Fifteen years from discovery to the bedside: hydroxy-propyl-β-cyclodextrin, where to now?

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Disclosures

- Cyclo Therapeutics, Inc. PI for Phase I trial; senior clinical advisory/safety monitoring board for international Phase I/II trial; Global and site investigator for Phase III trial; development of rater tools for clinical trials
- Orphazyme: Scientific Advisory Board; ReachMD educational programming
- NNPDF: Scientific Advisory Board, grant reviewer
- Australian NPC Foundation, Board member
- UPenn Orphan Disease Center: NPC grant reviewer
- Go4TheGoal: Grant reviewer

Translation of Preclinical Science to Clinical Evaluation

- Early understanding of MOA from preclinical studies
- Application in humans: route/dose/frequency and toxicities
- Does MOA in animals and humans correlate?
- Correlating biomarkers and clinical disease (progression and response)
- Understanding potential targets to restore cellular homeostasis including lipid transport and autophagy
- Genetic and phenotypic variability: Can we predict timing to symptom onset, rate of progression, absolute severity, and determine optimal time for intervention?

NPC: A Debilitating Disease with Fatal Outcomes

- Rare, fatal and progressive genetic disorder affecting notably the brain, liver, spleen and lungs.
- Defect in the NPC1 (95% of patients) or NPC2 (5%) protein affects cholesterol transport
- Accumulation of lipids (cholesterol, sphingomyelin, sphingosine and glycosphingolipids) within the endosome
- Impaired intracellular lipid trafficking in major tissues and organs
- Extreme clinical heterogeneity with variable hepatic, pulmonary, neurological, and psychiatric manifestations at presentation, including auditory loss
- Major impact on QoL

Incidence

1/100,000 (~35 per year in U.S.)

Age at Time of Diagnosis

~ 3% are age 3 and below
~ 97% are age 3 and above
~ 60% age 16 and above

Median Survival

Early Infantile (2m-2): 4.6y

Late Infantile (3-6): 9.4y

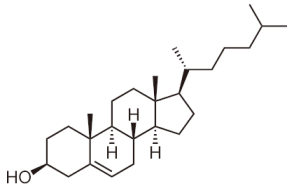
Juvenile (7-15): 15.4y

Adolescent/Adult (16+): 12.2y

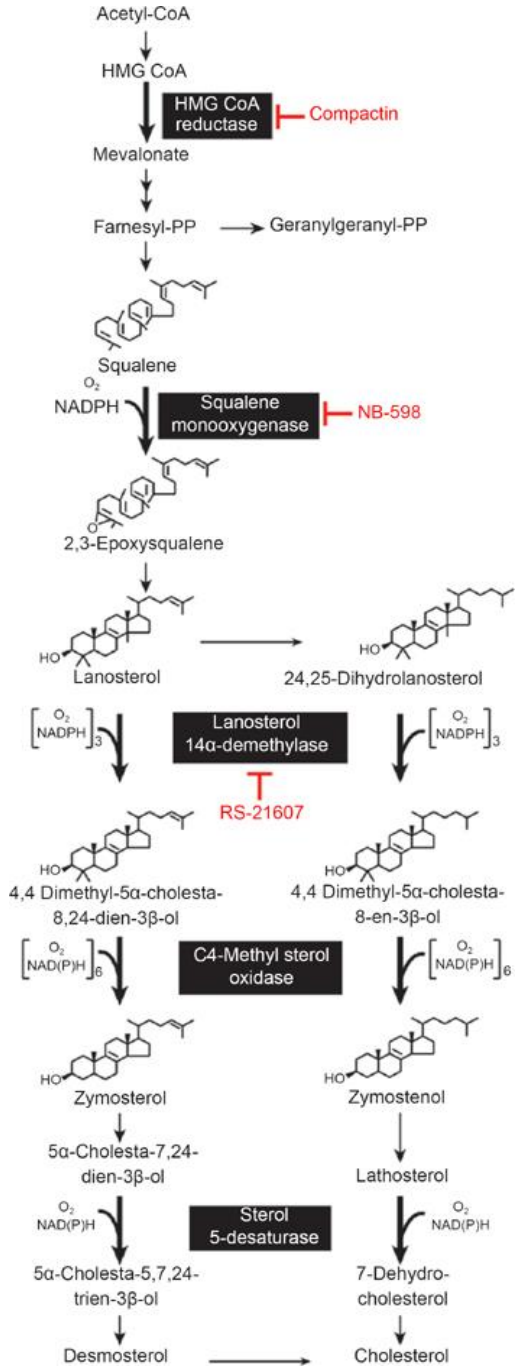
0 U.S. Approved NPC Therapies

1 EU Approved Therapy with no systemic effects

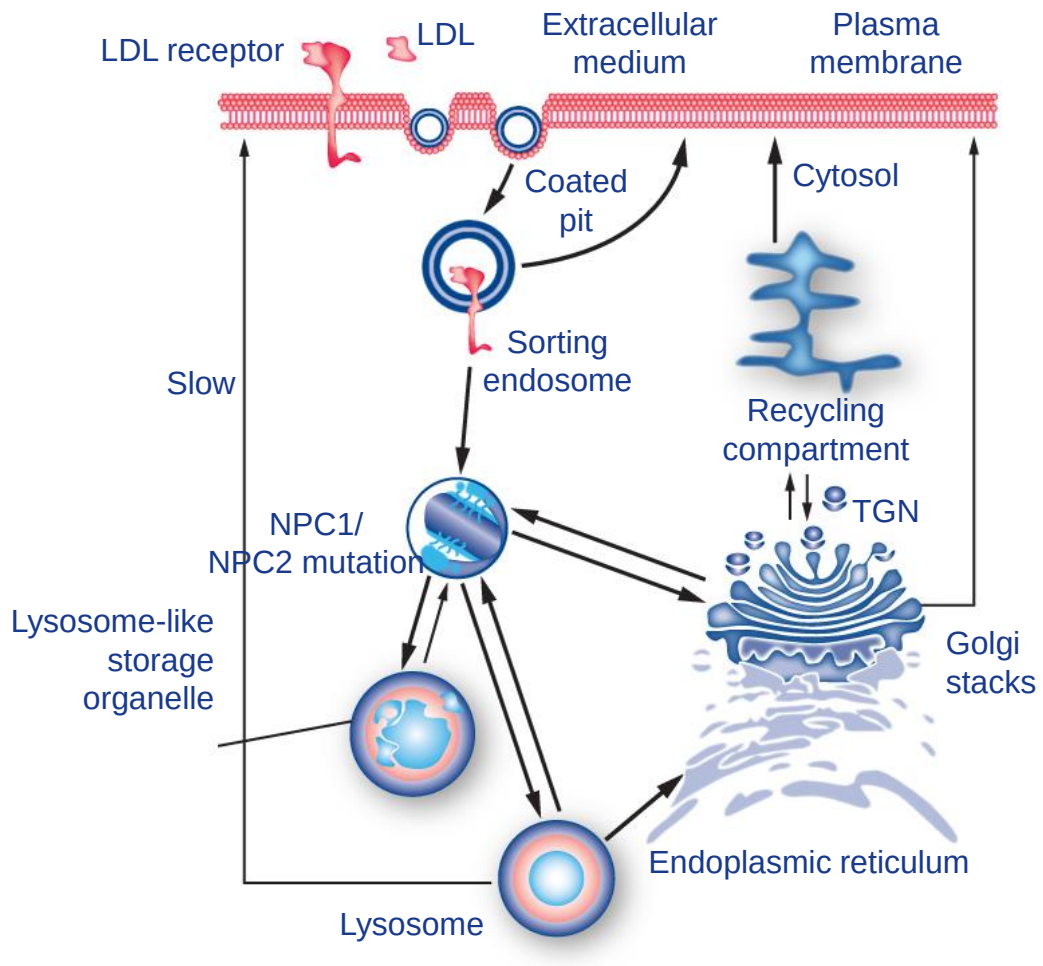
Cholesterol is Essential for Life



- Required to build and maintain cell membranes
- Used for synthesis of steroid hormones and bile acids
- Mammalian cells can make all the cholesterol they require
- Regulated process based upon the size of the metabolically active cholesterol pool within the cytosol
- Synthesis increased when cells sense a cholesterol deficiency
- Synthesis decreased when there is excess cholesterol in the cell



The Role of NPC1 and NPC2 in Cholesterol Transport



LDL = low-density lipoprotein

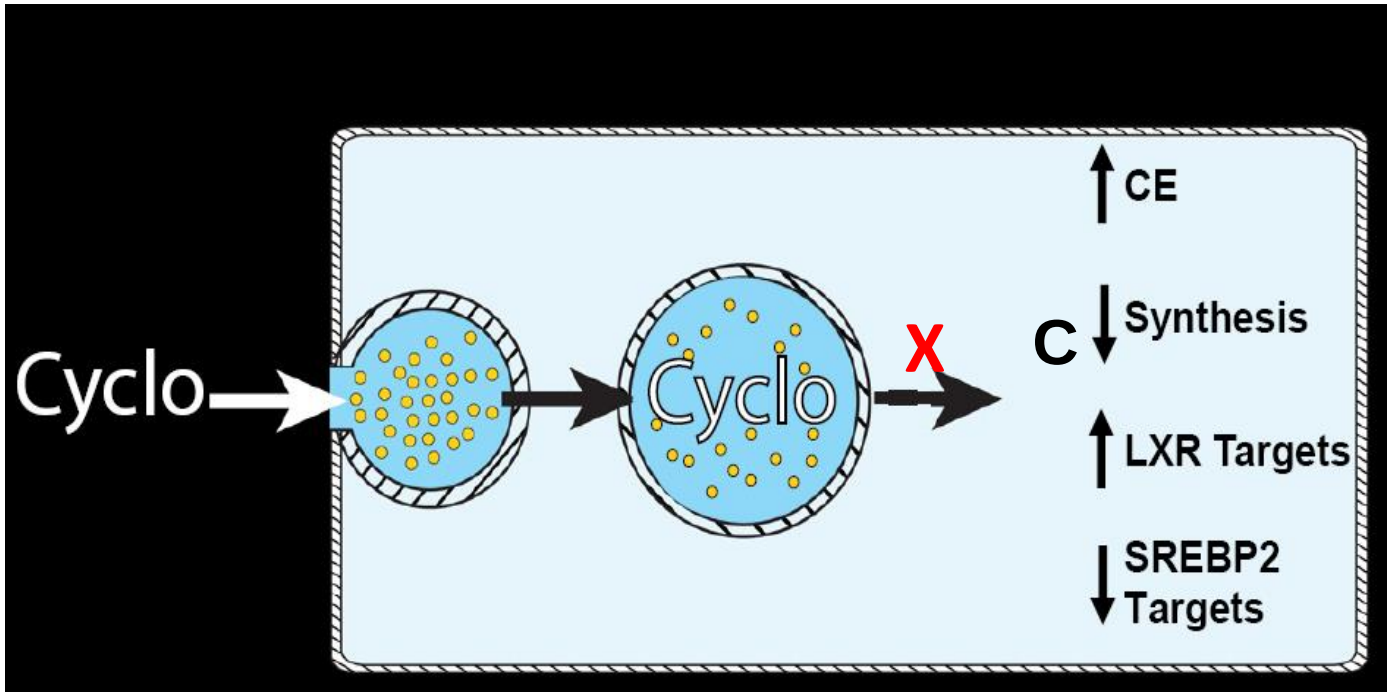
TGN = trans-Golgi network

Cyclodextrin – A Serendipitous Discovery

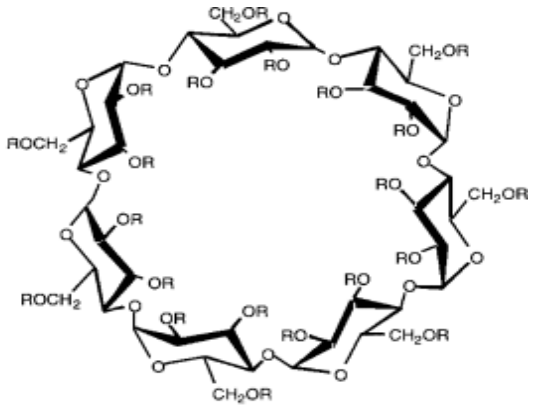
- Cyclodextrin extracts cholesterol from cell cultures
- 2005: Ahmad/Erickson report single injection allopregnanolone (in 20% solution of β-CD) delays myelination and enhances survival of NPC mice (based on Griffin/Mellon 2004)
- 2008: Liu/Dietschy; 2-HPβCD (not allopregnanolone) leads to prolongation of life and decrease in total cholesterol burden in all systems in7 day old npc1-/- mice
- 2009-: Multiple studies in mice and cats substantiating response with variable ages, dosing regimens, length and frequency, and routes of administration of 2-HPβCD

Proposed Mechanism of Action of HPβCD

- HPβCD is taken into cells via bulk phase endocytosis and into the late endosome/ lysosome
- Selectively allows the trapped unesterified cholesterol to be released from the late endosome/lysosome into the cytosol where it is then normally metabolized
- Levels 0.1 – 1.0 mM mobilize stored cholesterol in LE/L
- Functions as a shuttle at plasma concentrations ≤0.1mM, reduces C synthesis
- Extracts cholesterol from cell membranes at doses ≥1.0 mM, drives C synthesis
- Neurotoxicity/cell death at concentrations ≥10 mM



Trappsol® Cyclo™



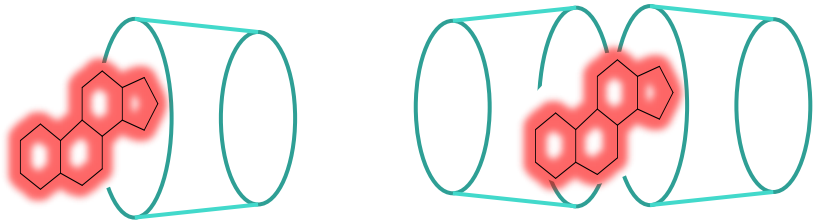
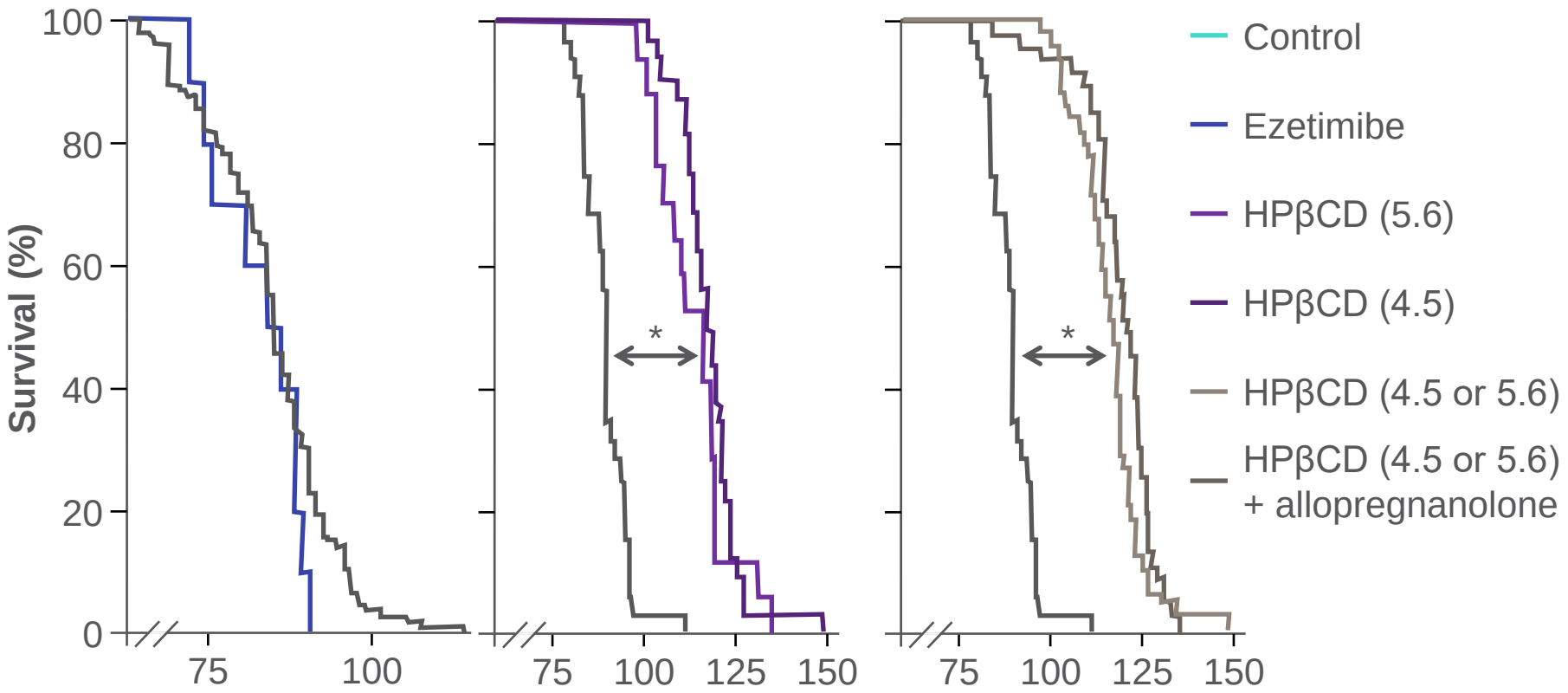


Figure is courtesy of David Begley, Kings College

- Proprietary formulation of hydroxypropyl-beta-cyclodextrin (HPβCD)
- Cyclic oligomer composed of 7 linked glucopyranose units, form hollow cone-like toroid structure
- Hydrophobic cavity, hydrophilic exterior
- Used to deliver drugs insoluble in aqueous solutions: serve as vehicles to improve solubility, stability, and bioavailability of many drugs
- Binds cholesterol – used to extract or enrich cholesterol in cell culture
- Cleared by glomerular filtration in the kidneys and excreted unchanged in the urine

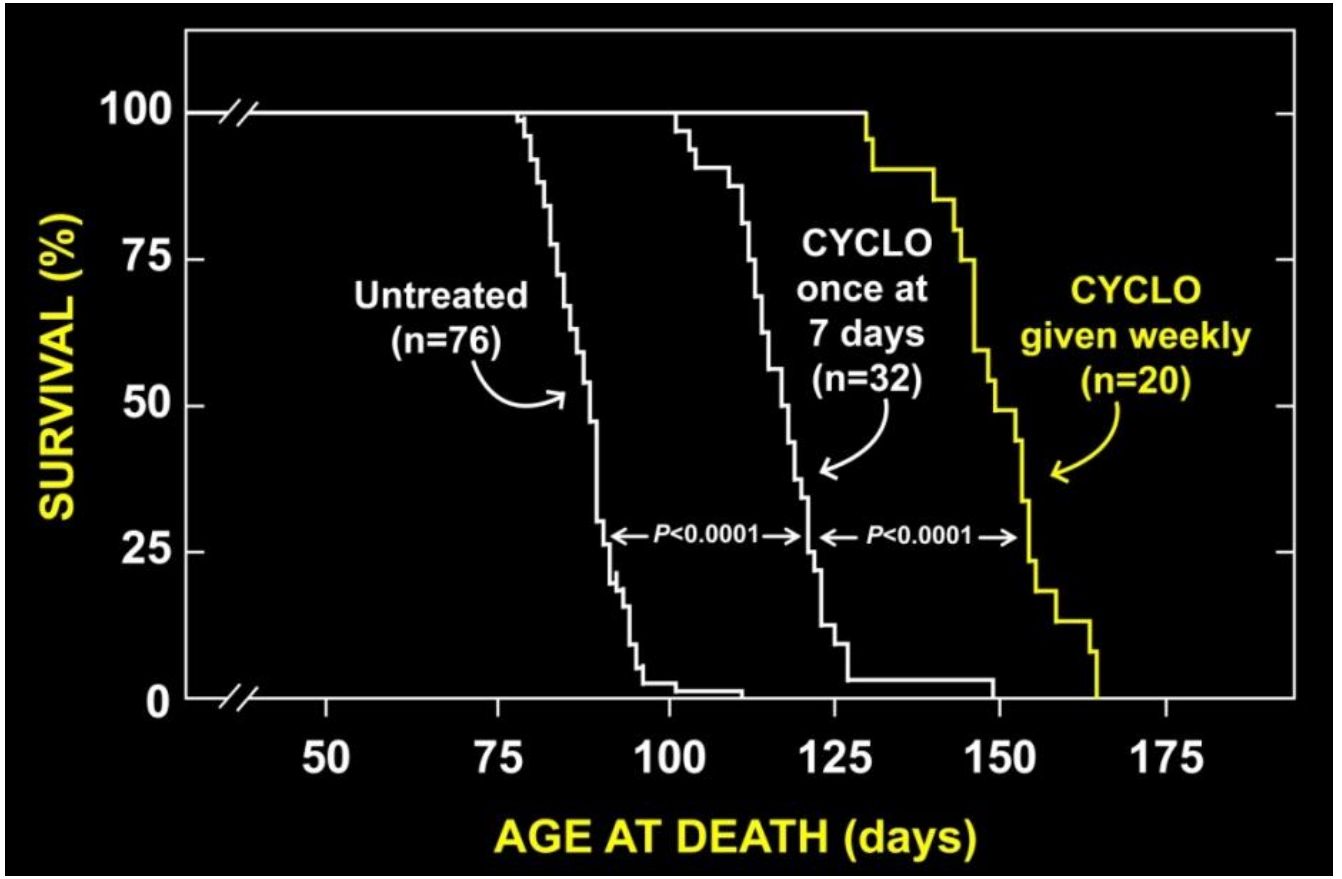
NPC mouse model: Peripheral HPβCD administration: Understanding effects on cholesterol burden and severity of disease



* p<0.0001

Liu et al. Proc Natl Acad Sci U S A 2009;106:2377-82

Weekly HPβCD Prolongs Lifespan in Mouse of NPC



Ramirez CM, et al. Ped Res. Vol. 68.No. 4 2010

SC Injection of HPβCD in Mouse Model

- Prolongs lifespan by 40% in the NPC mouse
- Delayed neurodegeneration, not completely prevented
- Decrease in Purkinje cell loss indication of crossing BBB in infant mice
- 24 hrs after HPβCD injection there is a selective release of the cholesterol trapped within the late endosome/lysosome
- At 24 hrs whole body cholesterol (C) burden is reduced
- At 6 weeks post injection there is further reduction of whole-body C
- There is no effect of HPβCD on *npc1*$^{+/+}$ (WT) mice
- Greater benefit with serial injections: increased longevity, decreased neuronal C/glycosphingolipid storage, normalization C content in tissues, reduction of C pools to normal levels

Initial INDs in the US, application 2008

- Development of formulation for safe human administration
- Goal to achieve similar serum PK as in preclinical model to promote cholesterol mobilization and avoid depletion leading to increased cholesterol synthesis (cholesterol sink)
- Determined dose based on known characteristics of HPβCD, blood volume; measured PK and aimed for **0.1 - 1mM** concentrations (Peake & Vance, J Biol Chem. 2012)
- As per preclinical model, aimed to test both systemic and CNS directed administration, placed Ommaya catheters and measured PK (calculated dose, total CSF volume and $t^{1/2}$ to determine dose) and indeed CSF concentrations were ≤1mM to avoid neurotoxicity. Separate calculations of dose for IT vs IO delivery.
- Measured urine/stool excretion, cholesterol metabolites, CSF Tau/inflammatory markers
- Safety assessments including liver, kidney and lung function, bone resorption, hearing (with CNS tx), neurotoxicity
- Measured clinical progression

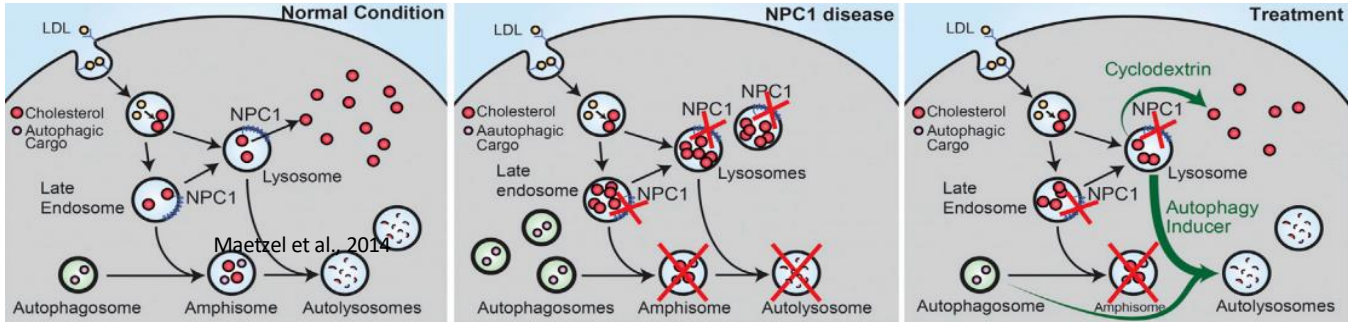
Expanded Access with Intravenous Hydroxypropyl-β-Cyclodextrin to Treat Children and Young Adults with Niemann-Pick Disease Type C1: A Case Report Analysis

Hastings C, Vieira C, Liu B, Bascon C, Goa C, Wang RY, Casey A, Hrynkow S, Orphanet J Rare Dis 2019

- IV HPβCD has been administered to >20 patients worldwide
  - Acceptable tolerability profile amongst patients treated to date
  - Safety profile enabling physicians to continue treatment >8 years
- Individual patients exhibit objective CNS/Systemic responses
  - Reduction in hepatic size and improvement in transaminases
  - Restoration of language skills
  - Resolution of interstitial lung disease
  - Improvement in fine and gross motor skills
  - Improvement of quality of life (communication, focus)
- Clinical experience warrants further investigation of intravenous HPβCD in the management of NPC
  - Treatment of clinical manifestations, systemic and neurologic
  - Halting or slowing the rate of disease progression
  - No added benefit of IT HPβCD (except hearing improvement in our 2 patients!)

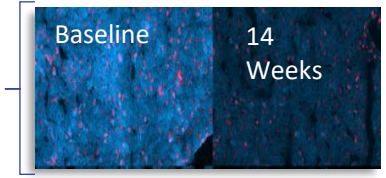
Trappsol® Cyclo™

Enables the Effective Transport of Cholesterol Out of Cells

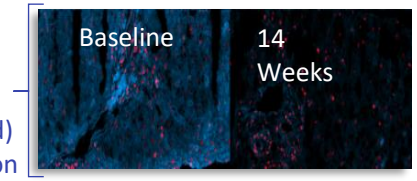


Cholesterol in liver biopsies as measured by Filipin staining at Baseline and after 7 doses over 14 weeks

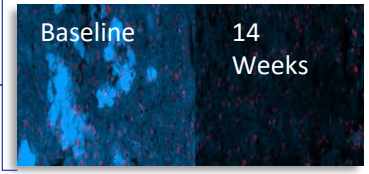
1500 Mg/kg (Mild) Reduction



2000 Mg/kg (Marked) Reduction



2500 Mg/kg (Marked) Reduction

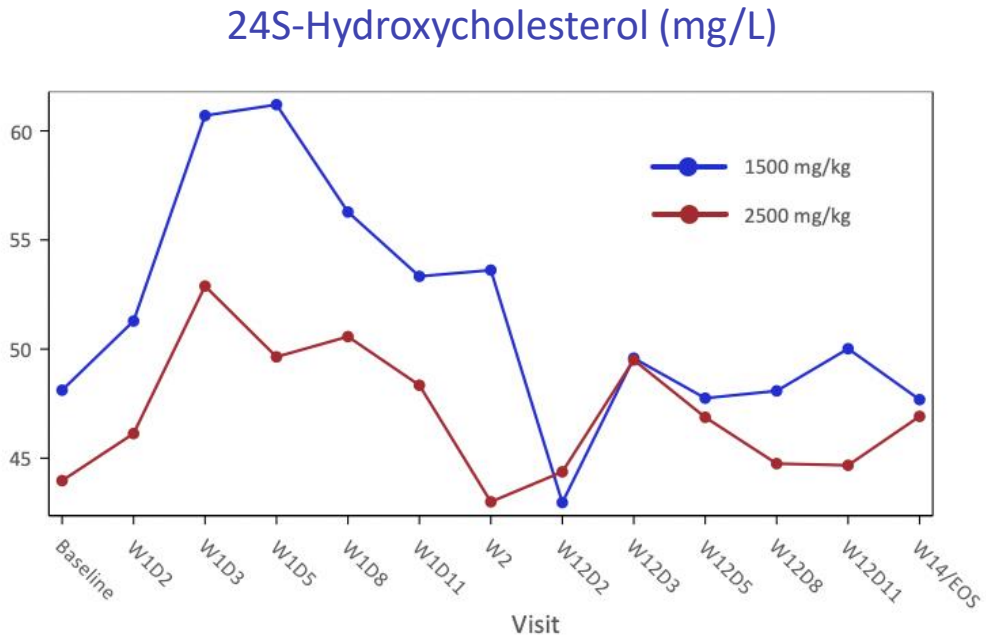


The lack of light blue represents the clearing of cholesterol from cells

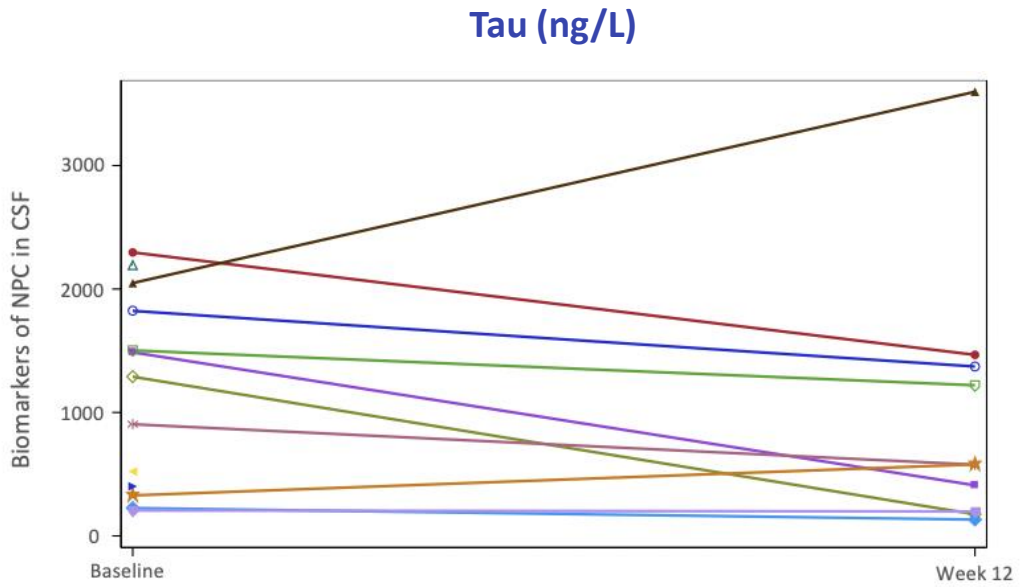
Maetzel et al., 2014
Source : Study 101

Increased Serum 24S-Hydroxycholesterol Levels Signals Removal of Excess Cholesterol From the Brain

- **24S-hydroxycholesterol**, a cholesterol metabolite from the CNS transported across the BBB, plays a major role in maintaining cholesterol metabolism in the brain
- Increases in serum following IV administration of HPβCD. Shown here are data after 1st dosing and 7th dosing.
- Signals effect on CNS cholesterol metabolism.



IV Trappsol® Cyclo™ Reduces Rate of Apoptosis of Cells in the CNS

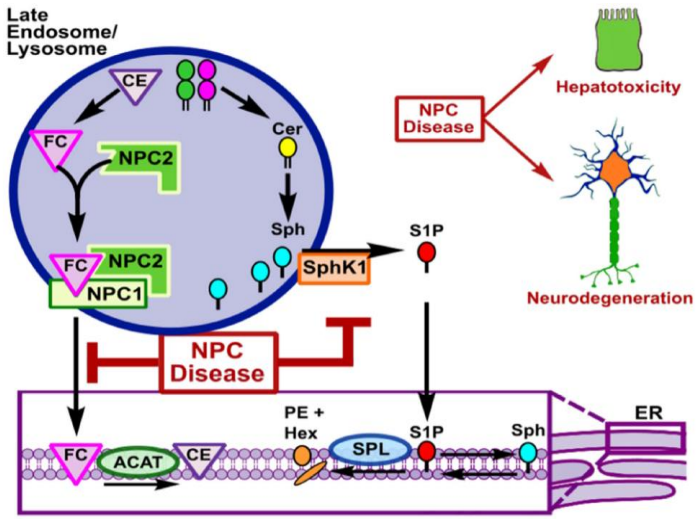


Source: CTD-TCNPC- Study 101

Tau: A protein related to onset and disease progression in NPC

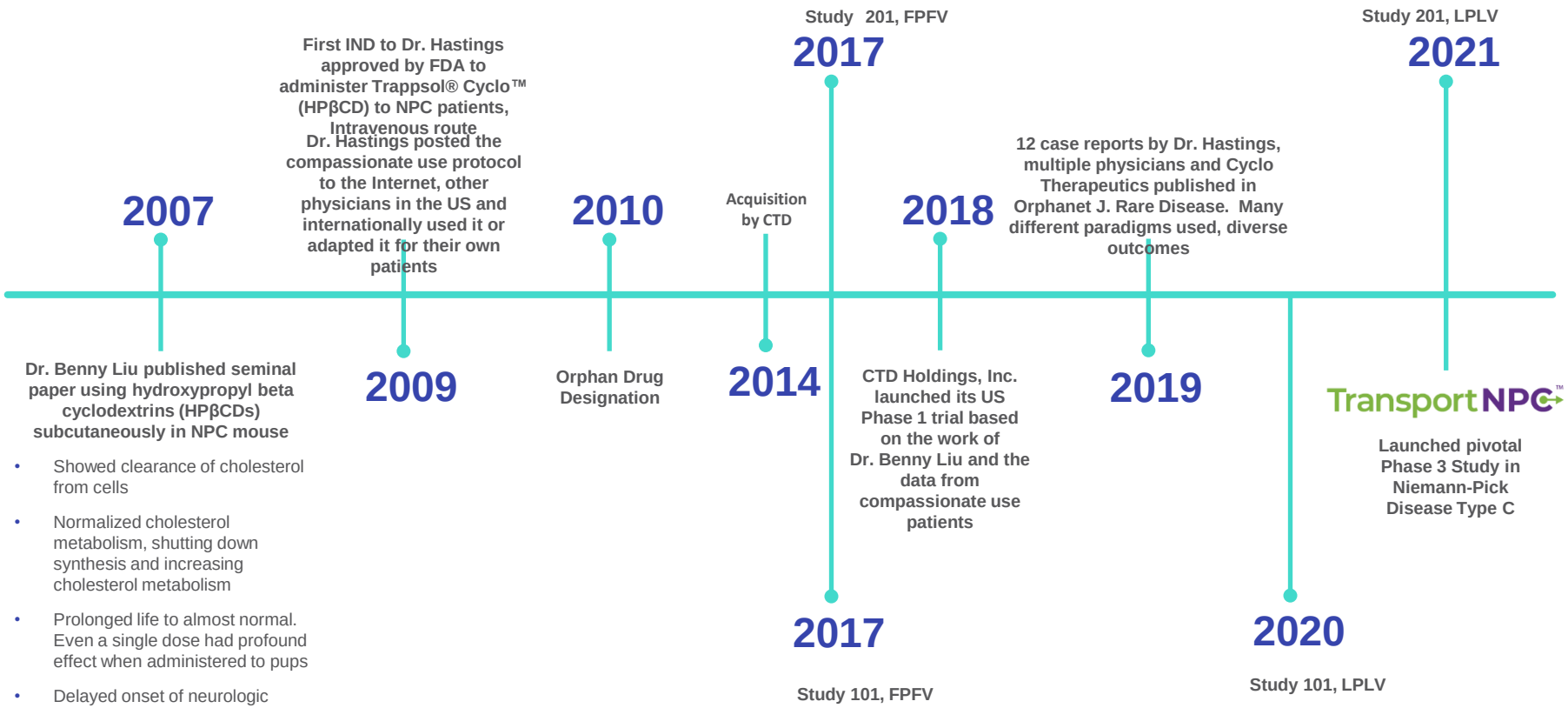
- **Tau** levels as measured in the CSF are shown here for 10 NPC patients who had lumbar punctures prior to treatment with HPβCD and after seven doses.
- 60% of patients had a reduction in Tau levels, 20% remained stable, and 20% increased, no dose response relationship.
- Suggestive of a neuroprotective benefit in CNS

Trappsol® Cyclo™ Targets Primary Pathophysiology of NPC



- Compelling direct and indirect data that Trappsol Cyclo releases accumulated cholesterol from cells in peripheral organs and the CNS and restores cholesterol homeostasis in NPC patients, and correlates with preclinical data
- The marked reduction in filipin staining in liver cells after treatment indicates the clearing of stored cholesterol
- Decrease in the serum level of the cholesterol precursor, lathosterol and increase in the cholesterol metabolite, 4β-hydroxycholesterol
  - Expected feedback mechanisms when the block in cholesterol trafficking relieved, and more cholesterol becomes available for cell metabolism
- Increased serum levels of the brain-specific cholesterol metabolite, 24S-hydroxycholesterol supports Trappsol Cyclo active in the brain and restores the normal export of cholesterol transport across the blood-brain-barrier

Where we are now…



Ongoing Pivotal Phase III Study in Niemann-Pick Disease Type C

TransportNPC™

Double-blind, Randomized, Placebo-controlled, Parallel-group study and
is currently the most advanced clinical research program underway to identify a treatment for NPC

Number of Subjects	93	
Current Sites	23 across 9 countries	United States, United Kingdom, Italy, Germany, Spain, France, Poland, Israel, and Australia
Duration	96-week trial, with Interim Analysis at 48 weeks	
Dose	2000 mg/kg via IV infusion	
Primary Endpoint	NPC Composite Severity Score	
Secondary Endpoints	SCAFI, Swallow, Vineland-2	
Exploratory Endpoints	Inclusive of Speech, Liver and Lung function	

Sub-Study in Patients < 3 years of Age-Trial Design, outside US only

- Sub-study requested by EMA to evaluate Trappsol® Cyclo™ as a potential preventative treatment and is being conducted ex-US only
- Safety and efficacy results from the sub-study to be analyzed separately form the main study cohort

Objective

To evaluate the safety, tolerability, and preliminary efficacy of Trappsol® CycloTM.

Population

Up to 12 subjects <3 years of age with confirmed NPC1, who may be symptomatic or asymptomatic, are eligible to receive open-label Trappsol® CycloTM for up to 4 years

Lessons Learned from preclinical and human studies in NPC exemplified by HPβCD

- Correlation of preclinical studies and early phase trials regarding mechanism of action, effect on cholesterol homeostasis and transport, effect of cellular/tissue concentrations, BBB signaling/permeability
- **Route is important**, differing tissue penetrability
- **Dose is important**, cholesterol homeostasis is dose dependent
- **Tissue concentration is important**…can drive cholesterol synthesis if too much depletion and can lead to direct neurotoxicity if tissue concentration is too high
- Need for therapeutics that treat all systems/tissues
- Benefits and toxicities may be species specific
- Risk vs benefit, what is acceptable?
- Some patients benefit and others do not, why?

Correlation of Biomarkers with Clinical Disease progression and response

- Critical step towards ensuring we have the right drugs and right dosing, routes, and frequency
- Biomarker assays must be easily accessible and reproducible
- Need for precise measurement tools that may need to be further refined for age/severity/affected domains to measure clinically relevant changes
- Do changes in biomarkers parallel changes in clinical response? Can one be a surrogate for the other? Is this necessary for establishment of a new intervention?
- Natural history studies may no longer be possible; confounding variables on assessing this direct relationship; reliance on the registry
- How do we define success in clinical outcomes?
- Remains a challenge

Understanding potential targets to restore cellular homeostasis including lipid transport and autophagy

- Regulation of lipolysis and autophagy are likely interrelated
- Autophagy – intracellular degradation pathway for damaged organelles and toxic proteins, essential for cellular homeostasis
- Autophagy regulates lipid metabolism and alterations in lipid content likely impacts the autophagy pathway
- High concentrations (supra-therapeutic) of HPβCD perturb autophagic flux in NPC1 hepatic cells with likely deleterious cellular consequences (Maetzel, Stem Cell Rep. 2014), consistent with neurotoxic effects on neurons (Peake & Vance, J Biol Chem. 2012), and clinical reports from IT studies

Genotypical and Phenotypical variability: clues to tailored therapy?

- Can we predict timing to onset of disease and subsequent rate of progression?
- Can we predict absolute severity and disease domains most affected?
- Influence of neuro-inflammation
- How can we determine optimal timing for intervention?
  - Newborn screening
  - Early vs late onset of clinical symptoms
  - Timing of diagnosis
  - Opportunity for response (neuronal plasticity, slow further damage/tx before damage becomes apparent/permanent)
- How do we develop tailored therapy for each patient with different approaches for genotype/phenotype expression?
  - Epigenetic modifiers, environmental influences

A Special Thank You

To all of the patients, families and physicians who support Cyclo Therapeutics, Inc. ongoing clinical trials and who provided their data from compassionate use programs early on, making our trials possible.