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Intravenous 2-hydroxypropyl-β-cyclodextrin (Trappsol® Cyclo™) demonstrates biological activity and impacts cholesterol metabolism in the central nervous system and peripheral tissues in adult subjects with Niemann-Pick Disease Type C1: Results of a phase 1 trial

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ABSTRACT

Background: Niemann-Pick Disease Type C1 (NPC1) is a disorder of intracellular cholesterol and lipid trafficking that leads to the accumulation of cholesterol and lipids in the late endosomal/lysosomal compartment, resulting in systemic manifestations (including hepatosplenomegaly and lung infiltration) and neurodegeneration. Preclinical studies have demonstrated that systemically administered 2-hydroxypropyl-β-cyclodextrin (HPβCD; Trappsol® Cyclo™) restores cholesterol metabolism and homeostasis in peripheral organs and tissues and in the central nervous system (CNS). Here, we assessed the safety, pharmacokinetics, and pharmacodynamics of HPβCD in peripheral tissues and the CNS in adult subjects with NPC1.

Methods: A Phase 1, randomized, double-blind, parallel group study enrolled 13 subjects with NPC1 who received either 1500 mg/kg or 2500 mg/kg HPβCD intravenously every 2 weeks for a total of 7 doses (14 weeks). Subjects were 18 years or older, with a confirmed diagnosis of NPC1 and evidence of systemic involvement on clinical assessment. Pharmacokinetic evaluations in plasma and cerebrospinal fluid (CSF) were performed at the first and seventh infusions. Pharmacodynamic assessments included biomarkers of systemic cholesterol synthesis (serum lathosterol) and degradation (serum 4β-hydroxycholesterol), secondary sphingomyelin storage (plasma lysosphingomyelin-509, now more accurately referred to as *N*-palmitoyl-*O*-phosphocholineserine [PPCS]), and CNS-specific biomarkers of neurodegeneration (CSF total Tau) and cholesterol metabolism (serum 24(*S*)-hydroxycholesterol [24(*S*)-HC]). Safety monitoring included assessments of liver and kidney function, infusion related adverse events, and hearing evaluations.

Results: Ten subjects completed the study, with 6 at the 1500 mg/kg dose and 4 at the 2500 mg/kg dose. One subject withdrew following the first infusion after experiencing hypersensitivity pneumonitis, and 2 subjects withdrew after meeting a stopping rule related to hearing loss. Overall, HPβCD had an acceptable safety profile. The observed pharmacokinetic profile of HPβCD was similar following the first and seventh infusions, with a plasma half-life of 2 h, a maximum concentration reached at 6 to 8 h, and no evidence of accumulation. Serum biomarkers of cholesterol metabolism showed reduced synthesis and increased degradation. Compared to Baseline, filipin staining of liver tissue showed significant reductions of trapped unesterified cholesterol at both dose levels at Week 14. Plasma PPCS levels were also reduced. HPβCD was detected at low concentrations in the CSF (maximum, 33 μM) at both dose levels and persisted longer in CSF than in plasma. Total Tau levels in CSF decreased in most subjects. Serum levels of 24(*S*)-HC, a cholesterol metabolite from the CNS that is exported

Abbreviations: 17D-NPC-CSS, 17-Domain Niemann-Pick disease Type C-Clinical Severity Scale; 24(*S*)-HC, 24(*S*)-hydroxycholesterol; HPβCD, 2-hydroxypropyl-β-cyclodextrin; AEs, adverse events; ALT, alanine aminotransferase; $AUC_{0-\infty}$, area under the concentration-time curve estimated from time zero to infinity; AST, aspartate aminotransferase; ABR, auditory brainstem response; CNS, central nervous system; CSF, cerebrospinal fluid; CRP, C-reactive protein; CK-MB, creatinine kinase MB isoenzyme; t1/2, elimination half-life; GFAP, glial fibrillary acid protein; ITT, Intent-To-Treat; INR, international normalized ratio; IND, investigational new drug; LC-MS/MS, liquid chromatography tandem mass spectrometry; C_{max}, maximum observed plasma concentration; NPC1, Niemann-Pick Disease Type C1; PP, Per protocol Population; PBMCs, peripheral blood mononuclear cells; SAEs, serious adverse events; SD, standard deviation; T_{max}, time to maximum concentration; TEAEs, treatment-emergent adverse events; UCSF, University of California San Francisco; URI;, upper respiratory infection; Vss., volume of distribution at steady state.

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across the blood-brain barrier and into the circulation, decreased after both the first and seventh doses. Hence, pharmacodynamic assessments in both peripheral and CNS-related tissue show target engagement. While not the aim of the study, subjects reported favorable impacts on their quality of life.

Conclusions: The plasma pharmacokinetics and pharmacodynamics of HPβCD administered at two intravenous dose levels to subjects with NPC1 were comparable to those observed in preclinical models. HPβCD cleared cholesterol from the liver and improved peripheral biomarkers of cholesterol homeostasis. At low CSF concentrations, HPβCD appeared to be pharmacologically active in the CNS based on the increased efflux of 24(*S*)-HC and reduction in CSF total Tau, a biomarker of CNS neurodegeneration. These data support the initiation of longer-term clinical trials to evaluate the safety and efficacy of intravenous HPβCD in subjects with NPC1. (ClinicalTrials.gov numbers: present trial, NCT02939547; open-label extension of the present trial, NCT 03893071; global pivotal trial, NCT04860960).

1. Introduction

Niemann-Pick Disease Type C (NPC) is a rare, pan-ethnic, autosomal recessive lysosomal storage disorder whose birth prevalence is estimated to be 1 in 90,000 to 100,000 worldwide [1–4]. The disease is caused by loss of function mutations in the *NPC1* (90% to 95% of subjects) or *NPC2* (4% of subjects) genes, resulting in impaired intracellular trafficking of cholesterol and lipids and leading to the toxic accumulation of unesterified cholesterol, glycosphingolipids, and GM2 and GM3 gangliosides [4–9]. Although the underlying pathological mechanisms are not fully understood, the accumulation of cholesterol and lipids in lysosomes and late endosomes is likely a crucial event in disease pathogenesis [4,10]. The symptoms associated with NPC vary in severity and with age of onset and include visceral manifestations (such as hepatomegaly, splenomegaly, and lung dysfunction), neurological manifestations (including hearing loss, cerebellar ataxia, dystonia, dysmetria, dysarthria, dysphagia, and vertical supranuclear gaze palsy), as well psychiatric symptoms and progressive cognitive impairment [1,5,6,11]. The disease is highly heterogeneous and may present from the perinatal period to the seventh decade of life, although most subjects develop symptoms in childhood and die between 10 and 25 years of age [1,4–6,12,13]. The diagnosis of NPC is often delayed in part due to the wide spectrum of clinical phenotypes, which are likely related to underlying genotypes (515 estimated pathogenic mutations) and epigenetic modifiers [5,6,14–16].

Currently, there is no treatment that ameliorates both the systemic and neurological manifestations of the disease [12]. Various therapeutic strategies have been attempted in the pre-clinical and clinical setting [6]. In the United States (US), the current standard of care is symptom-based management and palliative approaches including anti-epileptic, anticholinergic, antidepressant, and antipsychotic drugs [1,12]. Miglustat (Zavesca®), a glucosylceramide synthase inhibitor, is approved for the treatment of NPC in the European Union but not in the US, where it is commonly used off-label [12,17]. Miglustat reduces the accumulation of glycosphingolipids (glucosylceramide, lactosylceramide, and GM2 and GM3 gangliosides) in the brain, which slows the progression of the neurological symptoms in some subjects and increases median survival by approximately 10 years [12,18]. However, this drug does not impact the systemic manifestations of the disease, and the majority of subjects experience adverse events (AEs), most commonly diarrhea and tremor, that may preclude long-term use [19,20]. Therefore, there is a critical need for novel treatments for NPC that can impact disease progression, quality of life, and survival.

The cyclic oligosaccharide, 2-hydroxypropyl-β-cyclodextrin (HPβCD [Trappsol® Cyclo™]), has a hydrophilic exterior and a hydrophobic core, which allows it to form complexes with hydrophobic compounds and has led to its widespread use as a delivery vehicle to improve the solubility, stability, and bioavailability of various medicinal products [21,22]. Additionally, HPβCD is routinely used in cell culture systems to modulate cellular cholesterol content. In pre-clinical NPC1 mouse studies, HPβCD released trapped cholesterol from the late endosomal/lysosomal compartment in a dose-dependent manner, ameliorated hepatosplenomegaly and neurological symptoms, and prolonged survival [23–28]. Based on these pre-clinical studies, HPβCD was used in humans for the first time as an investigational new drug (IND) for named subject use in 2009. Treatment regimens were diverse in terms of dose level, frequency, and route of administration. In a case report study, 12 subjects ranging in age from 1 to 27 years with mild to severe disease were treated with intravenous HPβCD for over 7 years [29]. The treatment was well tolerated, and no serious AEs were attributed to the drug. In general, subjects experienced slowing of disease progression and reported improved quality of life.

These encouraging named subject data supported the initiation of a formal Phase 1 clinical trial of HPβCD administered intravenously in NPC1 subjects to evaluate its pharmacokinetics and pharmacodynamics as assessed by cholesterol metabolism and homeostasis in blood, liver, and the central nervous system (CNS) and additionally by biomarkers of sphingomyelin storage and neurodegeneration.

2. Materials and methods

2.1. Study design

This Phase 1, randomized, double-blind, parallel-group study enrolled adult subjects (18 years and older) with a confirmed diagnosis of NPC1 at the University of California San Francisco (UCSF) Benioff Children's Hospital Oakland in Oakland, California, US (ClinicalTrials.gov number, NCT02939547). The objective of the study was to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of two different doses of intravenous HPβCD (1500 mg/kg body weight and 2500 mg/kg body weight; Cyclo Therapeutics, Gainesville, FL), specifically through serum, plasma and cerebrospinal fluid (CSF) biomarkers and liver assessments after 7 infusions (14 weeks).

Eligible subjects had to meet the following inclusion and exclusion criteria. Subjects had to have an NPC1 diagnosis confirmed by one of the following: two *NPC1* mutations; one *NPC1* mutation and positive filipin staining (current or prior); vertical supranuclear gaze palsy plus either one or more *NPC1* mutations or positive filipin staining. The subject's clinical severity score based on the 17-Domain Niemann-Pick disease Type C-Clinical Severity Scale (17D-NPC-CSS) could not exceed 30 (severe impairment), with no more than 4 individual major domains scoring ≥3 (maximum score is 61, including 5 for each of the 9 major domains and 2 for each of the 8 minor domains) [30]. The major domains of the 17D-NPC-CSS are eye movement, ambulation, speech, swallow, fine motor skills, cognition, hearing (sensorineural), memory, and seizures. The minor domains are gelastic cataplexy, narcolepsy, behavior, psychiatric, hyperreflexia, incontinence, auditory brainstem response (ABR), and respiratory (history of pneumonia). The total score reflects the sub-totals of the major and minor domains [30]. Additionally, all subjects had at least one systemic manifestation of NPC1,

such as clinically detectable hepatomegaly and/or either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels above the normal range, clinically detectable splenomegaly, or impaired respiratory function due to NPC1. Female subjects of childbearing age had to have a negative pregnancy test prior to treatment and were required to use contraception during the study. Subjects with NPC2 mutations were excluded, as were subjects with grade 3 renal impairment or evidence of acute liver disease. Subjects using miglustat were permitted to enter the study provided that their dose was stable for at least 3 months prior to entry.

Subjects were scheduled to receive 7 intravenous infusions of HPβCD (Trappsol® Cyclo™, a proprietary formulation of HPβCD, Cyclo Therapeutics, Inc., at 1500 mg/kg [low-dose] or 2500 mg/kg [high-dose]; 25% diluted in 1000 mL) over 8–9 h every 2 weeks for a 12-week period, followed by further evaluation for 14 days after the final infusion (Week 14). Drug was administered using peripheral venous catheters. Subjects weighing >100 kg were infused a maximum volume of 1250 mL, and infusion times were lengthened to approximately 10–11 h.

The study was conducted at a single site in the US but was open to subject enrollment on an international basis. A Safety Review Committee met periodically to review AEs, study progress, and laboratory results. Informed consent was obtained from all subjects or their legal guardians prior to the initiation of treatment or the performance of assessments in accordance with the local Institutional Review Board and principles of ethical research according to the Declaration of Helsinki [31].

2.2. Pharmacokinetics

Plasma samples were collected to determine drug concentration and evaluate the time to maximum concentration (T_{max}), maximum observed plasma concentration (C_{max}), volume of distribution, and elimination half-life (t1/2) after the first and seventh (last) doses of HPβCD. Samples were collected at time 0 just prior to the infusion; at 2, 4, and 6 h after the start of the infusion; and at 0.5, 1, 2, 4, 8, and 12 h after the end of the infusion (approximately 20–21 h after the start of the infusion).

Lumbar spinal catheters were placed at the time of the first infusion to obtain sequential timed CSF samples for pharmacokinetic analysis. CSF samples after the first dose were collected at the start of the infusion (0 h, Baseline), 4 h after the start of the infusion, at the end of the infusion (approximately 8–9 h from the start), and 4 h after completion of the infusion (approximately 12–13 h from the start). Lumbar puncture was performed at the end of the seventh infusion (approximately 8–9 h from the start) to obtain the final CSF sample. All samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Medpace Bioanalytical Laboratories [MBL], Cincinnati, OH).

2.3. Pharmacodynamics

Plasma samples were collected at Baseline and at Weeks 2, 4, 8, 12, and 14 to assess inflammation (cathepsin S, lysozyme) by immunoassay (MBL) and lipid metabolism (plasma lysosphingomyelin-509, now more accurately referred to as *N*-palmitoyl-*O*-phosphocholineserine [PPCS]) by LC-MS/MS (Centogene, Rostock, Germany). CSF biomarkers of neurodegeneration and inflammation, including total Tau, interleukin-8, tumor necrosis factor-α, and glial fibrillary acid protein (GFAP), were measured by immunoassay (MBL) at Baseline and following the seventh dose of HPβCD. Serum cholesterol precursors (lanosterol, lathosterol, and desmosterol) and cholesterol metabolites/bile acid precursors (4β-, 24(S), 25-, and 27-hydroxycholesterol) were measured at Baseline and on Days 2, 3, 5, 8, 11, and 15 post doses 1 and 7 by gas chromatography (GC)-MS. The relative acidic compartment volume of peripheral blood mononuclear cells (PBMCs) was measured using the LysoTracker assay (Oxford University) assay at Baseline and on Day 15 post doses 1 and 7. Unesterified cholesterol was measured by fluorescence microscopy of filipin-stained sections of liver tissue obtained at Baseline and 2 weeks post dose 7. Liver biopsies were performed using standard ultrasound-guided needle or transjugular catheter procedures, and the tissue was flash-frozen in liquid nitrogen. Tissue sections (8 μm) were stained with either hematoxylin and eosin or filipin III (filipin; Sigma, St. Louis, MO, USA) or phosphate buffered saline (Oxoid, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All samples were digitally scanned using a Zeiss Axio Scan whole fluorescence slide scanner and Zeiss ZEN Lite software (Carl Zeiss Microscopy, Jena, Germany). Study-specific algorithms were developed to define weak, moderate, strong, and maximum intensity filipin staining using either the Halo® image analysis platform (Indica Labs, Albuquerque, NM, USA) or a qualitative assessment of mild, moderate, or marked change in filipin staining compared to Baseline was performed by a qualified technical reader at Histologix Ltd. (Nottingham, UK).

Exploratory assessments included hepatic elasticity, a measure of liver stiffness related to tissue composition [32] measured by ultrasonography at Baseline and Week 14, as well as filipin staining in skin tissue at Baseline and Week 14. Skin tissue obtained using a standard punch biopsy procedure was flash-frozen and processed using the same procedure as for liver tissue.

2.4. Clinical outcome measures

Clinical outcome measures were assessed at Baseline and Week 14 using the 17D-NPC-CSS total and individual domain scores. Liver and spleen sizes were assessed by ultrasound using the longest two-dimensional axis measurement and reviewed by a central reader.

2.5. Safety outcome measures

Subjects underwent routine local laboratory assessments, including complete blood counts, comprehensive metabolic panels, liver and renal function panels, creatinine kinase MB isoenzyme (CK-MB), C-reactive protein (CRP), prothrombin, international normalized ratio (INR), and urinalyses performed at Baseline and periodically throughout the study. Automated total cell counts (white and red blood cells) were performed on CSF samples obtained following doses 1 and 7, as per protocol. A 24-h urine collection was obtained at Baseline and Week 14 to measure urinary hydroxyproline as a biomarker of bone turnover (MBL).

Adverse events and concomitant medications were recorded at each visit. As hearing loss is associated with NPC natural disease progression, it is one of the 17 domains measured in the 17D-NPC-CSS, which is scored 0–5 based on the pure tone average across all measured frequencies (ranges tested, 0.5 to 8 kHz). Subjects underwent audiologic testing using behavioral assessment or the auditory brainstem response (ABR), if needed, at Baseline and at Weeks 2, 4, 6, 8, and 14 and as required for AE assessments. It should be noted that grade shifts in hearing may not result in a significant change in the pure tone average that changes the hearing score in the 17D-NPC-SS.

2.6. Statistical analysis

As this was an exploratory Phase 1 study with a blinded, randomized, parallel-group design, no formal statistical analysis was conducted. Instead, descriptive statistics are presented here. The sample size was determined on a practical basis with a plan to recruit 12 subjects randomized 1:1 to receive one of the two dose levels (1500 mg/kg or 2500 mg/kg; six subjects per dose level). The study population was described by demographics, baseline characteristics, medical history, and concomitant medications to assess the comparability of treatment groups using descriptive statistics and data listings.

Efficacy was evaluated on all randomized subjects (Intent-To-Treat [ITT] population). The Per Protocol population (PP) included all subjects

who completed the study and did not have major protocol deviations. Summary statistics included N, mean, median, standard deviation (SD), minimum and maximum for continuous data, and count and percentage for categorical data.

The pharmacokinetic analyses included subjects with sufficient data to enable estimation of key parameters (e.g., T_{max}, C_{max}, t1/2), with subjects grouped according to the treatment dose received. Individual and mean plasma and CSF HPβCD concentration versus time data were tabulated and plotted by dose level. The plasma and CSF pharmacokinetics of HPβCD were summarized by estimating T_{max}, C_{max}, and volume of distribution. It was not possible to measure the half-life in CSF at the last time point measured, as it was at the maximum or close to maximum concentration. For pharmacodynamic assessments, descriptive statistics and change from baseline measurements were used.

3. Results

3.1. Subject demographics and baseline disease characteristics

Subject accrual began in October 2017 and was completed in February 2020. The baseline demographics and disease characteristics of the 13 enrolled study subjects are summarized in Table 1. Subjects had a mean age of 35.7 years, and most were male (61.5%) and white (84.6%). The high-dose group was on average 20 years younger than the low-dose group (26 vs 46 years). Overall disease features were similar in both dose groups as determined by the total 17D-NPC-CSS: both dose groups were in the mild to moderate range in disease severity. Eleven (85%) of the 13 subjects had some degree of hearing loss at Baseline. Three (23%) subjects had a history of familial hearing loss that was not associated with NPC (two were siblings whose mother had otosclerosis, wore a hearing aid, and had a family history of hearing loss at young age due to otosclerosis; and one subject had a family history of hearing loss of unknown etiology). Two (15%) subjects wore hearing aids (both in the low-dose group, 1500 mg/kg, one with moderate and one with severe hearing loss), and an additional two (15%) subjects tested in the range for hearing aids (moderate hearing loss) at Baseline (one each in the low- and high-dose groups) and were due to receive hearing aids. Additionally, slight to mild hearing loss was noted in 3 (23%) subjects in the low-dose group and 4 (31%) subjects in the high-dose group. Two (15%) subjects had normal hearing at study entry, both in the low-dose group. Three (23%) subjects were receiving treatment with miglustat at the time of enrollment: 1 subject in the low-dose group for 4.6 years, and 2 subjects in the high-dose group for 6.9 years.

3.2. HPβCD safety profile

Both dose levels of HPβCD showed an acceptable safety and tolerability profile with no clinically significant events, changes, or trends noted across safety labs, physical examinations, vital signs, or electrocardiograms considered related to study treatment. Urinary hydroxyproline levels were unchanged from Baseline to the end of the study (data not shown), suggesting no effect on bone turnover for either dose group. Of the 13 subjects enrolled, 10 (77%) completed the study (6 in the low-dose group and 4 in the high-dose group). Three (23%) subjects discontinued prematurely: 1 after the first dose due to hypersensitivity pneumonitis, and 2 after multiple doses due to transient changes in hearing.

A total of 44 AEs were reported in the study, with 13 in the 1500 mg/kg group and 31 in the 2500 mg/kg group (Table 2). Of these, 40 were treatment-emergent adverse events (TEAEs), with 13 in the 1500 mg/kg group and 27 in the 2500 mg/kg group. The most common TEAE was hearing loss/reduction (deafness) occurring in 6 (46%) subjects: 1 subject (16.7%) in the 1500 mg/kg group and 5 subjects (71.4%) in the 2500 mg/kg group. The next most common TEAEs were vomiting, headache, and hematuria, which occurred in two subjects each (one in each dose group for vomiting and hematuria, both in the 1500 mg/kg group for headache). A total of 8 serious adverse events (SAEs) in 4 subjects were reported in the study, all in the 2500 mg/kg group.

Two subjects in the 2500 mg/kg group experienced a Grade 3 hearing loss (change from Baseline of 25 dB averaged over 3 contiguous frequencies) that was temporally related to the study drug infusion; the loss resolved within 2 weeks post-infusion in both subjects. After two further doses in both subjects, Grade 3 hearing loss was observed again, both of which improved significantly within 2 weeks. Both subjects were withdrawn from the study. The transient hearing loss in both subjects was at predominantly higher frequencies, and neither subject perceived a change in hearing. One of these subjects wore hearing aids prior to study enrollment.

One subject in the 2500 mg/kg group experienced hypersensitivity pneumonitis and a concurrent Grade 3 hearing loss with the initial dose. This subject was removed from the study, and 3 months later, the pulmonary symptoms resolved completely, and the hearing was

Table 1
Demographics and baseline disease characteristics.

		HPβCD 1500 mg/kg ($N = 6$)	HPβCD 2500 mg/kg ($N = 7$)	Total ($N = 13$)
Age (years)	Mean	46.7	26.3	35.7
	(range)	(20–69)	(18–40)	(18–69)
Sex	Male n (%)	4 (66.7)	4 (57.1)	8 (61.5)
	Female n (%)	2 (33.3)	3 (42.9)	5 (38.5)
Race	White n (%)	6 (100)	5 (71.4)	11 (84.6)
	Asian n (%)	0	2 (28.6)	2 (15.4)
	American Indian/Alaska Native n (%)	0	0	0
	Black/African n (%)	0	0	0
	Native Hawaiian/Pacific Islander n (%)	0	0	0
	Other	0	0	0
	Multiple	0	0	0
Ethnicity	Hispanic or Latino n (%)	0	1 (14.3)	1 (7.7)
	Not Hispanic or Latino n (%)	6 (100)	6 (85.7)	12 (92.3)
Hepatosplenomegaly		5	5	10
Hepatomegaly-only		0	1	1
Splenomegaly-only		1	1	2
17D-NPC-CSS	Mean (SD)	15.2 (4.49)	17.7 (7.36)	16.5 (6.10)

17D-NPC-CSS, 17-Domain Niemann-Pick disease Type C-Clinical Severity Scale; HPβCD, 2-hydroxypropyl-β-cyclodextrin (Trappsol® Cyclo™); SD, standard deviation.

Table 2
Summary of adverse events by treatment.

Parameter	HPβCD 1500 mg/kg ($N = 6$) n (%)	HPβCD 2500 mg/kg ($N = 7$) n (%)	Total ($N = 13$) n (%)
Total Number of AEs	13	31	44
Total Number of TEAEs	13	27	40
Common ($n \geq 2$) TEAEs by preferred term			
Deafness (hearing loss/reduction)	1 (16.7)	5 (71.4)	6 (46.2)
Vomiting	1 (16.7)	1 (14.3)	2 (15.4)
Headache	2 (33.3)	0	2 (15.4)
Hematuria	1 (16.7)	1 (14.3)	2 (15.4)
Total Number of Serious AEs	0	8	8
Number of Related Serious AEs	0	6	6
Total Number of Serious TEAEs	0	6	6
Number of Subjects with at Least One TEAE	4 (66.7)	7 (100)	11 (84.6)
Number of Subjects with at Least One Related TEAE	0	5 (71.4)	5 (38.5)
Number of Subjects with at Least One Severe (Grade ≥ 3) TEAE	0	4 (57.1)	4 (30.8)
Number of Subjects with at Least One TEAE Leading to Treatment Discontinuation	0	3 (42.9)	3 (23.1)
Number of Subjects with at Least One TEAE Leading to Death	0	0	0
Number of Subjects with at Least One Serious TEAE	0	3 (42.9)	3 (23.1)

AE, adverse event; HPβCD, 2-hydroxypropyl-β-cyclodextrin (Trappsol® Cyclo™); TEAE, treatment emergent adverse event.

back to baseline other than mild loss at very high frequencies. Another subject in the 2500 mg/kg group had hearing loss at the end of the study and an upper respiratory infection (URI) and congestion at that time; the audiology assessment was consistent with conductive rather than sensorineural hearing loss. This hearing loss also improved back to Baseline 4 weeks later. All AEs of hearing loss were detected by protocol-mandated audiometry and were not perceived by the subjects themselves or their families.

To summarize the results on hearing based on the 17D-NPC-CSS, 6/10 (60%) subjects who completed the study had hearing that was unchanged at Week 14 compared to Baseline (4 in the low dose group and 1 in the high-dose group; note that one subject in the low-dose group had Baseline scores of 4 in one ear and 3 in the other, while at the end of the study had a score of 4 in both ears); 3 (30%) subjects had worsened hearing scores compared to Baseline (all in the high-dose group, including the subject with the URI at time of assessment that reverted to baseline post-URI; note that none of these subjects required a change in their hearing aids during the trial period); and 1 (10%) subject had improved hearing scores compared to Baseline (low-dose group).

3.3. HPβCD pharmacokinetics in plasma and cerebrospinal fluid

HPβCD showed similar plasma pharmacokinetic profiles after the first and seventh infusions, with a mean t1/2 of 2 h and mean T_{max} of 6 and 9 h after the start of the infusion, respectively, followed by a rapid decrease in concentration after the end of the infusion (Fig. 1A). Based on the C_{max} (1500 mg/kg: 1.96 to 2.07 mg/mL; 2500 mg/kg: 2.66 to 2.45 mg/mL) and the area under the concentration-time curve estimated from time zero to infinity ($AUC_{0-\infty}$; 1500 mg/kg: 18.1 to 19.5 mg·h/mL; 2500 mg/kg: 22.5 to 22.4 mg·h/mL), systemic exposure was predicted to increase on average 1.2- to 1.5-fold for a doubling in dose. However, the increase in systemic exposure was less than dose-proportional across the dose range studied. The geometric mean terminal t1/2 was 1.93 to 2.38 h, the geometric mean clearance (76.9–112 mL/h/kg) was similar to the glomerular filtration rate (125 mL/h/kg), and the geometric mean volume of distribution at steady state (Vss) ranged from 236 to 268 mL/kg. The coefficient of variation for clearance and Vss was between 10.0% and 27.7%.

Albumin was not detected in the CSF pharmacokinetic samples, indicating no contamination from peripheral blood. HPβCD was first

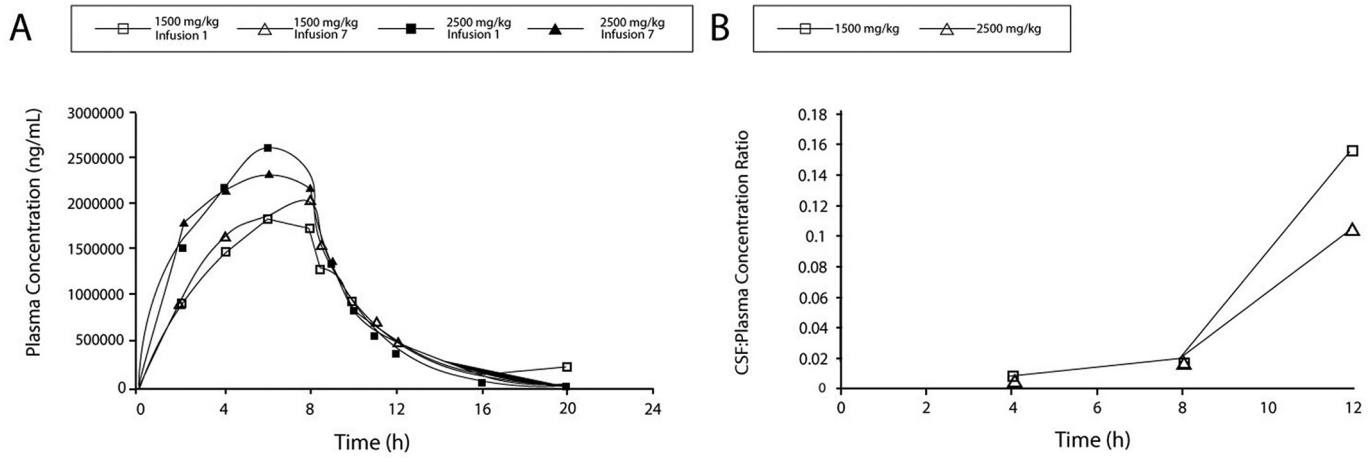

Fig. 1. HPβCD had a 2-h half-life in plasma and persisted longer in cerebrospinal fluid (CSF).
Mean HPβCD plasma concentration (ng/mL) curves over time (hours) after the first (squares) and seventh (triangles) infusions with low (1500 mg/kg; clear symbols) and high (2500 mg/kg; filled symbols) doses (A). Ratio of HPβCD concentration in the CSF and plasma over time (hours) after infusion with low (1500 mg/kg; squares) and high (2500 mg/kg; triangles) doses (B).

detected in the CSF 4 h following the start of the infusion (first timepoint measured) for both dose groups. The highest CSF concentrations measured were 48.3 μg/mL (mean at 12 h, 33 μM) for the 1500 mg/kg group and 37.8 μg/mL (mean at 12 h, 25 μM) for the 2500 mg/kg group. CSF samples were not collected after this time point, precluding the ability to calculate a t1/2 for the drug in CSF. The HPβCD concentration ratio between CSF and plasma increased from 1.7% at 8 h post start of infusion to between 11% and 16% at 12 h post start of infusion (Fig. 1B), suggesting that HPβCD persisted in the CSF for several hours after the IV infusion had ended.

3.4. HPβCD effect on peripheral tissue cholesterol

The pharmacodynamics of HPβCD was assessed by filipin staining for cholesterol in liver tissue sections and by serum measures of cholesterol homeostasis. All 10 subjects who completed 7 doses of study drug and had paired liver biopsies at Baseline and the end of study treatment showed reductions in filipin staining (Fig. 2A). Subjects had different degrees of reduction in filipin staining (Fig. 2B). All subjects who received the high-dose showed marked reduction, while in the low-dose group the reduction in filipin staining was more varied, from minimal to marked (Fig. 2C). Filipin staining in skin tissue was inconclusive with respect to change between Baseline and the end of study treatment. LysoTracker staining of PBMCs did not show a clear trend (data not shown).

Additionally, serum levels of lathosterol, a marker of whole-body cholesterol synthesis, decreased within days of the first HPβCD infusion, reaching a low of 54.1% ($n = 6$) of the baseline value of 1.687 mg/L at Week 1, Day 3 (Fig. 3A). The mean serum lathosterol level decreased with repeated infusions, but to a lesser extent. After the last infusion, the mean serum lathosterol levels were at 81.3% ($n = 10$) of the baseline levels, and then fluctuated around or above the baseline value. In contrast, serum levels of 4β-hydroxycholesterol, a marker of cholesterol catabolism, increased after the first infusion to a high of 175.5% (101.08 μg/L) of the baseline value of 61.57 μg/L at Week 1, Day 5 (Fig. 3B). This pattern was also observed to a lesser extent after the last infusion, with an increase in the mean metabolite level from 82.4% of the baseline value at Week 12, Day 2, to 105.1% at Week 12, Day 8. There was no evidence of any dose-relationship in these changes. Subjects also had a considerable and prolonged reduction in plasma PPCS (lysosphingomyelin-509) levels, the acyl-phosphorylcholine metabolite of sphingomyelin, which accumulates secondarily in NPC disease as a result of excess cholesterol storage in lysosomes. The lowest value, at Week 12, was 46.8% of the baseline value of 5.9 ng/mL (Fig. 3C).

3.5. HPβCD effect on the CNS

Serum levels of 24(*S*)-hydroxycholesterol (24(*S*)-HC), a cholesterol metabolite produced in the CNS that is transported across the blood-

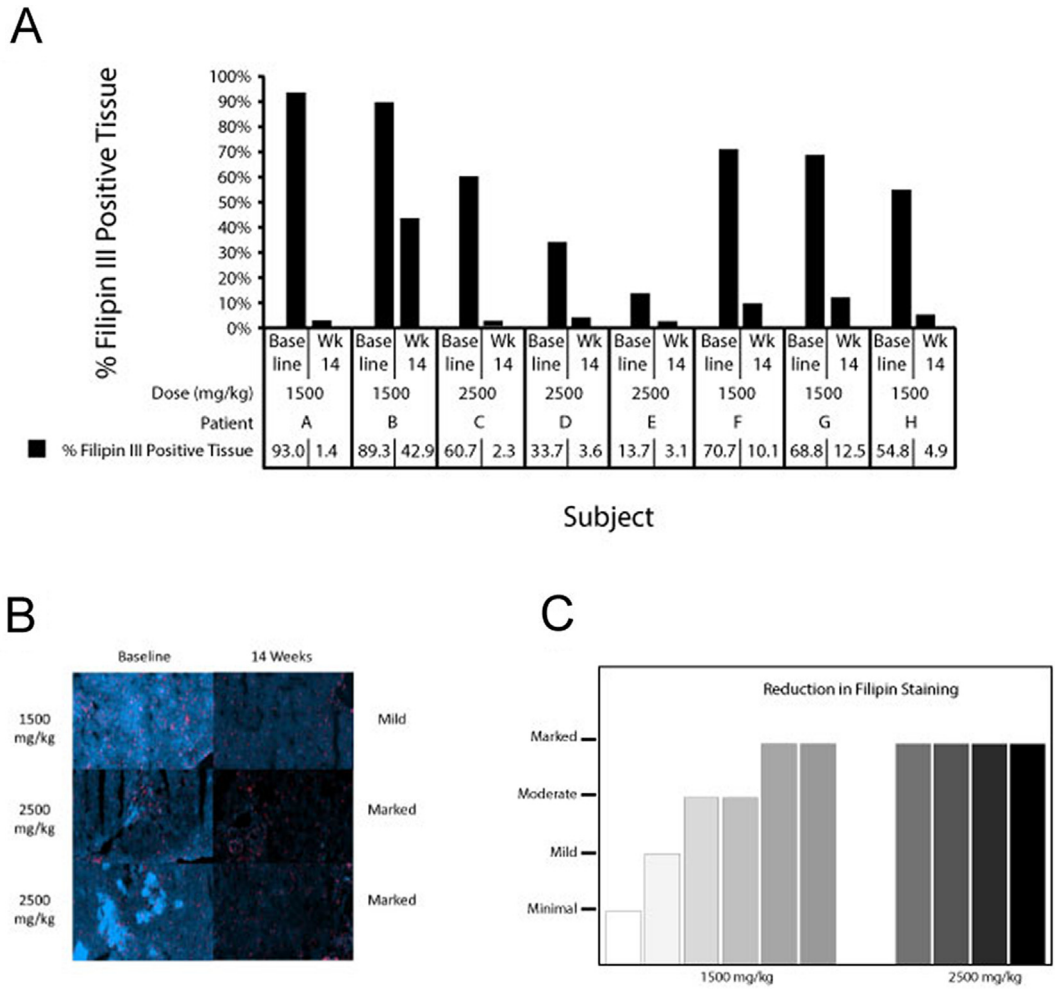

Fig. 2. Release of trapped liver cholesterol following treatment with HPβCD.
The percentage of filipin III (filipin)-stained positive tissue area in liver tissue samples from 8 NPC1 subjects at Baseline and 2 weeks after the seventh HPβCD infusion. Two samples represented in panel C were not available at the time of the quantitative assessment and were not able to be evaluated subsequently (A). Representative images of filipin staining of liver tissue at Baseline and 14 weeks post-treatment with low (1500 mg/kg) and high (2500 mg/kg) doses of HPβCD (B). Level of reduction (minimal, mild, moderate, and marked) in filipin staining for each subject (each bar represents a subject) according to the dose of HPβCD received (C).

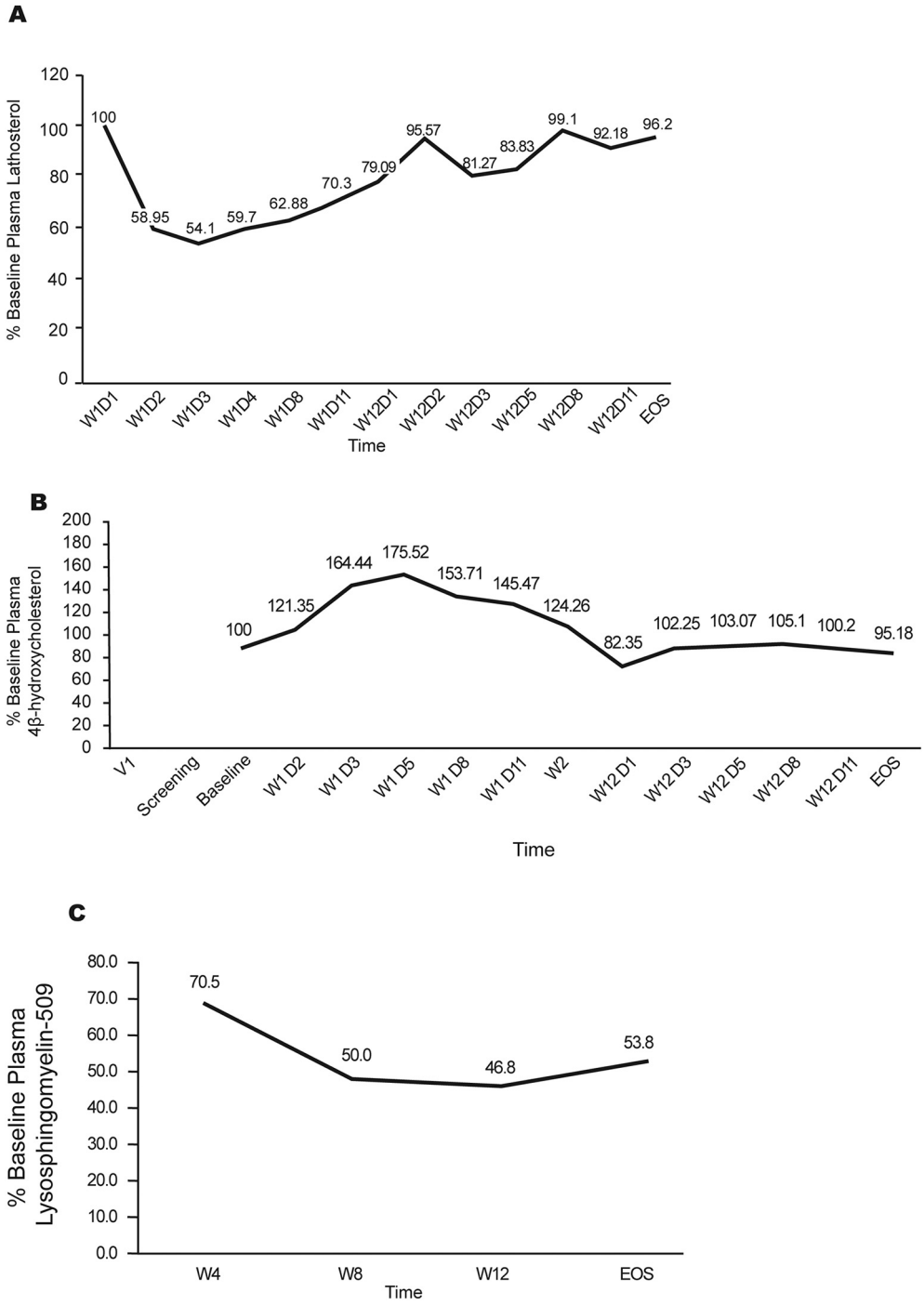

Fig. 3. Trapped cholesterol is released from lysosomes and metabolized following treatment with HPβCD.
Mean serum levels (as a % of the baseline mean value) of lathosterol (A), 4β-hydroxycholesterol (B), and PPCS (lysosphingomyelin-509) (C). Data combined across dose levels. D, day; W, week; EOS, end-of-study.

brain barrier into the circulation, increased following the first infusion with HPβCD, with a peak in the mean serum concentration at 128.02% of the baseline value of 60 ng/mL ($n = 13$) on Week 1, Day 3 (Fig. 4A). After the final dosing, the next time point of observation, a similar pattern of increase was observed albeit with a smaller peak.

Although a dose-response relationship could not be established, there was a tendency towards a post-treatment reduction in total Tau levels in the CSF in 6/10 (60%) subjects with the mean level increasing after the first infusion and decreasing after the last infusion in both dose groups (Table 3). Of the 10 subjects, 7 had a significant reduction, 1 had a mild reduction, and 2 had an increase in total Tau levels after 7 doses of HPβCD (Fig. 4B). Nine of 11 (82%) subjects for whom data were available showed an increase in CSF total Tau at the end of the first infusion (Table 3).

The mean serum 24(*S*)-hydroxycholesterol (ng/L) level increased in subjects from both dose groups after the first (Week 1 Day 2, W1D2) and seventh (Week 12 Day 3, W12D3) infusions with HPβCD and then returned to baseline levels. The peak following the seventh infusion was reduced compared to that of the first infusion (A). Total Tau levels measured in the cerebrospinal fluid collected from subjects following 7 doses compared to baseline pretreatment (B).

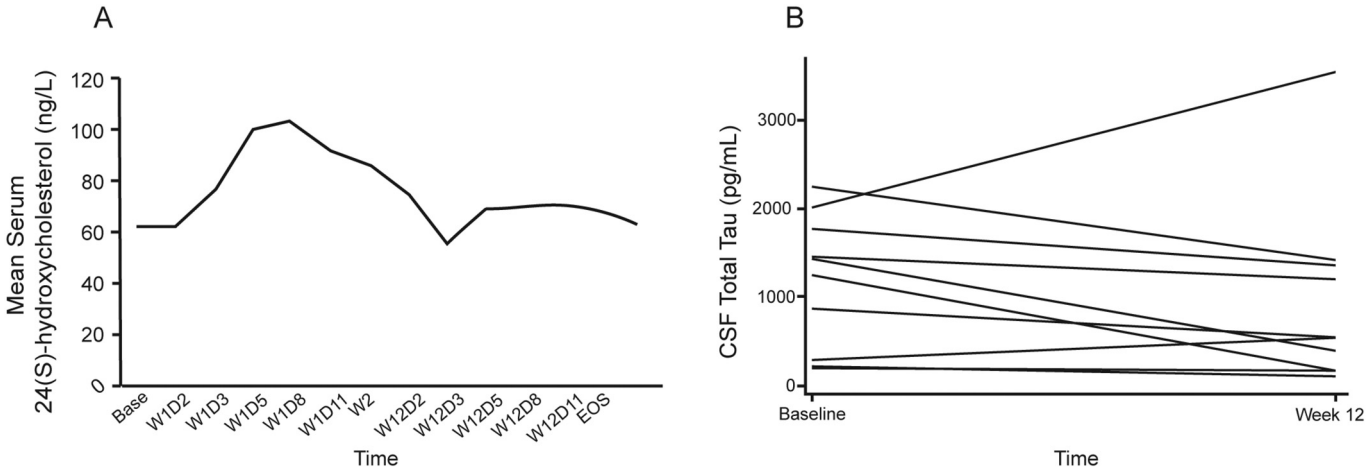

Fig. 4. HPβCD impacts cholesterol metabolism and a biomarker of neurodegeneration, total Tau, in the central nervous system.

3.6. Efficacy outcomes

There was little to no change in the sizes of the livers and spleens of subjects during the course of the study. In the low-dose group, the mean liver size increased from 16.88 cm to 17.55 cm, and the mean spleen size decreased from 16.42 cm to 16.33 cm. In the high-dose group, the mean liver size decreased from 17.34 cm to 17.18 cm, and the mean spleen size decreased from 14.94 cm to 13.50 cm. These changes did not reach statistical significance. Hepatic elasticity was unchanged in both dose groups.

The mean (SD) total 17D-NPC-CSS score decreased from 15.2 (4.49) to 14.8 (4.83) in the low-dose group (n = 6) and increased from 17.7 (7.36; n = 7) to 19.8 (6.34; *n* = 6) in the high-dose group (one subject with a very low score exited the study following dose 1, and no further scoring was done). Collectively, the baseline scores for all subjects averaged 16.5 (6.10; range 8 to 26) for 13 subjects and 17.3 (5.97; range 10 to 26) for 12 subjects at Week 14 or end of study. Three subjects showed improvements in the swallowing domain, 2 in the low-dose group (1 subject with a change of −2 and 1 subject with a change of −1) and 1 in the high-dose group (change of −1). During regular infusion visits, these subjects reported improvements within 24 h of dosing and lasting for up to a week before waning and appearing at the next dosing. These subjects, who reported acute improvements in swallow, also tended to have improvements in speech, though not to levels meeting criteria for a scoring change as measured by the 17D-NPC-CSS. Additionally, individual subjects and/or their caregivers reported improvement in gait as well as increased energy levels, ability to focus, and likelihood of social engagement, observations not able to be assessed on the 17D-NPC-CSS.

Table 3
% Change from Baseline in CSF total Tau levels.

Low-dose (1500 mg/kg)			High-dose (2500 mg/kg)		
Subject number	End of Infusion 1	End of Infusion 7	Subject number	End of Infusion 1	End of Infusion 7
A	+44%	−25%	J	+11%	
B	+43%	−36%	C	+10%	+76%
F	+23%	−42%	D		−72%
G		−36%	E	+10%	−86%
H	+4%	+77%	L	−7.0%	−19%
K	+12%	−4%	M	No change	
			N	+240%	
Mean	+26%	−11%	Mean	+44%	−25%

4. Discussion

The results from this Phase 1 study support the mechanism of action of systemically (intravenous) administered HPβCD in mobilizing intracellular cholesterol stores in subjects with NPC1 as demonstrated previously by preclinical studies [26,27]. Specifically, HPβCD showed positive pharmacodynamic effects in plasma (normalization of cholesterol homeostasis), liver (reduction in stored cholesterol), and CSF (reduction in total Tau), and the drug was well tolerated. As in a previous report [21], there was no significant change in plasma pharmacokinetic parameters or drug accumulation with repeated dosing, and the mean plasma clearance rate was consistent with elimination via renal filtration. Dosing of HPβCD by body weight (mg/kg) is supported by a volume of distribution that was slightly larger than the extracellular fluid compartment, which is proportionate to body weight (20%). HPβCD was detected in CSF at low concentrations (maximum, 33 μM) that persisted longer than in plasma. These results support the rationale for the use of this dosage regimen in future studies.

The dose and frequency of HPβCD administration for this study was determined based on pre-clinical data of *npc1*$^{-/-}$ mice treated subcutaneously with 4000 mg/kg 2HPβCD. At this dose 2HPβCD was cleared from the mice in 24 h [33], and the effective dose 50 (ED50) in mice was approximately 230 mg/kg [34]. It was also demonstrated that the duration of action of 2HPβCD was approximately 7 days [35]. Based on these data and the observation that cholesterol accumulation in humans is 13 times slower when compared to mice [36], as well as in vitro data on potential cellular toxicity at high concentrations of 2HPβCD [37], initial dosing in humans was calculated to achieve a serum concentration between 0.1 mM and 1.0 mM [29]. Pharmacokinetic data obtained in compassionate use patients confirm that a dose of 2500 mg/kg could achieve this serum concentration range, which had been determined in pre-clinical studies to be non-toxic to neurons and peripheral tissues and not to deplete cholesterol stores that would subsequently drive cholesterol synthesis [38]. In order to maximize clinical benefit and safety while considering the tolerability of undergoing 2HPβCD biweekly infusions of 1500 mg/kg and 2500 mg/kg were chosen.

Treatment with HPβCD released accumulated cholesterol from cells in peripheral organs and restored cholesterol homeostasis. Until recently, filipin staining in cultured fibroblasts or a liver biopsy was the gold standard test for diagnosing NPC [1]. With widespread availability of genetic testing, filipin staining is less in use today as a diagnostic tool, but it still holds value as a way to visualize accumulated cholesterol within lysosomes. The reduction in filipin staining of the liver biopsy samples provides direct evidence of the release of trapped cholesterol

from the liver cells of subjects with NPC1. The observed difference between filipin baseline levels in the two dose groups was not considered significant due to the genetic and clinical heterogeneity of the disease. The release of free cholesterol was accompanied by a reduction in the plasma levels of the cholesterol precursor, lathosterol, and an increase in the plasma levels of the cholesterol metabolite, 4β-hydroxycholesterol. That is, cholesterol synthesis was reduced, and cholesterol catabolism was increased, as expected with the restoration of intracellular cholesterol trafficking out of the late endosomal/lysosomal compartment and into other regions of the cells. The return to baseline levels with repeated infusions was expected as cholesterol turnover returned to normal and may be indicative of a decline in stored cholesterol or a slow build-up between infusions. In addition to this effect on stored cholesterol, there was also a consistent reduction in the plasma levels of PPCS (lysosphingomyelin-509), a fatty-acyl derivative of sphingolipid that secondarily accumulates in the plasma of NPC subjects and serves as a prognostic biomarker for disease severity [39]. The normalization of sterol levels in cells may lead to a reduction in cellular damage and functional recovery over time.

A key aspect that impacts the quality of life of subjects with NPC1 is the neurodegeneration caused by the disease. Therefore, any effective drug must have a therapeutic effect in the brain in addition to treating the visceral pathology [6]. Accordingly, the impact of HPβCD in the CNS was assessed in this study. While the volume of distribution was limited, detection of HPβCD in CSF suggests that systemically administered HPβCD has access the CNS. Although it is not clear how HPβCD reaches the CNS, it was detected at low levels in CSF (<0.1 mM) that are in the range of brain tissue concentrations associated with CNS efficacy in animal models of NPC1 [40], including preventing degeneration of Purkinje cells in adult animals [28]. A recent in vitro study using fibroblasts from NPC subjects showed that a 300 μM level of HPβCD affected the expression of over 400 genes [41], further supporting the statement that low levels of the drug in the CNS may underpin the clinical benefits observed in the present study and in compassionate use IV programs described elsewhere [29]. Further, CSF turns over completely on the order of 4 to 5 times daily, suggesting that molecules found in the CSF could persist for greater periods than in plasma [42]. The present finding that HPβCD is detected in the CSF at 4 h after the end of the IV infusion is consistent with its longer half-life in the CSF than in plasma.

Measurements of red blood cells and albumin levels in the CSF samples were made to determine if blood contamination from the lumbar puncture could explain any change in biomarkers or drug levels in the CSF. There was no evidence of blood contamination. Therefore, the HPβCD measured in the CSF was not introduced by the procedure.

Here, we show direct evidence that the drug is present in the CSF following intravenous administration. Whereas the plasma HPβCD levels declined rapidly after the end of the infusion, CSF levels were maintained longer, indicating that the drug persists in the CNS longer than the plasma, which was consistent with the Vss values measured. At low concentrations (<1 mM), such as those found in the CSF, HPβCD acts as a cholesterol shuttle, transporting cholesterol between membranes [25,43]. Therefore, the presence of HPβCD in the CSF, which gives the drug direct exposure to the brain surface even at low concentrations, indicates its potential to release unesterified cholesterol from neuronal cells, which may restore their function or prevent further loss of function. The baseline 24(*S*)-HC serum levels measured in this study (~60 ng/mL) are similar to those reported in the plasma (64 +/− 14 ng/mL) [44]. The rise in this brain-derived cholesterol metabolite (24(*S*)-HC) in serum correlated temporally with the IV infusion, peaking roughly at 3 days post dose for both doses studied before returning to baseline. Our interpretation is that HPβCD is active in the brain, and that it contributes to the reduction of stored cholesterol in the CNS.

Additionally, there was a post-treatment effect in total Tau levels in the CSF in most subjects. Tau is a microtubule-associated protein found primarily in axons of CNS neurons, and it is elevated in the CSF of some subjects with neurodegenerative disorders, including NPC, where its level correlates with neuronal degeneration and loss [12]. We observed an increase in the mean Tau levels after the first infusion, indicating the release of a Tau bolus from neurons. Nine of the eleven subjects for whom data were available showed an increase in some level in Tau at the end of the first infusion. This was followed by a reduction in the mean Tau levels after the last (seventh) infusion. Eight of ten subjects for whom data were available showed a reduction of some level of Tau at the end of the seventh infusion (the low-dose group averaged a reduction of 29%; and the high-dose group of 59%). The downward trend in total Tau levels observed suggests that HPβCD may be reducing the rate of apoptosis and degeneration of cells in the CNS. Subject heterogeneity is likely a key factor in the Tau findings, as some subjects had baseline levels of total Tau in CSF that were in the range of healthy adults (<200 pg/mL in CSF), while others had elevated levels. The observations showing an overall downward trend in CSF total Tau levels following the seventh infusion cannot be explained by concomitant use of miglustat, which has been shown in a pilot study to reduce levels of total Tau in NPC subjects [45], as only three subjects in the present study used miglustat. Furthermore, these 3 subjects had been taking miglustat for more than 4 years prior to the start of the study; therefore, it would be expected that any benefit from miglustat would have been realized prior to enrollment in the present study. This is consistent with results from the pilot study mentioned above where 2 subjects who were already being treated with miglustat at Baseline remained stable with respect to total Tau levels in CSF during the course of the study [45]. In the present study, in addition to the 3 subjects on stable doses of miglustat, 4 subjects not using miglustat showed levels of Tau reduction, compared to baseline, of between 19% and 42%. Finally, the rapid change in Tau levels after the first infusion of HPβCD in most subjects further supports its action on neurons in the CNS, apart from a presumed steady state effect of miglustat in the 3 subjects using that drug. The variability of results suggests that a longer observational period may be useful. In a Phase 1/2 study of HPβCD in NPC1 subjects that used the same administration schedule, reductions in CSF total Tau also were observed in the CSF after 24 and 48 weeks (manuscript in preparation; data presented at WORLD Symposium 2021) [46].

Preclinical studies suggest that little HPβCD penetrates the CNS through the blood-brain-barrier [47]. Presence of a drug in the CSF may also be a measure of drug transport across the choroid plexus, which forms the blood–CSF barrier and is leakier than the blood brain barrier [48]. Regardless of the mechanism of entry of HPβCD into the CSF after systemic administration, a previous study found that direct (intrathecal) administration of HPβCD to the brain did not result in additional improvements over what would have been expected from systemic administration alone, which showed signs of a CNS effect [29]. Therefore, it is possible that systemic administration results in sufficient amounts of HPβCD being present in the CSF to exert a beneficial effect. In this study, subjects in the high-dose group were more likely to experience transient hearing loss, providing further evidence of a CNS effect, even at low CSF drug concentrations. A high sensitivity of the inner ear hair cells to HPβCD, especially at high CSF drug concentration [49,50], may explain why all doses of intrathecally (IT) administered HPβCD (up to 1200 mg) in a Phase 1/2 study were associated with hearing loss as well as clinically significant post-administration fatigue and unsteadiness at doses >600 mg [51], supporting the systemic route of administration as being safer. A subsequent report on the long-term treatment of 3 NPC1 patients with IT HPβCD, through an investigational new drug application for expanded access, also reported permanent hearing loss while maintaining disease stability [52].

As this trial had a short duration and a small sample size, the full clinical effects of treatment could not be evaluated. The overall stability in the 17D-NPC-CSS for most subjects at the end of the study could therefore be related to benefits from the drug or to the short duration of the study. Nevertheless, 3 subjects showed an improvement in the swallowing domain of the 17D-NPC-CSS and reported acute changes in

speech and swallow after each dose. Many of the subjects reported improvements in quality-of-life areas such cognition, socialization abilities (increased focus and social interactions), and motor coordination (improved stance and gait). Considering the short duration of the trial, the clinical efficacy findings should be interpreted with caution.

None of the subjects in this trial experienced clinically significant effects, trends, or clinical safety laboratory findings. Given that another Phase 1/2 trial using HPβCD administered intrathecally led to permanent hearing losses at mid-to-high-frequencies in all participants [51], the present trial included close monitoring for hearing changes. A transient effect on hearing was observed in some subjects, particularly those in the high-dose group (2500 mg/kg). It is important to note that hearing loss is part of the natural history of NPC [4], and that 11 of the 13 subjects who enrolled in this study had hearing loss of some degree at Baseline. Given that 6/10 (60%) subjects who completed the trial had no change in hearing, one subject's hearing improved, and 3 subjects had high-frequency hearing loss that was imperceptible and showed evidence of recovery post dosing, we conclude that HPβCD administered intravenously at the doses studied does not pose a significant risk to subject hearing. Hearing should continue to be monitored in future clinical studies.

The results from this study suggest that intravenously administered HPβCD has the potential to treat both the systemic and neurological manifestations of NPC. Slowing down disease progression through cholesterol mobilization is an important consideration for subjects with established disease, who can expect to experience neurodegeneration without treatment. A natural history study of 18 subjects has shown on average a 1.4-point increase per year in the 17D-NPC-CSS, and a retrospective review of 19 subjects showed a 1.9 point increase per year [30]. Considering the progressive nature of NPC and the short duration of this study (14 weeks), the results presented here are very encouraging as the observed efficacy signals are likely to be only a proportion of the full treatment effect. The visceral effects of IV HPβCD may prove to be especially beneficial to NPC patients approaching liver failure. Indeed, a recent open label trial in NPC infants with liver disease administered low dose HPβCD (250 mg/kg, 500 mg/kg, and 1000 mg/kg) to 3 children (and an additional patient under an emergency investigational new drug study, who expired from the underlying progressive disease) showed encouraging biomarker changes and demonstrated safety in this small cohort [53]. US residents who completed the Phase 1 study and are presented here were eligible to enroll in an extension study, and all eight eligible subjects enrolled (ClinicalTrial.gov number, NCT03893071). Foreign-based participants were offered continuous access to HβCD through compassionate/named subject programs, and all 4 eligible subjects are either currently enrolled or in the process of enrolling. A Phase 1/2, 48-week study (ClinicalTrials.gov number, NCT02912793) has also been completed and the results will be presented in a future publication. Additionally, a multicenter, double-blind, randomized, placebo-controlled Phase 3 clinical trial to evaluate the long-term safety, tolerability, and efficacy of HPβCD is currently enrolling (ClinicalTrials.gov number, NCT04860960).

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Data availability

The data that has been used is confidential.

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