, 100 and 300 mg (maximum feasible dose) of tralesinidase alfa administered ICV into the lateral ventricle through an implanted ICV device, such as Ommaya reservoir, and a catheter. In Part 2, twenty-two (22) subjects were scheduled to receive 48 weekly administrations of tralesinidase alfa 300 mg through a similarly implanted device; 3 of these 22 subjects were the Part 1 subjects who transitioned into Part 2. Nineteen (19) subjects in study 250-201 were individuals previously recruited into observational study 250-901 as previously reported (6).

Study 250-201 Part 1 subject 9001 received 28 weekly doses of tralesinidase alfa 30 mg, followed by 10 doses at 100 mg and then followed by 11 doses at 300 mg before being recruited into study 250-201 Part 2. Subject 9002 received 8, 10 and 19 doses of 30, 100 and 300 mg of tralesinidase alfa, respectively, before being recruited into study 250-201 Part 2, while subject 9003 received 20, 10 and 9 doses of 30, 100 and 300 mg of tralesinidase alfa in study 250-201 Part 1.

Study 250-201 Part 2 characteristics are summarized in Table 1. Thirteen (13) males and 9 females affected by MPS IIIB were recruited; the average age at diagnosis was 33 months and the average age at the time of first dosing with tralesinidase alfa was 60 months; the average cognitive DQ score at baseline was 55 for an age equivalent (AEq) of 30 months, DQ being defined as AEq divided by age and multiplied by 100. Treatment compliance for study 250-201 Part 2 was 90% (median: 98%) of 48 doses of tralesinidase alfa scheduled to be delivered over 48 weeks of weekly ICV administrations; 2 subjects received only 17 and 57% respectively of the expected doses of tralesinidase alfa (Table 1). Ethnicity of the participants is listed in Table 1 but was not taken into account in our analyses.
No deaths occurred during the course of the study. One subject discontinued from the study following a serious adverse event (SAE) of subdural hygroma associated with increased intracranial pressure occurring after the 7th dose of tralesinidase alfa. This event was considered CTCAE Grade 3 and was assessed as unrelated to study drug or device by the site principal investigator. Clinical symptoms (headache, vomiting, listlessness) resolved within 24 hours with medical management. In total, this subject received only 8 doses of tralesinidase alfa. The most common treatment emergent adverse events (TEAEs) included vomiting, pyrexia, upper respiratory tract infection, headache, and CSF pleocytosis. SAEs assessed by investigators reported to be related to study drug were CSF pleocytosis, vomiting, angioedema, fluctuating consciousness and pyrexia. SAEs assessed by investigators to be related to the ICV device were infection, device malfunction, CSF leakage and wound infection. Overall, these TEAEs and SAEs are consistent with known complications of enzyme replacement therapy (ERT), ICV devices and/or neurodegenerative disease in a pediatric population.

Eight hypersensitivity events were reported in five subjects, five events of rash and one event each of angioedema, choking and maculopapular rash. Blood samples for total IgE, C4, serum tryptase and drug-specific IgE did not show evidence of anaphylactic reaction in any of these 5 subjects for any of the hypersensitivity events observed.

*Tralesinidase alfa pharmacokinetics (PK) in CSF and plasma.* Table 2 summarizes average drug exposure in CSF and plasma achieved after a single ICV administration of 30 mg (2 subjects, study 250-201 Part 1), 100 mg (3 subjects, study 250-201 Part 1) and 300 mg tralesinidase alfa (17 subjects, study 250-201 Part 2). The analysis of PK data is focused on the 300 mg dose in 17 subjects from study 250-201 Part 2 who were not previously exposed to tralesinidase alfa.

The total CSF exposure, i.e., area under the curve (AUC0-last), achieved for subjects 9002 and 9003 after a single dose of 30 mg tralesinidase alfa, were 1,130,000 and 1,620,000 ng/mL*hr for a maximum concentration (Cmax) of 403,000 and 470,000 ng/mL measured at 1-hr post drug
administration, respectively. The average CSF $AUC_{0-\text{last}}$ and $C_{\text{max}}$ were 19,100,000 ng/mL*hr and 3,440,000 ng/mL (median of 15,400,000 ng/mL*hr and 2,900,000 ng/mL), respectively, achieved for subjects (n=17) receiving the first ICV dose of tralesinidase alfa 300 mg in study 250-201 Part 2 (Table 2); the lowest CSF exposure ($AUC_{0-\text{last}}$) was 5,050,000 ng/mL*hr and the highest was 49,800,000 ng/mL*hr. Estimated $T_{1/2}$ and $T_{\text{last}}$ were 5 hr and 68 hr on average, respectively, suggesting that tralesinidase alfa is efficiently distributed throughout the brain and quickly absorbed via the CI-MPR receptor, as intended. The average $AUC_{0-\text{last}}$ and $C_{\text{max}}$ appear to scale linearly with dose suggesting the drug exposure achieved in the CSF of MPS IIIB subjects is dose-proportional (Table 2).

Kinetics of drug exposure in both CSF and plasma were analyzed for 20 of 22 subjects in study 250-201 Part 2 on weeks 1, 5, 12 and 36 (Figure 2A&B) along with anti-drug antibodies (ADAs) in the CSF and serum at weeks 1, 4, 12, 36, and 48 (Figure 2C&D); PK data were not available for 2 subjects. $AUC_{0-\text{last}}$ values remained very constant over time in the CSF with mean values of 19.1, 16.4, 19.9 and 19.3 mg/mL*hr for median values of 15.4, 16.9, 17.7 and 17.0 mg/mL*hr on weeks 1, 5, 12 and 36, respectively (Figure 2A). Seven days post administration, little to no tralesinidase alfa was detectable in the CSF prior to the subsequent dose; this observation along with the fact that tralesinidase alfa did not increase during the course of the study demonstrate that tralesinidase alfa does not accumulate over time with weekly dosing frequency.

Plasma exposure ($AUC_{0-\text{last}}$) significantly decreased over time from a mean value of 18,800 ng/mL*hr on week 1 to 10,900 on week 5, 9,700 on week 12 and 7,260 on week 36 (Figure 2B; p values of 0.05, 0.05 and 0.01 comparing week 5, 12 and 36 to week 1, respectively, using a one-way ANOVA with Dunnett’s multiple comparisons test). On week 1, drug exposure was on average 1,000-fold lower in plasma compared to CSF (range of 300 to 3,300) and dropped to 22,000-fold lower by week 36 (range of 500 to 170,000). Of note, decreases in plasma exposure were not substantial from week 5 to week 12 or week 36. Estimated $T_{\text{max}}$ in plasma was 7.5 hr on
week 1 (n=16) and remained around 10 hr among treated subjects with quantifiable drug exposure in plasma on weeks 5, 12 and 36, i.e., AUC₀⁻ₐₙₜ > 10,000 ng/mL·hr. For the other subjects, exposure was too low to estimate Cₘₐₓ and Tₘₐₓ.

**Immunogenicity.** ADA response was monitored in both CSF and serum during the course of study 250-201 Part 2 (Figure 2C&D). Total ADA titers were measured along with specific anti-IGF-I and anti-IGF-II antibodies. Serum biochemistry showed no evidence of abnormal levels of endogenous IGF-I or IGF-II, or any signs of hypoglycemia in subjects enrolled in study 250-201. Therefore, anti-IGF-I and anti-IGF-II antibody responses will not be discussed further as they did not provide additional information beyond the findings with total anti-tralesinidase alfa antibodies.

The only 3 subjects with ADA in serum at week 1 were the individuals who had already received tralesinidase alfa treatment in study 250-201 Part 1 (Figure 2D). In all subjects ADA titers were higher in serum than CSF by study 250-201 Part 2 completion (week 48). Three subjects had no detectable ADA in serum and 6 subjects had no ADA in CSF. Only 3 subjects had ADA endpoint titers > 1:3,645 (log₁₀ = 3.56) in CSF on week 36 and 48 (Figure 2C). Conversely, median serum ADA titers went from 1:405 (log₁₀ = 2.61) on week 4 to 1:3,645 (log₁₀ = 3.56), 1:98,415 (log₁₀ = 4.99) and 1:37,345 (log₁₀ = 4.57) on week 12, 36 and 48, respectively (Figure 2D), suggesting that by week 36, ADA titers had reached an apex and might stabilize or decrease beyond this timepoint. Presence of drug in analyzed samples did not confound ADA titration since trough levels prior to drug administration were in the 4-7 ng/mL range on average and often below level of quantification (data not shown).

The largest drop in plasma drug exposure occurred between week 1 and week 5 of study 250-201 Part 2 (Figure 2B) when the median ADA titer in serum was only 1:405 on week 4. *In vitro* cell-based studies suggested that antibodies detected in ADA positive CSF samples were not able to neutralize uptake of tralesinidase alfa (titers of neutralizing antibodies were <1:100 in ADA positive CSF samples).
Tralesinidase alfa 300 mg normalizes HS and HS-NRE. Total HS and HS-NRE concentrations in CSF were measured weekly over the course of study 250-201 Part 1 (Figure 3A&B). For reference, HS and HS-NRE concentrations were measured in the CSF, and also plasma, of non-affected subjects and 95th percentile values (148 and 10 ng/mL, respectively) have been used to identify elevated levels of these biomarkers. Subjects 9001, 9002 and 9003 had respective total HS values of 255, 208 and 457 ng/mL and HS-NRE values of 48, 48 and 105 ng/mL at study 250-201 Part 1 baseline. Tralesinidase alfa 30 mg administered ICV reduced levels of total HS and HS-NRE in both CSF and plasma in all 3 subjects within weeks of dosing; however, only 300 mg of tralesinidase alfa could sustain total HS normalization in all 3 subjects for at least 6 weeks and HS-NRE normalization in 2 of 3 individuals. HS-NRE levels in the CSF of subject 9003 remained in the 20-28 ng/mL range, above the 95% percentile of non-affected subjects but close to the maximum value measured in unaffected CSF samples (18.6 ng/mL, Figure 3B) after 6 weeks of tralesinidase alfa 300 mg ICV administration. There is no evidence that ADA interference explains the HS-NRE level observed in the CSF of subject 9003 considering the low level of neutralizing ADA in the CSF.

During the course of study 250-201 Part 2, HS-NRE levels in the CSF were normalized over the 48 weeks of dosing with levels after the first 4 weeks of treatment below the 10 ng/mL higher end of normal cutoff (Figure 3C and Supplemental Table 1). Total HS levels in CSF were similarly reduced after treatment with tralesinidase alfa (Supplemental Table 2). Of note, HS quantification was not affected by the presence of tralesinidase alfa in the sample because a) samples were collected prior to treatment and one week after last treatment and b) tralesinidase alfa would not be functional extracellularly and at the CSF neutral pH. Subject 9003 was the only individual for whom levels of HS-NRE levels in CSF never normalized and consistently fluctuated between 15-25 ng/mL (Supplemental Table 1). Two other subjects received only 17 and 57% of the intended dose of tralesinidase alfa, i.e., subjects 9017 and 9022, and were therefore excluded from Figure
Prior to treatment interruption, both of these subjects had had CSF and total HS-NRE levels within the normal range (Supplemental Tables 1&2).

Significant correlations were observed between total HS and HS-NRE levels in both CSF and plasma at different timepoints (Supplemental Tables 1-4). HS-NRE levels in plasma were significantly reduced to < 40 ng/mL in subjects after 4 weeks of administration of 300 mg tralesinidase alfa (Figure 3D). Six subjects had plasma HS-NRE levels that fluctuated between 15 and 24 ng/mL during the 48-week study (Supplemental Table 3). In general, the concentrations were close to the maximum value assessed in plasma for non-affected subjects, i.e., 21.3 ng/mL (Supplemental Table 3). Concentrations of total HS were also significantly reduced in plasma as the result of tralesinidase alfa administration (Supplemental Table 4).

**Weekly administration of tralesinidase alfa 300 mg resolves organomegaly.** Changes in liver and spleen volumes on week 1, 24 and 48 of study 250-201 Part 2 are depicted in Figure 4A&B; volumes are expressed as mL of tissue corrected for m² of body surface area (BSA); BSA were calculated using the Mosteller method (12). Compared to the liver volumes for non-affected children aged 2-10 years, estimated to be between 0.55 and 0.85 L/m² (12, 13), liver volumes in MPS IIIB children ranged from 1.13 to 1.83 L/m² of BSA for a mean value of 1.40 (median 1.39) at the baseline visit for Study 250-201 Parts 1 and 2 (Figure 4A). Of the four subjects with liver volumes > 1.0 L/m² of BSA on week 48, two appeared to have fast progressive disease based on changes in both cortical grey matter volume (CGMV) and cognitive score, and two were the subjects who received only 17 and 57% of the expected doses of tralesinidase alfa. By week 24 of study 250-201 Part 2, the mean liver volume was reduced to 0.82 L/m² (median 0.80; range 0.59-0.98) and by week 48, the mean value was 0.87 (median 0.81; range 0.71-1.34; Figure 4A). The reduction in liver volume was statistically significant (p<0.0001, two-tailed paired t-test) when comparing measurements at week 48 versus baseline.
At study 250-201 Part 1 or Part 2 baseline, spleen volumes in MPS IIIB children ranged from 0.15 to 0.44 L/m$^2$ of BSA for a mean value of 0.23 (median 0.21), while normal spleen volumes for 2- to 10-year-old children were estimated between 0.04 and 0.15 L/m$^2$ of BSA (13). By week 24 of study 250-201 Part 2, the mean spleen volume decreased to 0.18 L/m$^2$ (median 0.16; range 0.13-0.28) and this decrease was maintained through week 48 (median 0.17; range 0.12-0.28; Figure 4B). The reduction in spleen volume was statistically significant (p<0.0001, two-tailed paired t-test) when comparing the volumes at week 48 to volumes at baseline.

*Tralesinidase alfa stabilizes brain atrophy in MPS IIIB subjects.* CGMV is a measure of the region of the brain most affected by atrophy in MPS IIIB subjects within the age range included in Study 250-201 (6). MPS IIIB subjects enrolled in study 250-201 had an average CGMV of 471 mL (range 268-621; median 476, Figure 4C). Non-affected children would be expected to have CGMV ≥ 489 mL (14). At week 48, eight subjects had CGMV within normal range; 5 subjects had CGMV between 473 and 485 mL or close to normal; 7 subjects had CGMV below normal ranging from 384 to 455 mL; finally, the 2 oldest subjects in the study, 116 and 118 months-old at baseline, had CGMV of only 268 and 343 mL, respectively (Supplemental Table 5).

Subjects enrolled in study 250-201 lost on average 58 mL (median 68 mL) of CGMV from baseline to week 24 but only 2 mL from week 24 to week 48 for a total loss of 60 mL (median 63 mL) from week 1 to week 48 (p<0.0001, two-tailed, paired t-test in both cases). Every subject in Study 250-201, except two, lost CGMV during the course of study 250-201 (Supplemental Table 5). One of the two subjects who presented CGMV increase was the youngest one who gained 36 mL of CGMV from baseline to week 24. Meanwhile, the same subjects gained on average 12 mL (median 9.5 mL) in cerebral ventricle volume from baseline to week 24, but only 1 mL (median -2 mL) from week 24 to 48 (Supplemental Table 5); a gain of 13 mL of cerebral ventricular volume over 48 weeks is slightly higher than the gain of 8 mL previously observed in the natural history study (6).
Nineteen of 22 subjects treated with tralesinidase alfa had total cerebellar volume within normal range at baseline (Figure 4D and ref.(6)). Three subjects had cerebellar volumes above the normal range at baseline. The mean cerebellar volume increased to 149 and 153 mL at week 24 and week 48 respectively from 148 mL at baseline (Figure 4D). The increase in total cerebellar volume from week 1 to week 48 approached statistical significance (Figure 4D; p=0.0626, using a two-tailed paired t-test).

Correlations of tralesinidase alfa’s exposure on cognitive decline trajectory and plasma HS-NRE.

A treatment duration of longer than 48 weeks will be needed to establish whether there is a clinically meaningful benefit on cognition related to treatment with tralesinidase alfa. However, to elucidate if there is early evidence of an effect of tralesinidase alfa on disease trajectory in MPS IIIB subjects, correlations between change in cognitive AEQ and plasma drug exposure, defined as AUC\(_{0\text{-}\text{last}}\), on weeks 5, 12 and 36 of study 250-201 Part 2, HS-NRE biomarker levels, and CGMV over the 48-week study period were explored. There was a significant correlation between change in cognitive AEQ over the course of study 250-201 Part 2 and average tralesinidase alfa exposure (AUC\(_{0\text{-}\text{last}}\)) in plasma on weeks 5, 12 and 36 (Pearson r=0.62, two-tailed p=0.0034, n=20; Figure 5A). Three of 4 subjects with average AUC\(_{0\text{-}\text{last}}\) > 22 µg/mL*hr had an increase of 5 months or more in AEQ over 48 weeks while five of six subjects with AUC\(_{0\text{-}\text{last}}\) < 1 µg/mL*hr lost between 8 and 21 months of AEQ during the same period (Figure 5A).

A cumulative plasma HS-NRE concentration was calculated for each subject by integrating plasma HS-NRE concentrations measured every 4 weeks between weeks 12 and 36 of study 250-201 Part 2. The average drug exposure, i.e., AUC\(_{0\text{-}\text{last}}\), in plasma on weeks 12 and 36 inversely correlates with cumulative plasma HS-NRE concentrations over the same period, i.e., average of week 12 and 36 (Pearson r=-0.77, two-tailed p=0.0002, n=18). Eight of 12 subjects with plasma drug exposure AUC\(_{0\text{-}\text{last}}\) < 6 µg/mL*hr had HS-NRE cumulative concentrations above normal range, i.e., between 360 and 540 ng/mL*week, in plasma (Figure 5B). Five out of 5
subjects with HS-NRE cumulative concentrations between 170 and 240 ng/mL*week, i.e., within normal range, from week 12 to 36 had plasma AUC_{0-last} > 15 µg/mL*hr. Subjects with the highest plasma drug exposure were subjects with the lowest plasma HS-NRE concentrations measured repeatedly between week 12 and 36 of study 250-201 Part 2, and vice-versa.

*Correlations of cognitive decline trajectory compared to plasma HS-NRE and cortical grey matter volume.*

An inverse correlation was also observed between cumulative plasma HS-NRE concentration and change in cognitive AEq from week 1 to week 48 of study 250-201 Part 2 (Figure 5C). There was a trend that showed 4 out of 5 subjects with positive change in AEq from week 1 to week 48 had cumulative plasma HS-NRE concentrations within the normal range between 260 and 380 ng/mL*week from week 8 to 48 (r=-0.35, p=n.s., n=22).

A correlation was observed between change in cognitive AEq over the course of study 250-201 Part 2 and change in CGMV during the same period (Figure 5D; Pearson r=0.59, p=0.0082, n=19). All four subjects with change in CGMV ≥ 0 mL from week 1 to week 48 of study 250-201 Part 2 had a change in AEq score ≥ 0, while the subject who received only 17% of their intended dose of compound lost 54 mL of CGMV, and a loss of 21 months on the cognitive AEq scale.
Discussion

In the present study, we show that, within weeks of weekly ICV administration, tralesinidase alfa 300 mg can effectively compensate for NAGLU enzymatic activity as demonstrated by the normalization of HS and HS-NRE in both CSF and plasma. Consequently, resolution of hepatomegaly was observed in most MPS IIIB subjects within 24 weeks of tralesinidase alfa administration. The occurrence of ADA had no influence on HS normalization, hepatomegaly resolution or rate of cognitive decline. Longer treatment duration will be needed to fully evaluate the capacity of tralesinidase alfa to positively impact the life of subjects affected by MPS IIIB. Safety data for tralesinidase alfa (300 mg) administered weekly via ICV infusion are in line with other ERTs, especially those administered via ICV dosing such as cerliponase alfa (15).

The study of 22 patients with MPS IIIB aged 25 to 118 months at baseline establishes an effective dose of 300 mg of tralesinidase alfa administered ICV as necessary to achieve and sustain normalization of both total HS and disease-specific HS-NRE. The normalization of total HS and HS-NRE in the CSF is the first evidence that tralesinidase alfa can be potentially beneficial to MPS IIIB subjects. While HS-NRE normalization in CSF is critical and a prerequisite for potential efficacy, the concentration of both tralesinidase alfa and the greater dynamic range of measurement of HS-NRE in plasma might be indicative of the efficacy of tralesinidase alfa in the CNS (Figure 5). We hypothesize that plasma drug exposure and HS-NRE may indirectly indicate how well the glymphatic system is working.

It is classically assumed that the CSF flow is unidirectional and therefore proteins directly administered in the CSF should be rapidly transported out of the brain and into the periphery (16). According to this model, ICV delivery should result in little distribution of tralesinidase alfa in the brain. Our preclinical data have established that tralesinidase alfa can be effectively distributed throughout the brain of NAGLU-deficient mice and dogs, thereby preventing disease manifestations (10, 11). Data in the dog model of MPS IIIB have demonstrated that HS-NRE
levels in CSF and brain correlate with each other; the normalization of HS-NRE in CSF predicts benefits (11). Others have also recognized the value of CSF HS as a predictive marker of clinical efficacy (17); unfortunately, intravenous delivery of recombinant N-sulfoglucoamylase sulfohydrolase resulted in no resolution of hepatosplenomegaly and no clear clinical benefits in a clinical study recruiting six subjects suffering from MPS IIIA. Although significant, the reduction in HS level in the CSF of these subjects was probably too slow and too little to translate into clinical benefits (17). Induction of ADA has been suggested as an explanation for the lack of clinical efficacy (17); if true, it would make efforts to develop enzyme replacement therapy for neurological disorders based on peripheral delivery, or so called molecular trojan horse approach, challenging (16).

Our data favors an alternate explanation based on a recently identified pathway regarding the exchange of fluids and solutes from the CSF to the brain; the existence of the glymphatic system has now been established in human brain (18). The importance of a defective glymphatic system on the brain accumulation of toxic amyloid-beta and tau proteins has been shown in animal models (19) and suggested as an explanation for the poor efficacy of immunotherapy in Alzheimer’s patients (20). This dural lymphatic system and the active pumping of CSF into the periarterial spaces likely provide a way by which ICV-dosed tralesinidase alfa can be effectively distributed from the CSF to the brain (21); plasma hyperosmolality and sleep cycle have been described as parameters that could modulate the delivery of macromolecules to the brain (22). Subjects with the highest drug and greatest sustained drug exposure, i.e., AUC$_{0\text{-last}}$, in plasma were the subjects who had the highest gain in AEq score from baseline to week 48 of study 250-201 Part 2, and vice versa (Figure 5A); subjects with the highest gain of AEq score also tended to have the lowest, cumulative plasma HS-NRE concentrations from week 8 to 48 (Figure 5C). A significant inverse correlation was also observed between plasma drug exposure and cumulative plasma HS-NRE concentration (Figure 5B).
The use of both a sedative and a flushing solution could facilitate tralesinidase alfa distribution into the brain, possibly through the perivascular Virchow-Robin spaces (23, 24). The correlations between change in cognitive AEq score and both plasma HS-NRE and plasma drug exposure (Figure 5A&C) might reflect the ability of a given MPS IIIB subject to effectively circulate macromolecules and metabolites from the brain to the periphery. A better preservation of their brain functionality and fluid exchanges with the periphery might provide some MPS IIIB subjects, in particular the youngest ones, with an advantage and greater efficacy of treatment with tralesinidase alfa. Over time, more subjects might benefit from tralesinidase alfa treatment through a potentially slower but sustained improvement in their blood-CSF, brain-CSF and brain-blood exchanges. We believe that tralesinidase alfa must be administered to the CSF via the ICV route to circumvent the blood brain barrier so that sufficient drug concentrations can reach deep brain regions. However, plasma concentrations of drug and HS-NRE following ICV treatment appear to be indirect indicators of sufficient brain uptake and glymphatic function.

Advances in magnetic resonance imaging (MRI) procedures are enabling new and exciting opportunities to understand the exchange of fluids and solutes from the CSF to the periphery in patients (25, 26). For now, the cost and procedural difficulties make advanced MRI impractical for standardized medical practice. In the future, however, it would be interesting to understand the dynamics of CSF/plasma exchange in MPS IIIB patients and its consequence on drug efficacy and resolution of pathology. Understanding of the dynamics of CSF/plasma exchange may inform and improve treatment procedures and increase the chance of success.

Our data shows ADAs have no impact on the pharmacokinetics and pharmacodynamics of tralesinidase alfa, unlike what has been reported by others (16, 17); most likely, direct ICV administration of tralesinidase alfa explains this distinctive and advantageous observation. There was no correlation between plasma drug exposure on week 5 and serum ADA titer on week 4 (Pearson r=-0.29, p=n.s.). Neutralizing antibody testing for positive ADA samples in the CSF were
low; <1:100 was the highest titer measured (data not shown) suggesting no impact of ADA on drug exposure and efficacy in the brain tissue, the primary organ target. The majority of tralesinidase alfa-treated subjects (19 of 22; 86%) had normalized HS and HS-NRE levels in both CSF and plasma during the course of study 250-201 Part 2, regardless of their ADA titers (Figures 1C&D and 2C&D), again arguing that ADA have no impact on tralesinidase alfa function. Even if serum or CSF ADA could interfere with plasma drug exposure and plasma HS-NRE clearance, there is no evidence that ADA influenced changes in CGMV during the course of study 250-201 Part 2. ADA had clearly no impact on resolution of hepatomegaly (Figure 4A).

We believe that the initial accelerated loss of CGMV in the first 24 weeks of study 250-201 Part 2, i.e., 58 mL on average, versus 22 mL for the same subjects in natural history study 250-901 (6), reflects an HS clearance out of the brain while the average loss of only 2 mL from week 24 to 48 demonstrates the ability of tralesinidase alfa to stabilize brain atrophy, especially since 9 subjects had higher CGMV at week 48 than week 24 of study 250-201 Part 2. One hypothesis is that HS clearance as a result of tralesinidase alfa treatment allows for the build-up of a denser dendritic network among neurons which leads to stabilized, and perhaps increased, CGMV (27). Considering the importance of HS in neurogenesis, it would be nice to imagine that, by restoring a natural physiological cycle for HS, tralesinidase alfa could favor postnatal neuronal cell division or even promote the differentiation of neural stem cells, especially in the youngest subjects (28).

Data presented here and in our natural history (6) indicate that preservation of CGMV is linked to preservation of cognitive development. It is known that HS build-up in the lysosomes can cause neuronal cell death (29) and brain atrophy in turns leads to cognitive loss. While normalizing biochemical function is likely a prerequisite for clinical efficacy, additional factors are expected to contribute to the best clinical outcomes, such as the patient’s age and degree of brain atrophy at treatment onset, the duration of treatment, and the fraction of the delivered dose taken up by the
brain versus CSF drainage to the periphery. While our data suggest favorable results, longer treatment duration is needed to assess long term effects of tralesinidase alfa on cognition.

The present analysis has focused on the pharmacokinetics of tralesinidase alfa, the normalization of HS in CSF and plasma following tralesinidase alfa administration and its impact on hepatosplenomegaly and stabilization of brain atrophy. In addition, our data shows a positive impact of tralesinidase alfa on cognitive function in subjects with the highest plasma drug exposure and the lowest level of HS in plasma. Yet, we recognize the limitations of the current study. In particular, the number of enrolled subjects were limited due to the ultra-rare nature of the disease. The treatment duration was only of 48 weeks for the great majority of the subjects; however, our ongoing clinical study seems to confirm a long-term effect of tralesinidase alfa on cognitive and communication skills. Other long-term effects on sleep behavior, hearing, and other quality of life measures are being monitored and will be reported in a future publication. Nonetheless, the analyses described in the current study can be applied to other forms of MPS and more broadly to other LSDs with neurological manifestations.

In conclusion, the ability of tralesinidase alfa 300 mg to normalize HS and HS-NRE in CSF, resolve hepatomegaly and stabilize brain atrophy in MPS IIIB subjects has been demonstrated. ADA did not interfere on tralesinidase alfa efficacy. Tralesinidase alfa safety profile is consistent with what is expected of a drug administered by ICV. Plasma drug exposure and plasma HS-NRE levels may indirectly indicate glymphatic function, which may play a role in improved efficacy. Considering the severity of MPS IIIB, it would be unexpected for tralesinidase alfa to provide clinical benefits to every subject, at least in terms of cognitive function. However, the early effect observed in a few subjects within only 48 weeks of treatment is very encouraging and warrants further evaluation. In light of the poor and eventually fatal prognosis and the absence of disease-modifying therapies, tralesinidase alfa has a potential to change the course of the disease in children living with MPS IIIB.
Methods

Study designs. Interventional study NCT02754076, i.e., study 250-201, was a phase 1/2, open-label, dose escalation (Part 1), and stable dose (Part 2) study in males and females between ≥ 1 and < 11 years of age (12 and 132 months) with a confirmed diagnosis of MPS IIIB. Three subjects, i.e., 9001, 9002 and 9003, enrolled in study 250-201 Part 1 and received weekly dosing at escalating dose levels, i.e., 30, 100, and 300 mg of tralesinidase alfa via an ICV device such as an Ommaya reservoir and a catheter implanted in the lateral ventricle. Twenty-three patients, 19 of whom were rolled over from a natural history study (6), were enrolled in study 250-201 Part 2 with the intent of receiving 48 weekly infusions of tralesinidase alfa 300 mg; one patient discontinued after consent but prior to reservoir implantation, and one patient discontinued on the recommendation of the Data Monitoring Committee (DMC) following a serious adverse event of subdural hygroma associated with subdural hematoma and increased intracranial pressure. In total, 23 subjects were recruited in 6 different countries. Tralesinidase alfa was administered in a 10-mL solution of artificial CSF followed by flushing of the infusion line with a flushing solution. Composition of the artificial CSF was sodium phosphate monobasic, monohydrate 0.4mg/mL, sodium phosphate dibasic, heptahydrate 0.19 mg/mL, sodium chloride 8.66 mg/mL, potassium chloride 0.22 mg/mL, magnesium chloride, hexahydrate 0.16 mg/mL and calcium chloride, dihydrate 0.21 mg/mL.
The sample size was determined by the number of subjects who rolled over from Study 250-901 into Study 250-201. Based on natural history data for untreated MPS IIIA patients, 10 points of yearly decline in cognitive DQ score was assumed for the natural course of MPS IIIB and the standard deviation (SD) of the difference in yearly decline between pre- and post-treatment periods within a subject was assumed to be 10. Under these assumptions, a sample size of 20 subjects was estimated to provide greater than 90% power to detect an 8-point difference in yearly decline between pre- and post-treatment, using a paired t-test at a 5% significance level. A total of 21 patients completed study 250-201; patients were sedated, if needed, during the treatment procedure.

The primary objectives were to 1/evaluate the safety and tolerability of tralesinidase alfa administered to patients with MPS IIIB by an implanted ICV reservoir and catheter, and 2/evaluate the impact of tralesinidase alfa on cognitive function as assessed by DQ. Additional objectives were to 1/evaluate the impact of tralesinidase alfa on cognitive function as assessed by AEq, 2/characterize the PK of tralesinidase alfa in CSF and plasma, 3/characterize the immunogenicity of tralesinidase alfa in CSF and serum, 4/evaluate the impact of tralesinidase alfa treatment on CSF, serum and urine GAGs, 5/evaluate the impact of tralesinidase alfa treatment on brain structure, liver size, and spleen size assessed by MRI.

Inclusion and exclusion criteria. All study participants had deficient NAGLU enzyme activity, had mutations in NAGLU gene confirmed by genetic testing, and all presented with signs/symptoms consistent with MPS IIIB. Individuals were excluded from this studies if they 1) had another neurological illness that may have caused cognitive decline, 2) received stem cell transplantation, gene therapy, or ERT for MPS IIIB, 3) received any investigational medication within 30 days prior to the baseline visit or were scheduled to receive any investigational drug during the course of the study, 4) presented a medical condition or extenuating circumstance that, in the opinion of the investigator, might compromise the subject’s ability to comply with protocol requirements, the
subject’s well-being or safety, or the interpretability of the subject’s clinical data, 5) participated in another natural history study, 6) had contraindications for neurosurgery, 7) had contraindications for MRI scans, 8) had a history of poorly controlled seizure disorder, 9) were prone to complications from intraventricular drug administration, including subjects with hydrocephalus or ventricular shunts, or 10) required ventilation support, except for noninvasive support at night.

Cognition. Cognitive function was measured using the nonverbal scales of the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) (30) or the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) (31); the choice of test was determined at study screening and for further visits using a predefined algorithm (Supplement 1). AEq scores from published normative data were obtained from raw scores, and DQ was derived by dividing the AEq by chronological age and then multiplying by 100. Use of AEq scores circumvents the “floor effect” of standardized scores when applied to children with severe cognitive impairment (32).

The BSID-III is a validated and standardized developmental tool comprised of five domains (cognitive, language, motor, social-emotional, and adaptive functioning) intended to assess developmental function in children ages 1 to 42 months (30). Cognitive assessment is administered individually by a qualified rater to capture the development of critical skills such as processing speed, problem solving and play. Importantly, the cognitive assessments do not require the child to respond verbally; as a result, this test is particularly useful for assessing cognitive function in conditions such as MPS IIIB where expressive language might be limited. Raw scores, i.e., the numbers of correct responses, allow the generation of an AEq score and a DQ; these latter scores for the cognitive domain are presented hereafter. Mean raw scores were also analyzed but data are not presented.

The KABC-II is a validated and standardized clinical psychological diagnostic test for assessing cognitive development (31). The subtests that comprise the KABC-II nonverbal index include Conceptual Thinking, Face Recognition, Story Completion, Triangles, Pattern Reasoning and
Hand Movements. In addition to the nonverbal index subtests, the knowledge cluster subtests (riddles, expressive vocabulary, and verbal knowledge) were administered to subjects who had language. As with the BSID-III, raw scores associated with different ages allow for generation of AEq scores and a DQ. Mean age equivalent scores were averaged over the administered domains; these scores for the nonverbal index are presented hereafter.

BSID-III or the KABC-II instruments were performed every 12 weeks in study 250-201. Baseline, week 24 and week 48 visits involved 2 days of testing, with the cognitive, adaptive, and behavioral testing occurring on the first day (morning preferred), and MRI, lumbar puncture and laboratory testing occurred on the second day to avoid confounding effects of sedation or general anesthetic administration.

*Magnetic resonance imaging (MRI).* Head and abdominal MRIs were used to assess regional brain, liver and spleen volumes at baseline, week 24 and week 48 of study 250-201. Brain volumetric analysis was performed on three-dimensional T1-weighted image using FreeSurfer Image Analysis Suite version 5.3 (Martinos Center, Harvard University, Boston, Massachusetts)(33) as described previously (34, 35). This analysis yields surface-based cortical parcellation and volume-based morphometric segmentation.

Brain volumes were expressed without normalization, similarly to previous publications (6, 34). Regional brain volumes of non-affected children (controls) measured by the same procedures and the same central reader were used for comparison (14).

Liver and spleen volumes were calculated by ICON (Farmingdale, NY). Because liver and spleen volumes increase linearly versus both age and BSA, these volumes were normalized to BSA calculated using the Mosteller formula to allow for comparison among subjects ranging from 2 to 9 years of age (12). Normal ranges for BSA-corrected liver and spleen volumes were estimated based on literature (13, 36, 37).
Laboratory testing. Tralesinidase alfa quantification in CSF and K2EDTA plasma was based on an electrochemiluminescent immunoassay (ECLA) technique using a standard-bind 96-well Meso Scale Discovery (MSD) streptavidin-coated plates. Samples were collected at predose, 0.5, 4, 10, 24, 48, 72, 96 and 168 hr past drug administration on weeks 1 (baseline), 5, 12 and 36 of study 250-201 Part 2. CSF samples were collected from the lateral ventricle through the ICV port. Methods were validated, and sample analysis was conducted by Charles River laboratories, Skokie, IL, USA.

Anti-drug antibodies (ADA) were measured in CSF and serum using an ECLA method utilizing MSD technology. Samples were diluted in a Master Mix with an equal concentration of ruthenylated (sulfo-tagged) tralesinidase alfa and biotinylated tralesinidase alfa in the wells of polypropylene plates. Detection of ADA is based on the bivalent characteristics of the antibodies; ADA bind to both sulfo-tagged and biotinylated molecules to form an antibody complex bridge. Samples are dispensed onto streptavidin-coated MSD assay plates to allow binding of biotinylated tralesinidase alfa to the streptavidin in the wells. Only the samples that contain ADA bound to both the biotinylated and sulfo-tagged tralesinidase alfa generate an ECL signal. In the presence of tripropylamine, ruthenium produces a chemiluminescent signal that is triggered when voltage is applied. The signal produced is proportional to the amount of ADA present. Endpoint titer determination estimates the relative levels of ADA for those positive samples. ADA assays were validated, and samples were run by ICON Laboratories Service Inc., Whitesboro, NY, USA.

Neutralizing antibodies were quantified in CSF using a cell-based assay which measures the ability of ADA to interfere on CI-MPR-mediated uptake of tralesinidase alfa by the human Jurkat cell line. Tralesinidase alfa conjugated to Alexa 647 is monitored and quantified by flow cytometry using median fluorescence intensity (MFI) as the readout. Method was validated and samples were analyzed by Eurofins Pharma Bioanalytics Services US Inc, St Charles, MO, USA.
Levels of tralesinidase alfa-specific IgE were quantified using tralesinidase alfa covalently coupled to ImmunoCAP®. After washing away non-specific IgE, a β-galactosidase-labeled mouse monoclonal antibody against human IgE was added to form a complex. After incubation, unbound enzyme anti-IgE was washed away and the bound complex was incubated with the ImmunoCAP® Development Solution containing 4-methylumbelliferyl-β-D-galactoside, a β-galactosidase fluorogenic substrate. The fluorescence measured in the eluate is directly proportional to the concentration of drug-specific IgE in the patient sample. Method was validated and samples were analyzed by Viracor Eurofins Clinical Diagnostics, Lee’s Summit, MO, USA.

Total HS and HS non-reducing end (HS-NRE) measurements were performed by ARUP Laboratories (Salt Lake City, UT) on CSF and plasma using a previously described method (38). The lower limit of quantification (LLOQ) of the assay was 5 ng/mL for HS-NRE and 100 ng/mL for total HS. Cut-off values for non-affected subjects in CSF (n=60) and plasma (n=91) were established at 148 and 323 ng/mL for total HS, and 10 and 15 ng/mL for HS-NRE, in CSF and plasma, respectively. NAGLU genotyping and enzyme activity testing were performed by Greenwood Genetic Center (Greenwood, SC).

Statistical analysis. No corrections or carry-forwards were made for missing values. Two-tailed, paired t-tests and linear correlation analyses were performed using GraphPad Prism 9.3.1.

Study approval. Written informed consent from parent or legal guardian and assent from subject, if required, was obtained prior to conducting any study specific assessments. Study was approved by the Institutional Review Board affiliated with each individual clinical site.
Author contributions

AJS and SMM designed the clinical protocol. NM, AK, KvC, IO, FE, PH, MJdCL, MLC, SPL, SB, MC, and MS recruited subjects, performed and supervised clinical practice. IN designed and performed the brain MRI analysis. B Kuca supervised the clinical study execution and was responsible for regulatory submissions. EZ, JK and B Kaufman performed the data analysis. EZ and JK designed the data analysis and wrote the original draft. All authors reviewed and edited the manuscript.
Acknowledgements

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References


Figure 1. Flow diagram of study 250-201. In part 1, 3 subjects, i.e., 9001 to 9003, were recruited and treated with escalating doses of 30, 100 and 300 mg of tralesinidase alfa as described in Results section. These 3 subjects were eventually recruited into Part 2 and treated for an additional 48 weeks. In addition, 19 subjects, i.e., 9004 to 9015 and 9017 to 9023, previously observed in natural history study 250-901 (6), were treated with tralesinidase alfa 300 mg. One subject, 9016, withdrew from the trial prior to the first drug administration. In all cases, treatment was weekly through ICV administration.
Figure 2. Drug exposure in plasma and CSF and anti-drug antibody response in serum and CSF. (A) Subjects were treated ICV weekly from week 1 to 48 (n=22). Total exposure in CSF was calculated as AUC_{0-last} by collecting samples at 0.5, 4, 10, 24, 48, 72, 96 and 168 hr past drug administration on week 1 (baseline), 5, 12 and 36 of study 250-201 Part 2. (B) Similarly, total exposure in serum was calculated as AUC_{0-last} on week 1 (baseline), 5, 12 and 36 of study 250-201 Part 2. Anti-drug antibodies were measured in CSF (C) and serum (D) on week 1 (baseline) and week 4, 12, 36 and 48 of study 250-201. Quantification of tralesinidase alfa and titration of anti-drug antibody response were done as described in the Methods section. NT: not tested, i.e., samples were not collected for PK analysis on week 48. Data are represented as scattered plots with median value.
Figure 3. Tralesinidase alfa 300 mg administered weekly by ICV normalizes total HS and HS-NRE in CSF and plasma. In study 250-201 Part 1, 3 individuals, i.e., 9001, 9002 and 9003, were treated with 30, 100 or 300 mg of tralesinidase alfa weekly. Total HS (A) and HS-NRE (B) were quantified weekly in the CSF of treated subjects using a Sensi-Pro assay as described in the Methods section. Black dots labeled 100 or 300 indicate the weeks when treatment increased for each subject from 30 to 100 or from 100 to 300 mg. In study 250-201 Part 2, subjects (n=22) were treated weekly for 48 weeks with 300 mg of tralesinidase alfa. HS-NRE was quantified in the CSF (C) and plasma (D) of treated subjects weekly or at least every 4 weeks using the Sensi-Pro assay as described in the Methods section. Two subjects were excluded from graphs (C) and (D) because they received only 17 and 57%, respectively, of the tralesinidase alfa doses expected to be administered from baseline to week 48 of study 250-201. Data in panels C&D are expressed as mean +/- 95% CI; individual values for each datapoint and each subject are listed in Supplemental Tables 1&3.
Figure 4. Changes in liver, spleen, cortical grey matter, and cerebellum grey matter volumes over the course of study 250-201 Part 2. Liver (A), spleen (B), and brain subregions (C, D) were measured by MRI on weeks 1 (baseline), 24 and 48. Ranges for non-affected subjects were defined based on previous publications (13, 14, 36, 37). MRI data were collected as described in the Methods section. Liver and spleen volumes in panels A&B were adjusted for body surface area (BSA). Individual values in panels C&D for each subject at part 1 week 1 and part 2 weeks 1, 24 and 48 are listed in Supplemental Table 5. Boxes represent 5-95th percentile with median; dots represent values outside the 5-95th percentile. P values comparing week 1 (baseline) to week 48 were calculated using a two-tailed, paired t-test as provided by GraphPad Prism version 9.3.1; n=22 for liver and spleen; n=19 and 20 for cortical grey matter and cerebellum respectively.
Figure 5. Correlation analyses looking at cognitive AEeq, plasma drug exposure, plasma HS-NRE concentration and cortical grey matter volume (CGMV) over 48 weeks of tralesinidase alfa treatment. (A) Change in cognitive AEeq from study 250-201 Part 2 week 1 to week 48 versus average plasma drug exposure, i.e., $AUC_{0-\text{last}}$, on week 5, 12 and 36 of study 250-201 Part 2. (B) Plasma HS-NRE cumulative concentrations from week 8 to 48 of study 250-201 versus average plasma drug exposure, i.e., $AUC_{0-\text{last}}$, on week 5, 12 and 36 of study 250-201 Part 2; HS-NRE LLOQ was defined as 200 ng/mL*week, i.e., 5 ng/mL/week times 40 weeks while the 95th percentile (95%ile) for non-affected subjects was defined as 15 ng/mL/week times 40 weeks. (C) Change in cognitive AEeq from study 250-201 Part 2 week 1 to week 48 versus plasma HS-NRE cumulative concentrations from week 8 to 48 of study 250-201 Part 2. (D) Change in cognitive AEeq from study 250-201 Part 2 week 1 to week 48 versus change in CGMV from study 250-201 Part 2 week 1 to week 48. Correlation Pearson r and p values were calculated using GraphPad Prism 9.3.1; n=20, 18, 22 and 19 for figures A, B, C and D respectively. LLOQ- lowest level of quantification, AEeq- Age equivalent.
Table 1. Study 250-201 Part 2 subjects’ characteristics (n=22)

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<td></td>
<td>13 males - 9 females</td>
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<tr>
<td>Ethnicity (as defined by the participants)</td>
<td>86.3% Caucasian, 9% Asian, 4.5% multiple (Native)</td>
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Table 2. CSF and plasma drug exposure in subjects dosed with a single dose of 30, 100 or 300 mg of tralesinidase alfa ICV

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<tr>
<th>Tralesinidase alfa (mg)</th>
<th>No of subjects</th>
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<th>CSF C_{max} (ng/mL)</th>
<th>Plasma AUC_{0-last} (ng/mL*hr)</th>
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<td></td>
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<td>ND</td>
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<td>3,800-3,870</td>
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*Week 1 of Study 250-201 Part 2; ND: not determined