Trappsol®Cyclo™ (HPβCD) for the Long-Term Treatment of NPC1: Efficacy and Safety Data from Four Clinical Studies and the Ongoing Expanded Access Program

Caroline Hastings[1], Fatih Ezgu[2], Roberto Giugliani[3], Beata Kieć-Wilk[4], Lukasz Pawlinski[4], Eugen Mengel[5], Elena Martin-Hernandez[6], Sema Kalkan Ucar[7], Reena Sharma[8], Nicholas Smith[9], Yin-Hsiu Chien[10], Mark Walterfang[11], Moeen AlSayed[12], Ozlem Goker-Alpan[13], Leonardo Oliveira Mendonça[14], Julian Raiman[15], Ronen Spiegel[16], Rita Barone[17], Alberto Burlina[18], Cristian Calandra[19], Jordi Gascón[20], Heidi Peters[21], Orna Staretz Chacham[22], Bryan Murray[23], Andreas Brecht[24], and Joseph Mejia[24]

[1]UCSF Benioff Children's Hospital, Oakland, CA, USA, [2]Gazi University, Ankara, Turkey, [3]Hospital de Clinicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil, [4]Szpital Uniwersytecki w Krakowie, Krakow, Poland, [5]SphinCS GmbH, Hochheim, Germany, [6]Hospital Universitario 12 de Octubre, Madrid, Spain
[7]Ege University Medical School, Izmir, Turkey, [8]Salford Royal Hospital NHS Foundation Trust, Manchester, UK, [9]Women's and Children's Health Network, Adelaide, South Australia, AUS, [10]National Taiwan University Hospital, Taipei, Taiwan, [11]Royal Melbourne Hospital, Melbourne, Victoria, AUS
[12]King Faisal Specialist Hospital & Research Center, Riyadh, Kindgom of Saudi Arabia, [13]Lysosomal and Rare Disorders Research and Treatment Center, Fairfax, VA, USA, [14]Hospital Nove de Julho, Sao Paulo, Sao, Brazil , [15]Birmingham Children's Hospital NHS Foundation Trust, Birmingham, UK, [16]Emek Medical Center, Afula, Israel
[17]University Hospital of Catania, Catania, Italy, [18]Università di Padova, Padova, Italy, [19]Hospital El Cruce, Buenos Aires, Argentina, [20]Bellvitge University Hospital, Barcelona, Spain, [21]The Royal Children's Hospital Melbourne, Victoria, AUS, [22]Ben-Gurion University of the Negev, Beer Sheva, Israel[23]Boyd Consultants Ltd, Crewe, Cheshire, UK
[24]Cyclo Therapeutics, Gainesville, FL USA

Abstract

Background: HPβCD is a well-established compound that accelerates its movement within cells, operating independently of NPC1 and NPC2 proteins. Nonclinical studies and animal models have consistently shown HPβCD's positive impact on cholesterol homeostasis and transport. Studies in the NPC mouse model showed that HPβCD cleared cholesterol from cells including from CNS neurons confirming that HPβCD penetrates the BBB in mature mice. These intracellular effects demonstrate the therapeutic potential of HPβCD as an effective treatment for patients with Niemann-Pick disease type C (NPC), offering hope for patients battling this challenging condition.
The development program for Trappsol® Cyclo™ (HPβCD) represents the largest series of NPC patients treated to date. Phase 1 and Phase 1/2 studies have shown that biweekly IV administration of HPβCD mobilizes intracellular cholesterol stores, supporting its preclinical mechanism of action. These studies have consistently demonstrated signs of efficacy and an acceptable safety profile.

Methods: An ongoing open-label extension (OLE) study is evaluating the long-term effects and safety of HPβCD at 1500 mg/kg IV biweekly, with an additional 20+ patients in an international Compassionate Use Program further substantiating its safety and clinical benefits. A double-blind Phase 3 study is ongoing, assessing the long-term safety and efficacy of biweekly IV HPβCD (2000 mg/kg) compared to placebo.

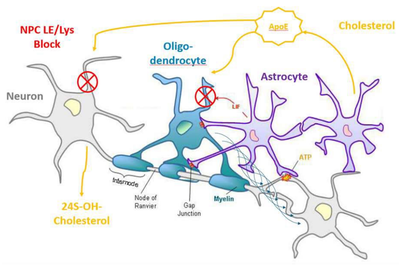
Results: More than 150 patients ages < 1 yr to 65 with mild to severe disease presentation at baseline have been exposed to Trappsol® Cyclo™ across all studies and Compassionate Use, some reaching a cumulative exposure of >5 years of therapy with clinically meaningful effects compared with natural disease progression. Of the OLE, 3 patients remain on treatment with the longest having reached up 1963 days of treatment (Sept 2024).

Conclusion: Data continues to be generated strengthening Trappsol® Cyclo™'s potential benefits from its expected effects on both the systemic and neurological manifestations of the disease, offering a promising therapeutic option for patients.
Contact: Lori J McKenna; +1.508.410.0104; patients@cyclotherapeutics.com; clinicaltrials.gov NCT04860960

Importance of the BBB Penetration in NPC

Overcoming the challenge for therapy delivery over the blood-brain barrier

- Cholesterol metabolism in the CNS is separate from the visceral system
- From a young age, most CNS cholesterol is synthesized by astrocytes and delivered to neurons and oligodendrocytes
- In NPC disease cholesterol delivery to these cells through the lysosomal pathway is defective
- Adequate penetration of blood-brain barrier is critical in therapies addressing diseases with CNS involvement
- Studies in the NPC mouse model showed that Hydroxypropyl-Beta-Cyclodextrin (HPβCD) cleared cholesterol from cells including from CNS neurons indicating penetration of the blood-brain barrier (BBB)
- HPβCD detected in CSF after IV application - confirms penetration of the BBB by HPβCD in humans - leads to increased excretion of cholesterol metabolite from CNS
- Since its inception, the purpose of treating with HPβCD has been to affect the deleterious impact of NPC in the CNS

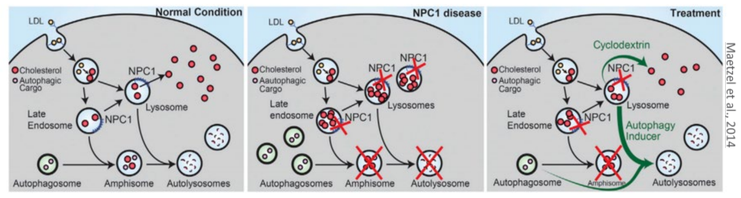


Lanciotti et al., Translational Neuroscience 2013 (modified)

Camargo F. et al., Life Sci. 2001; Liu B. et al., J. Lipid Res. 2008; Davidson C. et. al., PLoS One 2009; Liu B., J. Clin. Lipidol. 2012, Hastings C. et al., Mol. Genet. Metab. 2022

TransportNPC™

Mechanism of Action of HPßCD



Maetzel et al., 2014

- HPβCD is taken into cells via bulk phase endocytosis and into the late endosome/ lysosome (LE/L)
- Selectively allows trapped unesterified cholesterol to be released from the LE/L into the cytosol from where it enters cellular cholesterol processing
- Levels up to 1.0 mM mobilize stored cholesterol in LE/L
- Neurotoxicity/acute cell death at concentrations of > 10mM
- During infusion HPßCD reaches plasma concentration of about 1 mM
- Transfer over the BBB happens by micropinocytosis with a transfer rate of 0.1 – 0.2 µl / g tissue / minute
- Assumed peak concentration of HPßCD in brain tissue is 0.04 – 0.08 mM; confirmed by values obtained in lumbar CSF (0.02 – 0.03 mM)

Administration of HPβCD

Intravenous (IV)

- IV HPßCD (Trappsol® Cyclo™) is well tolerated
- Slow infusion used to control peak level of the drug
- Sufficient transfer across the BBB needs to be achieved
- Data from preclinical studies as well as completed and ongoing clinical studies suggest Trappsol® Cyclo™ has potential to treat both systemic and neurological symptoms of NPC. Data from Cyclo Therapeutics' early phase studies and OLE are currently being confirmed in the Phase 3 Study
- The most comprehensive randomized, placebo-controlled pivotal, phase 3 trial is ongoing with data readout end of H1 2025

Intrathecal (IT)

- Access from CSF to brain parenchyma varies widely, affecting therapeutic outcome and toxicity.
- In the cat model, a significant concentration gradient in the normal brain is observed with values over 80 times higher in the cortex region and over 40 times higher in the cerebellum pointing to an uneven tissue penetration of cyclodextrin towards innermost organ tissues using IT route of administration[1)]
- Dosing at lumbar end of spinal channel leads to concentration gradient towards brain
- **Under compassionate use, no further improvements of Trappsol® Cyclo™ therapy were observed when adding IT to IV [2)]**

Oral

- Bioavailability of Trappsol® Cyclo™ is not sufficient
- Oral administration is not an option

[1)] Kao ML et al., J. Inherit. Metab. Dis. 2019
[2)] Hastings C et al., Orphanet J. Rare Dis. 2019

Abbreviations: IV= intravenous; IT= intrathecal; BBB= blood-brain barrier; OLE= open label extension (study); H1=first half; CSF=cerebrospinal fluid

NPC1 Clinical Trial Program

Cumulative subject exposure within the clinical development program for IV HPβCD in NPC

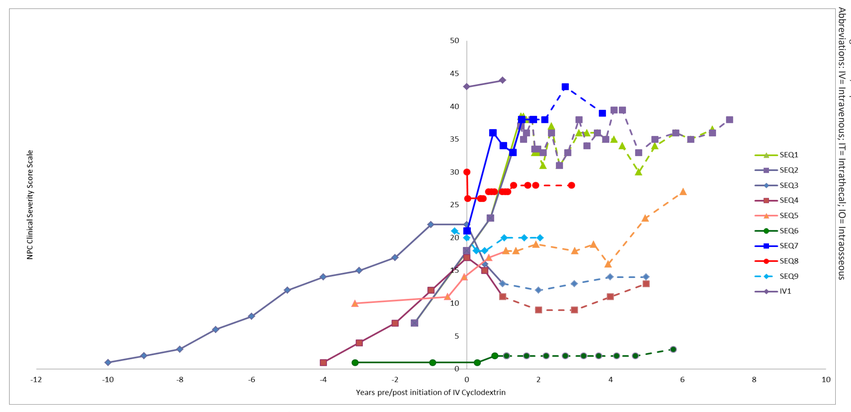
Study	Population	Blinded	HPβCD	Dose of IV HPβCD	Placebo	N of infusions*	Total days of treatment	Total patients
CTD-TCNPC-101	NPC-1 subjects, 18 years of age and older	N/A	13	Randomized to receive either 1500mg/kg or 2500mg/kg	N/A	80	1080	13
CTD-TCNPC-102	NPC-1 subjects, 18 years of age and older	N/A	8	Randomized in a prior study to receive either 1500mg/kg or 2500mg/kg	N/A	283	13,998*	8[a]
CTD-TCNPC-201	NPC-1 subjects, 2 years of age and older	N/A	12	Randomized to receive either 1500mg/kg, 2000mg/kg or 2500mg/kg	N/A	248	3574	12
CTD-TCNPC-301	NPC-1 subjects, 3 years of age and older	94	N/A	Randomized to receive either 2000mg/kg or placebo at a 2:1 ratio	N/A	2655	41,055**	94
	Sub-study in subjects less than 3 years of age	N/A	10	Sub-study is an open-label study to receive 2000mg/kg	N/A	299	4480**	10
International compassionate use program (ICUP)	NPC-1 subjects, pediatric to adult	N/A	23	Dosing regimen varies per individual treating physician	N/A	Incomplete data	Incomplete data	23[b]
Total								152[c]

[a)] These 8 subjects completed the phase 1 (101) study and were then randomized and exposed in the open label extension (102) study: they are not counted twice in the total exposure.
[b)] Cumulatively, 23 patients with NPC have been treated with Trappsol® Cyclo™ through the ICUP/Named Patient Program in different countries across the world. Not all clinicians and patients have consented to share/provide information.
[c)] 8 out of 13 subject who completed the 101 study were also randomized and exposed in the 102 study but were not counted twice in the total exposure.

Abbreviations N/A= not applicable; NPC-1= Niemann-Pick disease Type C1.
*Data on file; Ongoing OLE study. Cut-off date: Jan 2024
**Data on file; Ongoing phase 3 study. Cut-off date: Jan 2024

Studies and Clinical Findings

Clinical observations in 10 patients on HPβCD compassionate use



[1)]Hastings C et al., Orphanet J. Rare Dis. 2019
Abbreviations: IV= Intravenous; IT= Intrathecal; IO= Intraosseous

- For consistency NPC Clinical Severity Scores (NCSS) reported at approximate 6-month intervals
- Only descriptive data shown however statistical significance demonstrated in 4/10 patients
- Retrospective scores were calculated in some patients to establish rate of disease progression
- Each patient served as their own control for comparison of severity scoring
- Patients with severe clinical manifestations (typically NCSS over 30) demonstrate progression of disease with increasing scores over time and then appear to plateau
- Two less severely affected patients had notable decreases in their scores (SEQ3, SEQ4) with initiation of IV therapy. Some progression seen years later. Patients never reached the pre-infusion level of clinical severity scores
- Data suggests no added benefit when IT (or IO) therapy was added to the IV treatment

NPC Clinical Severity Scores: Pre- and post-IV infusion. The center vertical bar represents the time each patient began treatment with IV treatment. Scores obtained prior to treatment initiation are shown to the left of this bar. Solid lines to the right of treatment initiation represent IV only and dotted lines represent the addition of IT treatment for the sequentially treated patients[1)]

Studies and Clinical Findings

Phase 1 study: Clearance of Trapped Liver Cholesterol Following 14 Week of IV Treatment with Hydroxypropyl-Beta-Cyclodextrin

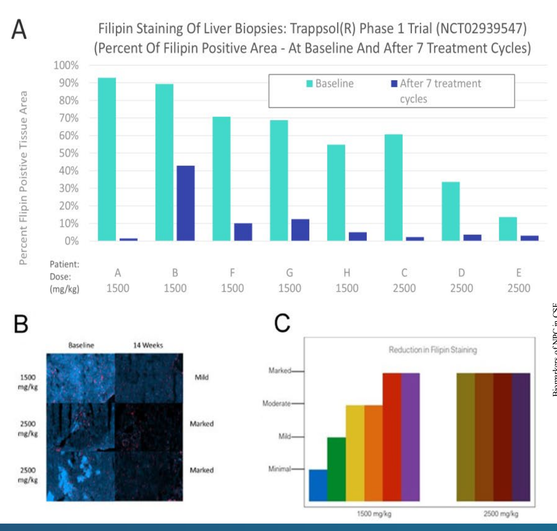
A. Hepatic tissue samples from 8 NPC1 subjects at Baseline and 2 weeks after the seventh HPβCD infusion, showing meaningful effect of hydroxypropyl-beta-cyclodextrin on peripheral tissue cholesterol

B. Representative images of filipin staining of liver tissue at Baseline and after 14 weeks of bi-weekly treatment at low-dose (1500 mg/kg) and high-dose (2500 mg/kg) doses of HPβCD showing overall reductions in filipin staining indicating clearance of cholesterol storages

C. 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

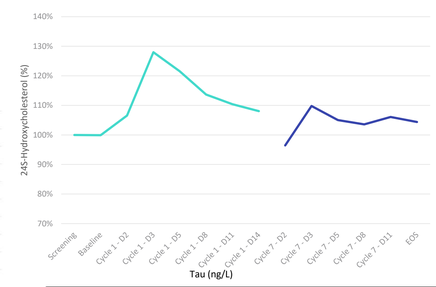
Further results* observed over 14 weeks of treatment (marked after infusion # 1, attenuated after infusion # 7):
- Reduction in cholesterol synthesis (liver)
- Increase in cholesterol metabolism (liver)
- Increase in cholesterol metabolism (brain)

*Data associated with further results not shown here



Hastings C. et al., Mol. Genet. Metab. 2022

Phase 1 study: Impacting CNS markers in NPC patients

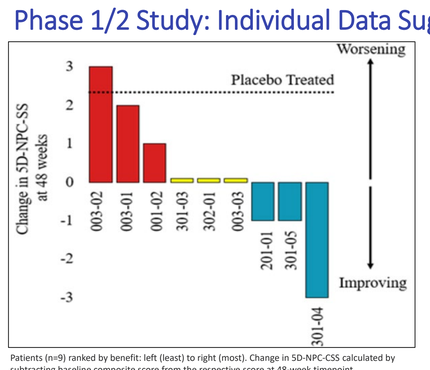


The mean serum **24S-hydroxycholesterol** (%) level increases in NPC patients following IV administration of HPβCD, signaling effect on CNS cholesterol metabolism. Shown here are data after 1st infusion cycle (week 1) and 7th infusion cycle (week 13) [1)] . Note: In humans the majority of 24S-hydroxycholesterol is produced in the ER of CNS neurons; cholesterol stored/trapped in the endo/lysosomal compartment is not accessible for this transformation [2)]

Tau levels as measured in the CSF are shown here for 10 NPC patients who had lumbar punctures prior to treatment with HPβCD and after seven doses. Six of 10 patients showed a reduction in Tau levels, two remained stable, and two increased, no dose-response relationship [1)]

[1)] Hastings C. et al., Mol. Genet. Metab. 2022
[2)] Mahley R., Arterioscler. Thromb. Vasc. Biol. 2016
Abbreviations: SE=standard deviation; D=day; EOS=end of study

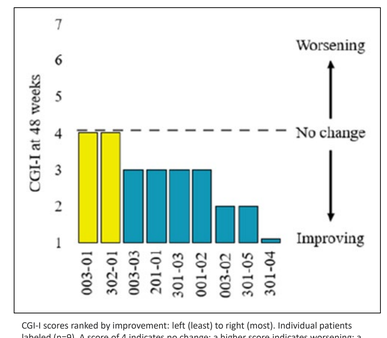
Phase 1/2 Study: Individual Data Suggestive of Potential Clinical Benefit



Patients (n=9) ranked by benefit: left (least) to right (most). Change in 5D-NPC-CSS calculated by subtracting baseline composite score from the respective score at 48-week timepoint. (based on Mengel et al, 2021)

Data Included

- 9 completers (2 patients in the 1500 mg/kg group; 4 patients in the 2000 mg/kg group; 3 patients in the 2500 mg/kg group).
- Three patients were excluded from this analysis due to 1 only having baseline data and 2 patients discontinued prior to Week 48 for non-safety reasons.



CGI-I scores ranked by improvement: left (least) to right (most). Individual patients labeled (n=9). A score of 4 indicates no change; a higher score indicates worsening; a lower indicates improvement.

Key Outcomes

- In a disease characterized by linear / steady disease progression, treatment with Trappsol Cyclo resulted in the stabilization or improvement in 6 of 9 (67%) completer patients on the 5D-NPC-SS
- 8 of 9 (89%) completer patients showed improvements over disease progression (2.15 points per annum based on Mengel E. et al., 2021) in the 5D-NPC-SS
- 9 of 9 (100%) completer patients exhibited stabilized or improved CGI-I ratings, a clear indicator of efficacy in NPC-1 patients

Sharma R., Hastings C. et al., Mol. Genet. Metab. Rep. 2023
Abbreviations: 5D-NPC-CSS= 5-domain Niemann-Pick disease type C clinical severity scale; CGI-I= Clinical Global Impression –Improvement

Clinical Trial Program (former and ongoing)

Current Progress of the Phase 3 Study*

Core Study (>3 years of age)		Sub-Study (0 – 3 years of age)	
Age range in years (mean)	3 – 65 (20.2)	Age range in years (average)	0y7mo – 2y7mo (1y8mo)
Male	49	Male	8
Female	45	Female	2
Randomized	94	Enrolled	10
Ongoing	60	Ongoing	8
End of treatment (EOT)ϕ	6	End of Treatment (EOT)ϕ	2
Transitioned to OLE (of these before week 96)	28 (6)		
No Miglustat	17	No Miglustat	7
ϕ Non-safety related withdrawals			

Core Study (>3 years of age) ITT at 96wks	
January 2025	29
February 2025	33
March 2025	37
April 2025	38
June 2025	39
August 2025	43
September 2025	50
October 2025	53

*Data on file, cut-off date: Dec 2024
Abbreviations: ITT= Intent to treat; OLE=Open label extension

Summary of AE/SAE: Phase 1, Phase 1/2, Phase 3 (main) and 102 (OLE)

Severity	Total	Mild / Grade 1	Mod / Grade 2	Severe/ Grade 3	Life-Threat / Grade 4	Fatal / Grade 5	Serious Adverse Events
Phase 1	44	18	17	9	0	0	8
OLE*	67	38	19	6	1	3	13
Phase 1 / 2	203	169	19	13	2	0	15
Phase 3**	1115	839	229	47	0	0	47
Phase 3 Sub-study**	107	82	24	1	0	0	19
Total AE and SAE	1536	1146	308	76	3	3	102

Abbreviations: AE= adverse events; SAE= serious adverse events; OLE= Open-label extension study; Life-Threat= life threatening
*Data for OLE is based on available medical records
**Safety data for phase 3 main and sub-study, cut-off date: Dec 2024

Summary of AE/SAE for Sub-Study Only*

Adverse Event Summary	Total	Mild / Grade 1	Moderate / Grade 2	Severe / Grade 3
Total Number AEs	107	82	24	1
Considered Possibly Related or Related by the Investigator	1[1]	1	0	0
Serious (SAE)	19[2]	11	8	0
Considered Possibly Related or Related by the Investigator	0	0	0	0

[1]-Just 1 AE considered as being Possibly Related; i.e., vomiting
[2]-19 non-study treatment related SAE's

Serious Adverse Events (SAE)		
Mild	**N = 11**	RSV bronchiolitis; Gastroenteritis / rotavirus x 2/ campylobacter, Fever x 3; Post surgical bleed (tonsillectomy), Seizure exacerbation;
Moderate	**N = 8**	Fever; Lymphadenitis; Gastroenteritis, UTI; URI; Malnutrition x 2 ; Parainfluenza infection
Severe	**N = 0**	

Abbreviations: AESI= adverse events of special interest; UTI= urinary tract infection; URI= upper respiratory infection
*Cut-off date: Dec 2024

Conclusions and Ongoing Efforts

- Intravenous administration of Trappsol® Cyclo™ (HPβCD) reaches both peripheral and central (crosses the BBB) compartments
- The liver showed a significantly increased level of stored unesterified cholesterol at baseline. It was down to normal levels after 14 weeks of treatment indicating a significant mobilization of lysosomal liver cholesterol over 14 wks
- The mean serum 24S-hydroxycholesterol (%) level increases in NPC patients following IV administration of HPβCD, signaling effect on CNS cholesterol metabolism
- In a disease characterized by steady disease progression, treatment with HPβCD in the phase 1 / 2 resulted in stabilization or improvement in 6 of 9 (67%) completer patients on the 5D-NPC-SS
- An OLE study (open to US patients who completed the phase 1 study) of the long-term safety and efficacy of IV HPβCD in NPC is ongoing. Three patients remain with the longest having reached 296 weeks of treatment as of January 8th, 2025
- With over 5 years of data for those continuing the OLE study, individual patient data for the 5D-NPC-CSS has demonstrated a possible reduction in disease progression
- Despite the progressively neurodegenerative disease course of NPC and the time commitment required of both patients and families to remain actively involved in the 96-week Phase 3 trial, only 6 percent of patients have withdrawn from the study for non-safety related reasons including 1 non-study treatment related death
- Use of HPβCD in the trial program has reliably demonstrated an acceptable safety profile with the majority (94%) of the adverse events considered mild or moderate in severity. Three fatal outcomes occurred in the OLE, none considered related to study drug
- Overall, the study treatment in the Phase 3 study has been well tolerated across all age ranges with an AE/SAE profile consistent or similar with that of the completed Phase 1 and Phase 1/2 studies as well as the pediatric sub-study and the other ongoing study programs (OLE and ICUP)
- In addition to the clinical studies, the program continues to offer expanded access to patients globally in the US, Europe, Asia, South America, Australia and Israel with more than 20 patients having received or are still (N = 11) receiving treatment to date
- A 48-week comparative interim analysis of our pivotal, phase 3 study is planned for the end of first half of 2025