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Clinical pharmacokinetics and pharmacodynamics of cerliponase alfa, enzyme replacement therapy for CLN2 disease by intracerebroventricular administration

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Abstract

Cerliponase alfa is recombinant human TPP1 delivered by intracerebroventricular (ICV) infusion for CLN2, a pediatric neurodegenerative disease caused by deficiency in lysosomal enzyme TPP1. We report the PK and PD of cerliponase alfa, the first ICV enzyme replacement therapy, characterized in a Phase 1/2 study. Escalating doses (30-300 mg every two weeks, Q2W) followed by 300 mg Q2W for ≥48 weeks were administered in 24 patients aged ≥3 years. Concentrations peaked in CSF at the end of ~4-hour ICV infusion and 8 hours thereafter in plasma. Plasma exposure was 300-1000 fold lower than in CSF, with no correlation in the magnitude of C_{max} or AUC between body sites. There was no apparent accumulation in CSF or plasma exposure with Q2W dosing. Inter- and intra-patient variability of AUC, respectively, were 31-49% and 24% in CSF versus 59-103% and 80% in plasma. PK variability was not explained by baseline demographics, as gender, age, weight, and CLN2 disease severity score did not appear to impact CSF or plasma PK. No apparent correlation was noted between CSF or plasma PK and incidence of adverse events (pyrexia, hypersensitivity, seizure, and epilepsy) or presence of antidrug antibodies in CSF and serum. There was no relationship between magnitude of CSF exposure and efficacy (change in CLN2 score from baseline), indicating maximum benefit was obtained across the range of exposures with 300 mg Q2W. Data from this small trial of ultra-rare disease were leveraged to adequately profile cerliponase alfa and support 300 mg ICV Q2W for CLN2 treatment.

1. Introduction

CLN2 disease is an ultra-rare, inherited, pediatric neurodegenerative disorder that belongs to the family of neuronal ceroid lipofuscinoses (NCL; number indicates the form of NCL), collectively and more familiarly known as Batten disease.[1,2,3] Classified by the underlying gene defect, CLN2 specifically is caused by mutations in the gene encoding the lysosomal enzyme, tripeptidyl peptidase 1 (TPP1). TPP1 deficiency results in lysosomal accumulation of abnormal storage material (called ceroid and lipofuscin) in neuronal cells, leading to cell damage and cell death of mainly the central nervous system (CNS) and the retina. This rapidly progressing, irreversible disease typically appears as epilepsy and slowing of psychomotor development at age 2-4 years, followed by standstill then regression of psychomotor development, blindness, and death by age 12 years. All forms of NCL are fatal and therapy has been limited to symptomatic treatment or palliative care.[3]

Cerliponase alfa is a recombinant proenzyme form of human TPP1 (rhTPP1) that is delivered by intracerebroventricular (ICV) infusion to replace the deficient enzyme in affected nervous tissues of CLN2 disease. It is activated upon uptake into the acidic pH environment of the lysosome *in vivo*, to become the mature, proteolytic form of rhTPP1 that catabolizes lysosomal storage material. Drug administration is direct, bypassing the blood-brain barrier (BBB), into the cerebral ventricles via a surgically implanted ICV access device.[4] In 2017, cerliponase alfa was approved by the US Food and Drug Administration and the European Medicines Agency based on evidence of slowing or halting disease progression in CLN2 patients.[3,5]

CLN2 represents approximately 25% of all NCL disorders, with an ultra-rare prevalence of <1 per million population estimated; whereby in comparison, the prevalence threshold to qualify as a rare disease is approximately <500 patients per million.[6,7,8,9] Granted orphan drug status by regulatory bodies, clinical development of cerliponase alfa comprised of 24 patients in two clinical trials: one first-in-human Phase 1/2 study and one Phase 1/2 extension study in progress. The completed initial study was designed to evaluate the safety and efficacy of cerliponase alfa 300 mg ICV every two weeks (Q2W) after at least 48 weeks of treatment.[10] The clinical dose regimen of 300 mg Q2W was pre-determined based on pharmacology and pharmacokinetic studies in dog and monkey, animal

species chosen for their likeness in CNS parameters to humans. Herein, we describe the clinical pharmacokinetics and pharmacodynamics of cerliponase alfa, the first ICV-administered enzyme replacement therapy (ERT), as characterized in a single study of CLN2 patients.

2. Methods

2.1 Study Design and Patients

This was a Phase 1/2, open-label study in children with CLN2, conducted at five study centers in four countries.[10] The study protocol was approved by an Institutional Review Board or Independent Ethics Committee at each clinical site. Written informed consent from a parent or legal guardian of each patient was obtained, and if appropriate, written assent was obtained from the patient. Data from this initial study served as the basis for our analysis; data from the thereafter extension study were not included as PK data were not collected.

All study participants had a documented diagnosis of CLN2 disease, determined by TPP1 enzyme activity and CLN2 genotype analysis. Since CLN2 is exclusively a pediatric disease, eligible patients were 3-15 years of age at time of enrollment. All patients had mild to moderate disease progression, measured by a combined score of 3-6 on the motor and language domains of the CLN2 Clinical Rating Scale (range 0-6; 0 representing no function and 3 representing normal function for each of the two domains) and a score of at least 1 in each of the two domains.[10]

A total of 24 patients were enrolled in the study. Ten patients were assigned to three 3-subject cohorts for the dose escalation phase, evaluating 30, 100, and 300 mg Q2W by ICV administration. One patient in the third cohort discontinued the study after one dose due to unwillingness to continue with study visits and procedures, and was replaced, resulting in four patients assigned to the third cohort. Dose cohorts were enrolled to the next higher dose level upon approval by an independent data and safety monitoring committee (i.e., patients starting at 30 or 100 mg were escalated to 300 mg). Nine patients entered the stable dose phase upon completion of the dose escalation phase, along with 14 additional patients who enrolled directly into the stable dose phase. The stable dose phase comprised of 300 mg ICV Q2W (the target clinical dose regimen) for 48 weeks. Twenty-four patients

were enrolled in the study and 23 patients completed at least 48 weeks of therapy (i.e., 24 infusions) at the 300 mg dose level.

2.2 Administration of Cerliponase Alfa

Prior to study drug administration, an ICV reservoir and catheter was surgically implanted. The patency, location, and skin integrity of the ICV access device (reservoir plus catheter) was evaluated by the investigator at each study drug administration. Because hypersensitivity reactions may be associated with ERT administration, patients were pretreated with age-appropriate doses of antihistamine. All patients were administered cerliponase alfa by ICV infusion Q2W. The study drug was infused ICV at 2.5 mL/hour to deliver 10 mL over approximately 4 hours. Following infusion of cerliponase alfa, a flushing solution was administered at 2.5 mL/hour for 30 minutes, for a complete infusion duration of 4.5 hours.

2.3 Pharmacokinetic Analysis

CSF and blood (plasma) samples for pharmacokinetic analysis were collected following the initial dose, the first dose at each new dose level during the dose escalation phase, and Week 5 and Week 13 of the stable dose phase. Samples were collected pre-dose (within 0.25 hours prior to start of infusion), and 0.25, 4, 8, 20, 72, and 120 hours after end of infusion. Additional CSF and blood (plasma) samples were collected pre-dose at the start and every 4 weeks of the stable dose phase whenever serial samples were not collected. CSF samples were obtained from the lateral ventricle of the brain using the ICV port.

CSF and plasma samples were assayed for concentrations of cerliponase alfa by validated electrochemiluminescence immunoassay (ECLA) methods (BioMarin Pharmaceutical Inc., Novato, CA, US). The lower limit of quantitation (LLOQ) was 20 ng/mL in CSF and 16 ng/mL in plasma. Both inter-assay accuracy (% relative error) and precision (% coefficient of variation) of quality controls were ≤13.2% in CSF and ≤17.2% in plasma throughout sample testing runs.

PK parameters were estimated based on concentration-time data in CSF and plasma by non-compartmental analysis (NCA) using Phoenix WinNonlin 6.4 (Pharsight Corporation, Cary, NC, USA). Maximum concentration (C_{max}) and time of maximum concentration (T_{max}) were recorded directly from the observed data. Other PK parameters estimated were elimination half-life ($t_{1/2}$); area under the concentration-time curve from time 0 to the time of last measurable concentration (AUC_{0-t}), estimated by the linear trapezoidal rule; area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$); clearance of the absorbed fraction (CL); volume of distribution based on the terminal phase (V_z); and steady-state volume of distribution (V_{ss}).

2.4 Efficacy and Safety Evaluations

The primary efficacy variable was an aggregate score in the domains for motor and language function on the CLN2 Clinical Rating Scale; each domain scoring from 0 (no function) to 3 (normal function) for a maximal possible score of 6 combined. Testing was performed at baseline, start of the stable dose phase (300 mg Q2W), and every 8 weeks thereafter over the 48-week study phase. Trained raters administered the clinical rating scales in a standardized manner. Safety was assessed by the incidence, severity, and relationship to study drug of treatment-emergent adverse events (AEs), which were monitored continuously for study duration. Full clinical efficacy and safety data from this study have been previously reported.[10]

2.5 Immunogenicity Analysis

CSF and blood (serum) samples for immunogenicity were collected at baseline, every 4 weeks during the dose escalation phase, and at the start and every 4 weeks thereafter of the stable dose phase. CSF and serum samples were tested for total anti-drug antibodies (TAb) specific to cerliponase alfa by validated bridging electrochemiluminescence assays (BioMarin Pharmaceutical Inc., Novato, CA, US). TAb-positive samples in CSF were further characterized using a validated cell-based flow cytometry assay (BioMarin Pharmaceutical Inc., Novato, CA, US) for neutralizing antibodies (NAb) that block the uptake of cerliponase alfa into the lysosome. NAb testing was performed only in CSF samples as the target site of action is the CNS, and TAb-positive samples were tested for NAb response. Full immunogenicity methods and results from this study have been previously reported.[11]

2.6 Statistical Analysis

Demographic characteristics were summarized for the PK population. PK parameters were summarized descriptively by biological matrix, dose group, and study visit. The relationship of PK parameters to demographic characteristics, immunogenicity, safety, and efficacy parameters were assessed graphically since analyses of the PK population were not powered to assess statistical significance. For analyses without time as a covariate, mean PK parameters for each patient was used as the representative measure of an individual patient's exposure during treatment with 300 mg Q2W. Mean PK parameters were derived by calculating the arithmetic means of C_{max} and $AUC_{0\text{-}t}$ values across study visits with 300 mg Q2W dosing and intensive PK sampling (i.e., first dose at 300 mg, and Weeks 5 and 13 of the stable dose phase).

3. Results

3.1 Patient Characteristics

Total of 24 patients were enrolled in the study (safety population), and 23 patients completed treatment at 300 mg Q2W for at least 48 weeks (efficacy population). Baseline demographics (reported as median (range) or percentage (number)) of patients (n=24) were: age 4 (3 to 8) years, weight 17.5 (14.5 to 26.0) kg, 38% (n=9) male versus 62% (n=15) female, and 96% (n=23) White versus 4% (n=1) Asian. By the time of initiation of the 300 mg Q2W regimen, median (range) CLN2 scores of patients (n=23) were 3 (1 to 6).

PK parameters were estimated in all patients, over various dose levels and study visits. At 300 mg Q2W, there were 24 patients with evaluable PK data in CSF versus 15 patients with evaluable PK data in plasma.

3.2 Pharmacokinetics

Single-Dose Pharmacokinetics

Single-dose PK data were available from patients who received an initial dose of 30 (n=3), 100 (n=3), or 300 mg (n=17; 4/4 from dose escalation phase and 13/14 enrolled directly into stable dose phase) of cerliponase alfa (**Error! Reference source not found.**).

In CSF, peak concentrations were observed at the first sampling time point after the end of 4-hour infusion and appeared to decline in a biphasic manner. CSF exposure increased less than dose proportional with approximately 5 to 7-fold increase in median C_{max} and AUC versus the 10-fold increase in dose from 30 to 300 mg. One patient in the 100 mg group had high exposure following their initial dose, and accordingly, exposure parameters for the 100 mg dose level were highly variable due to small sample size. C_{max} and AUC for this patient (shown by the maximum value reported for the 100 mg group) were higher than median values for the 300 mg group. Investigation into potential explanations for the high CSF exposure during their Day 1 visit was performed but did not reveal conclusive findings. Despite this outlier exposure, CSF exposures for this patient following subsequent infusions of 300 mg were less than their exposure following the initial 100 mg dose (data not shown).

During the initial stages of study conduct, plasma PK samples were stored outside of the stability range, and thus, no data are available for the 30 mg group and available for only one patient in the 100 mg group. Based primarily on the 300 mg dose level, concentrations in plasma peaked between 8 to 20 hours after the end of 4-hour ICV infusion and appeared to decline in a biphasic manner, remaining above LLOQ through 72 hours.

Multiple-Dose Pharmacokinetics

Multiple-dose PK data were evaluated from patients enrolled directly into the stable dose phase (n=14), who received 300 mg of cerliponase alfa Q2W throughout the study (**Error! Reference source not found.**; **Figure 1**).

PK parameters in CSF were similar between Day 1, Week 5, and Week 13 visits. While variable, plasma T_{max}, C_{max}, and AUC_{0-t} were comparable with no discernible trends across visits. With ICV

administration of 300 mg Q2W, median C_{max} in plasma was approximately 1000-fold lower than in CSF and median AUC_{0-t} in plasma approximately 300 to 1000-fold lower than in CSF. There was no apparent correlation between the magnitude of either C_{max} or AUC_{0-t} in CSF versus in plasma based on patient- and visit-matched PK (**Figure 2**). Inter-individual variability of C_{max} and AUC_{0-t}, respectively, were 26-73% and 31-49% in CSF versus 54-89% and 59-103% in plasma across visits. Intra-individual variability of C_{max} and AUC_{0-t} across visits, respectively, were 33% and 24% in CSF versus 69% and 80% in plasma.

3.3 Pharmacokinetics and Patient Characteristics

The potential impact of baseline patient characteristics on cerliponase alfa PK was evaluated for the 300 mg Q2W regimen. Mean estimates of C_{max} and AUC_{0-t} were used to represent an individual patient's exposure over the course of therapy and was deemed appropriate due to the lack of drug accumulation or time-dependent PK with 300 mg Q2W. There was no apparent effect of baseline gender, age, bodyweight, or CLN2 score on the exposure of cerliponase alfa in CSF or plasma (**Figure 3** and **Figures S1-S3**). There was a slight trend of increasing plasma C_{max} with decreasing age but was not exhibited of plasma AUC_{0-t} (**Figure 3**).

3.4 Pharmacokinetics and Immunogenicity

TAbs against cerliponase alfa were detected in CSF of 5/24 (21%) patients and in serum of 19/24 (79%) patients over the study duration. CSF TAb response was first detected at Week 13 of the stable dose phase, while serum TAb response was detected at the earliest time point sampled, Week 5 of the dose escalation phase. NAbs were not detected in CSF of any of the 5 patients with CSF TAb positivity.

To determine whether cerliponase alfa PK is affected by the development of anti-drug antibodies (ADA), C_{max} and AUC_{0-t} on visits with positive TAb response were compared to visits with negative TAb response. Visit-matched exposure parameters and ADA status (i.e., on Day 1, stable dose Week 5, and stable dose Week 13) were assessed from all patients who initiated treatment at 300 mg and had evaluable PK and ADA data. Eighteen patients with CSF data (4/4 from dose escalation phase

and 14/14 enrolled directly into stable dose phase) and 14 patients with plasma/serum data (1/4 from dose escalation phase and 13/14 from stable dose phase) were included for analysis.

As displayed in **Figure 4**, there was no discernible trend in CSF C_{max} and AUC_{0-t} by CSF ADA status across patients. CSF C_{max} and AUC_{0-t} values on visits with positive ADA response were well within the distribution of exposure values with negative ADA response. For the two patients with visit-matched PK and CSF ADA positivity, CSF AUC_{0-t} was lower by 17-27% on the ADA-positive visit compared to ADA-negative visits. No association was observed between plasma C_{max} and AUC_{0-t} with serum ADA status, across and within patients (**Figure 4**). Plasma exposure on visits positive for serum ADA spanned the range of exposure on ADA-negative visits within an individual patient.

3.5 Pharmacokinetics and Efficacy

In brief, patients treated with cerliponase alfa 300 mg Q2W demonstrated a statistically significant improvement in the time to and rate of decline in the motor-language scale as compared to historical controls.[10] Response was defined as the absence of either an unreversed two-point loss (the expected decline for an untreated population) or a combined score of zero (no function) in the motor and language domains of the CLN2 scale.

Here, the relationship between cerliponase alfa PK and efficacy outcomes was evaluated using the change in motor-language score from the start of 300 mg Q2W to the end of study. Of 23 patients in this analysis, 2 had a one-point gain, 13 had no change, 5 had a one-point loss, and 3 had a two-point loss after 48 weeks of treatment for an overall responder rate of 87% (20/23). A patient's change in motor-language score at Week 48 did not correlate with the individual mean C_{max} and AUC_{0-t} in CSF (**Figure 5**). Patients with a decline in score had CSF exposure parameters within the distribution of those with no change or gain in score. Similarly, no correlation was shown when assessed by the maximum decrease in a patient's score during 48 weeks of treatment (data not shown).

3.6 Pharmacokinetics and Safety

The incidence of adverse events in all 24 patients was assessed relative to the representative average exposure of each patient, in both CSF and plasma. All patients reported at least one adverse event while on study; 23 of 24 patients experienced an adverse event deemed to be related to cerliponase alfa by the investigator. Study drug-related events that occurred in at least 10% of the study population were included for analysis: pyrexia 46% (11/24), hypersensitivity 33% (8/24), seizure 33% (8/24), epilepsy 17% (4/24), headache 13% (3/24), and vomiting 13% (3/24). Between patients with and without pyrexia, hypersensitivity, seizure, or epilepsy, C_{max} and $AUC_{0\text{-}t}$ in CSF or plasma were not significantly different (**Figures S4-S7**). There were slight trends of higher CSF exposure for patients with headache and higher exposure in both CSF and plasma for patients with vomiting compared to those without (**Figures S8-S9**). Exposure in patients with headache or vomiting generally did not exceed the highest exposure observed in patients without either of the events.

4. Discussion

Cerliponase alfa was developed as ERT for CLN2 disease using the ICV route, administering directly into the cerebrospinal fluid of ventricles in the brain. Unlike the successes in other lysosomal storage diseases (LSD), systemically administered ERTs are unable to breach the BBB to access target tissues for CNS-centric diseases like CLN2 disease. ICV access to the CSF is advantageous for CLN2 patients as the rhTPP1 is administered in close proximity to the choroid plexus, the major site of CSF production, and thus carried to brain tissues downstream by CSF flow (rather than against CSF outflow with an intrathecal-lumbar route) to result in greater brain exposure.[12] Cerliponase alfa is the first ICV-delivered ERT, and the first and currently only approved treatment for any of the NCL types, specifically CLN2.[5] The pharmacokinetics of cerliponase alfa and the relationship to safety and efficacy were studied in this first-in-human Phase 1/2 trial of twenty-four CLN2 patients.

Data from nonclinical pharmacology studies supported the clinical dose regimen of 300 mg Q2W by 4-hour ICV infusion. In juvenile TPP1-null dogs, 16 mg Q2W by ICV infusion resulted in significant attenuation of disease and improvement in translatable pharmacological endpoints, including motor function, gait, cognitive function, brain morphology, and life span.[13] The human equivalent dose to

the efficacious 16 mg in dog was calculated by scaling brain mass across species to ~300 mg for a pediatric human [Hammon et al., unpublished data]. Concentrations in CSF were above the lysosomal k_{uptake} (concentration at which lysosomal uptake is half-maximal, ~2 nM or ~120 ng/mL) for 2-3 days following ICV infusion in dog and monkey, enabling widespread distribution to CNS tissues.[12,14,15] The concentration gradient between CSF and CNS tissues is expected to lead to tissue penetration by the administered rhTPP1, followed by lysosomal uptake and activation.[12] The lysosomal half-life of rhTPP1 was estimated to be ~12 days in TPP1-deficient human fibroblasts, and together with the CNS tissue half-life of 3-15 days in monkeys, supported a biweekly dosing interval for ICV administration.[12,14]

Cerliponase alfa demonstrated less than dose proportional increase in CSF exposure following initial ICV infusions of 30, 100, and 300 mg. CSF C_{max} across the single-dose range (2.08×10^5, 6.65×10^5, and 1.42×10^6 ng/mL respectively) were generally consistent with expected values for the amount of ICV dose administered to ~100 mL of CSF within a human brain (3.00×10^5, 1.00×10^6, and 3.00×10^6 ng/mL respectively).[16] At 300 mg Q2W, there was no apparent accumulation or time-dependence in CSF or plasma PK based on comparable C_{max}, AUC, CL, and V_{ss} across study visits. This is in line with the CSF half-life of 6.2-7.7 hours across patients and the calculable plasma half-life in one patient of 11.8 hours, given the biweekly dosing frequency. It should be emphasized that CSF half-life does not directly reflect the target site, for which the CNS tissue half-life (from assessment in monkeys) and lysosomal half-life (from *ex vivo* human fibroblasts) of days to weeks are most pertinent to the rationale for therapeutic biweekly dosing. In patients, CSF concentrations were greater than the lysosomal k_{uptake} for ~4 days, which based on animal data, suggest widespread distribution of the enzyme to CNS tissues. This was supported by estimates of CSF volume of distribution (median V_{ss}, 245 mL), which exceeded the typical CSF volume of ~100 mL. Direct administration of cerliponase alfa to the internal CSF spaces of the brain resulted in approximately three orders of magnitude greater exposure than in the periphery, with no correlation in the magnitude of C_{max} or AUC between CSF and plasma; indicating plasma PK is not a good surrogate for CSF PK. Plasma T_{max} was 8 hours after completion of 4-hour ICV infusion compared to CSF T_{max}, which occurred immediately after end of infusion. The blood-CSF barrier is leaky compared to the BBB,

and thus, ICV-administered drug is transported out of the brain through CSF flow tracks and absorbed into the peripheral bloodstream across the arachnoid villi.[17]

Variability in CSF and plasma PK between patients was not explained by patient demographics, as intrinsic factors did not appear to correlate with cerliponase alfa exposure. The ICV dose of cerliponase alfa is fixed and was designed according to brain mass and thus, CSF exposure would not be expected to change considerably for the range of age (3-8 years) and bodyweight (14.5-26.0 kg) in this study. The human brain on average achieves about 75% of adult mass by age 2 and 100% by age 5, with progressively decreasing brain-to-body weight ratio during development.[18] Between ages 3 to 8-9 years, non-diseased human brains weigh on average 1.09-1.18 kg for females and 1.27-1.37 kg for males, compared to bodyweights of 14.1-26.0 kg and 15.6-27.5 kg, respectively.[19] The change in brain weight is thus only 8% across the age range in this study versus an 80% change in bodyweight. Of note, the slight trend of increasing plasma C_{max} with decreasing age is likely attributable to the disproportionate change in body versus brain weights during early childhood. While matched in age by brain weight, the fixed amount of ICV dose is absorbed into a significantly smaller bodyweight and correspondingly smaller blood volume, resulting in more concentrated systemic exposure.

The between-patient variability in CSF exposure may rather be attributable to differences in disease severity than variability inherent to the ICV-administered enzyme, since within-patient variability was far less (24% for CSF AUC_{0-t}) than between-patient variability (31-49% for CSF AUC_{0-t} across visits). Although no association between CSF exposure and baseline CLN2 score was shown, there may be pathological effects to the CNS that are reflected in CSF PK but not translated to a change in clinical rating score. As systemic absorption occurs thereafter, inter- and intra-patient variability in plasma PK were both considerably higher than in CSF, contributed in part by the insufficient number of plasma samples with quantifiable concentrations.

Based on patient- and visit-matched analysis of 300 mg Q2W, the presence of ADA in CSF and serum did not appear to have an impact on PK in CSF and plasma, respectively. Majority of treated patients developed ADA in serum, suggesting that plasma exposure of cerliponase alfa will likely lead

to ADA positivity in serum, consistent with other ERTs as patients are deficient in endogenous protein.[20] Development of an ADA response in this study was previously demonstrated not to be predictive of an adverse safety profile or poor treatment outcome.[11] For the most common adverse events related to cerliponase alfa, there were no apparent correlations between CSF or plasma exposure and the incidence of pyrexia, hypersensitivity, seizure, or epilepsy. Slight trends of increased exposure with incidences of headache and vomiting were limited for interpretation, given the low frequency amidst a small sample size (3/24 patients) for both events.

Response to treatment, as measured by change in CLN2 score after 48 weeks of 300 mg Q2W, did not appear to correlate with magnitude of CSF exposure, indicating maximum benefit was obtained across the range of exposures with 300 mg Q2W. Of note, CSF exposures in CLN2 patients exceeded those associated with the efficacious 16 mg dose in TPP1-null dogs, suggesting these exposures were within the plateau of an exposure-response relationship.[13,15] Despite the inter- and intra-patient PK variability, 91% (62/68) of visits with reportable CSF $AUC_{0\text{-}t}$ at the clinical 300 mg dose had values above the mean CSF $AUC_{0\text{-}t}$ in TPP1-null treated dogs (6.45×10^6 ng-hr/mL).[15] Expression of low levels of TPP1 have been shown to dramatically attenuate disease in a CLN2-mutant mouse study, where just 6% of normal TPP1 activity in the brain increased the lifespan to almost that of a wild-type mouse.[21] Taken together, these nonclinical and clinical data indicate that cerliponase alfa delivered ICV at 300 mg Q2W provides sufficient TPP1 exposure to the CNS for a meaningful therapeutic benefit.

In this small clinical trial of ultra-rare disease, data were strategically obtained and used to characterize the pharmacokinetics and pharmacodynamics of cerliponase alfa. Because of the irreversible nature of disease, animal models were used to define the target therapeutic dose and rapid within-patient escalation to target dose was performed in lieu of a dose-finding study design. Despite the limited patient numbers in the study and limited PK data available (sampling restricted based on feasibility), data were leveraged to enable clinical utility of cerliponase alfa and support 300 mg ICV Q2W for CLN2 treatment. This is the first characterization of clinical CSF and plasma pharmacokinetics of an ICV administered protein. While numerous approaches are currently in

clinical study to address neurological manifestations of LSD, this work furthers the understanding of this novel route of administration to address an unmet medical need.

Study Highlights

- **What is the current knowledge on the topic?**

  Enzyme replacement therapy (ERT) has successfully treated systemic disease of lysosomal storage disorders but not central nervous system (CNS) disease due to inability to cross the blood-brain barrier. Cerliponase alfa is the first ERT administered by intracerebroventricular (ICV) infusion and indicated for CLN2, an ultra-rare, neurodegenerative disorder caused by deficiency in lysosomal enzyme TPP1.

- **What question did this study address?**

  What is the pharmacokinetics of cerliponase alfa and the relationship to safety and efficacy in CLN2?

- **What does this study add to our knowledge?**

  ICV cerliponase alfa at 300 mg every two weeks (Q2W) appears to provide sufficient TPP1 exposure to CNS for meaningful efficacy. With ICV administration, exposures in cerebrospinal fluid (CSF) were ~1000-fold higher than plasma. Plasma PK was not a good surrogate for CSF PK. High inter- and intra-patient variability were observed, more so in plasma than CSF PK.

- **How might this change clinical pharmacology or translational science?**

  Data from this small trial of ultra-rare disease were leveraged to enable clinical utility of cerliponase alfa, demonstrating strategic orphan drug development.

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Author Contributions

A.K. and J.H. wrote the manuscript; D.J. designed the research; A.S., N.S., E.D.L.R., and P.G. performed the research; A.K., A.G., K.H., G.D.H., P.S., A.C., T.A., and J.H. analyzed the data.

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Figure 1: Mean concentration-time profile of cerliponase alfa in CSF and plasma following 300 mg Q2W by ICV infusion. Time 0 represents the start of infusion. SD=stable dose phase.

Figure 2: Patient- and visit-matched CSF versus plasma exposure of cerliponase alfa at 300 mg ICV Q2W

Figure 3: Patient baseline age and cerliponase alfa PK in CSF and plasma
*Individual patient data shown as circles. Box represents the interquartile range (IQR) between first (Q1) and third (Q3) quartiles, bar within the box represents the median, and whiskers represent the minimum and maximum values excluding outliers (i.e., values outside the standard span of data defined as the range from Q1-1.5*IQR to Q3+1.5*IQR; open circles).*

Figure 4: Individual visit-matched PK of cerliponase alfa and ADA status, in **a)** CSF and **(b)** plasma/serum
Open circles shown for patients with negative ADA response at study visit and red, closed circles for patients with positive ADA response at study visit.

Figure 5: Change from baseline in the combined score for motor-language function of the CLN2 Clinical Rating Scale and cerliponase alfa PK in CSF
*Individual patient data shown as circles. Box represents the interquartile range (IQR) between first (Q1) and third (Q3) quartiles, bar within the box represents the median, and whiskers represent the minimum and maximum values excluding outliers (i.e., values outside the standard span of data defined as the range from Q1-1.5*IQR to Q3+1.5*IQR; open circles).*

Supplementary Files

1. Supplementary Figs

Table 1: Median (range) PK parameters for cerliponase alfa following single doses of 30, 100, and 300 mg by ICV infusion

Parameter	30 mg	100 mg	300 mg
CSF			
N	3 [a]	3	17 [c]
T_{max}, hr	4.50 (4.33, 8.08 [b])	4.50 (4.42, 4.50)	4.50 (4.25, 5.75 [d])
C_{max}, µg/mL	208 (186, 269)	665 (646, 5640)	1420 (359, 4380)
AUC_{0-t}, µg-hr/mL	1660 (1180, 1950)	5740 (3620, 62700)	9210 (3660, 19000)
$AUC_{0-\infty}$, ng-hr/mL	1810 (1660, 1950) [a]	5740 (3630, 62700)	9290 (3660, 19000)
CL, mL/hr	16.7 (15.4, 18.0) [a]	17.4 (1.59, 27.6)	32.3 (15.8, 81.9)
V_z, mL	286 (151, 420) [a]	296 (11.0, 409)	311 (159, 1920)
$t_{1/2}$, hr	11.5 (6.81, 16.2) [a]	10.3 (4.79, 11.8)	6.13 (4.13, 16.3)
Plasma			
N	0 [f]	1 [f]	13 [f, g]
T_{max}, hr	NA	24.0	12.0 (4.25, 24.5)
C_{max}, µg/mL	NA	0.357	0.944 (0.176, 3.87)
AUC_{0-t}, µg-hr/mL [e]	NA	11.4	11.4 (1.12, 69.9)
$AUC_{0-\infty}$, µg-hr/mL	NA	NA	14.3 [g]
CL, mL/hr	NA	NA	21000 [g]
V_z, mL	NA	NA	356000 [g]
$t_{1/2}$, hr	NA	NA	11.8 [g]

NA=not available

[a] CSF $t_{1/2}$, $AUC_{0-\infty}$, CL, and V_z reported for N=2 in the 30 mg cohort due to insufficient quantifiable samples during the terminal phase for 1 patient.

[b] CSF T_{max} for this patient was at 8.08 hr, collected 4 hr post-dose instead of the planned 0.25 hr post-dose assessment.

[c] CSF samples were not collected on Day 1 for 1/18 patients in the 300 mg cohort.

[d] CSF T_{max} for this patient was at 5.75 hr, collected 1.5 hr post-dose instead of the planned 0.25 hr post-dose assessment.

[e] Plasma AUC_{0-t} was estimated at times using less than three quantifiable concentration-time points.

[f] Plasma samples were improperly stored for 3/3 patients at 30 mg, 2/3 patients at 100 mg, and 5/18 patients at 300 mg.

[g] Plasma $t_{1/2}$, $AUC_{0-\infty}$, CL, and V_z were not estimated for nearly all patients in the 300 mg cohort due to insufficient quantifiable samples during the terminal phase, except for 1 patient.

Table 2: Median (range) PK parameters for cerliponase alfa following 300 mg Q2W by ICV infusion

Parameter	Day 1	Week 5	Week 13
CSF			
N	13 [a]	14	13 [a]
T_{max}, hr	4.50 (4.25, 5.75)	4.25 (3.83, 4.50)	4.25 (4.00, 4.50)
C_{max}, µg/mL	1260 (359, 4380)	1630 (376, 4670)	1390 (1110, 2340)
AUC_{0-t}, µg-hr/mL	9290 (3660, 19000)	12400 (4620, 26200)	10500 (7000, 18200)
$AUC_{0-\infty}$, µg-hr/mL	9290 (3660, 19000)	12400 (4620, 26200)	10500 (7000, 18200)
CL, mL/hr	32.3 (15.8, 81.9)	24.2 (11.4, 64.9)	28.7 (16.5, 42.9)
V_{ss}, mL	245 (78.4, 909)	196 (85.4, 665)	186 (131, 257)
V_z, mL	316 (178, 1920)	254 (148, 573)	263 (160, 443)
$t_{1/2}$, hr	6.15 (5.49, 16.3)	7.35 (3.33, 9.53)	7.65 (5.05, 9.43)
Plasma [b]			
N	12 [d]	12 [d]	9 [e]
T_{max}, hr	12.0 (4.25, 24.5)	12.0 (7.50, 24.2)	12.3 (4.25, 75.9)
C_{max}, µg/mL	1.28 (0.176, 3.87)	1.92 (0.222, 4.27)	0.962 (0.0295, 2.57)
AUC_{0-t}, µg-hr/mL [c]	16.2 (1.12, 69.9)	40.1 (11.1, 78.9)	9.50 (0.239, 51.6)
$AUC_{0-\infty}$, µg-hr/mL	14.3 [b]	NA	NA
CL, mL/hr	21000 [b]	NA	NA

V_z, mL	356000 [b]	NA	NA
$t_{1/2}$, hr	11.8 [b]	NA	NA

NA=not available

[a] CSF samples were not collected for 1/14 patients on Day 1 and Week 13.

[b] Plasma $t_{1/2}$, $AUC_{0-\infty}$, CL, and V_z were not estimated for nearly all patients due to insufficient quantifiable samples during the terminal phase, except for 1 patient on Day 1.

[c] Plasma AUC_{0-t} was estimated at times using less than three quantifiable concentration-time points.

[d] Plasma samples were improperly stored for 2/4 patients on Day 1 and Week 5.

[e] Plasma samples were either hemolyzed or not collected for 5/14 patients on Week 13.

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