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PIONEERS IN CYCLODEXTRIN-BASED INNOVATION

GETTING THE BEST OUT OF CYCLODEXTRINS

Cyclodextrins for Treatment of Niemann-Pick disease type C

Niemann-Pick disease

- Niemann-Pick is a rare lipid storage disorder, in which sphingomyelin (a lipid that can be found mainly in the cell membrane of the nerve axon cells) accumulates in lysosomes.
- It is a genetically-inherited disease caused by a deficiency in the lysosomal enzyme acid sphingomyelinase, which causes the accumulation of sphingomyelin in spleen, liver, lungs, bone marrow, and brain, causing irreversible neurological damage.
- There are several types of the Niemann-Pick disease, the most common one is type 'C'.

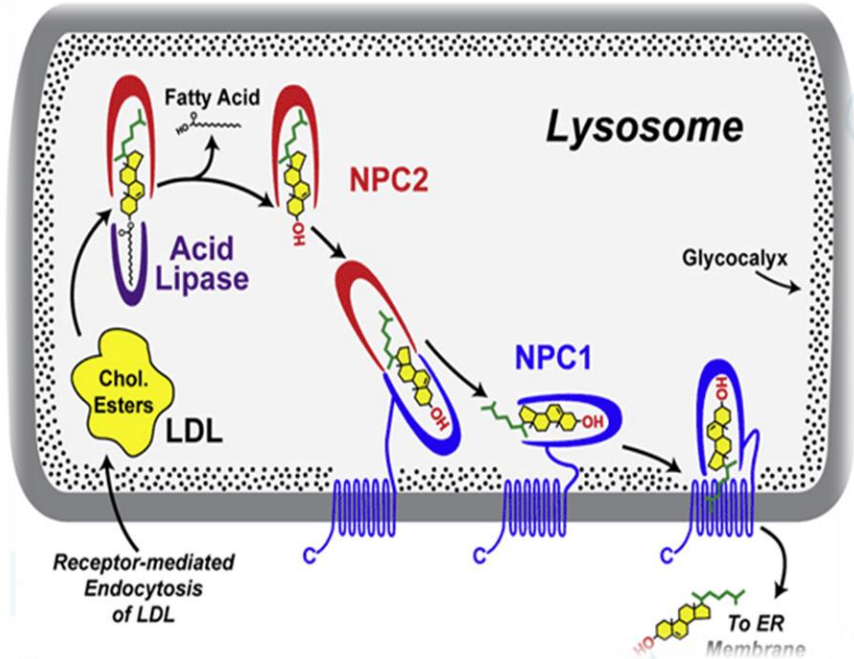
Niemann-Pick type C (NPC)

The mutations of NPC1 and NPC2 genes are associated with NPC.

NPC differs from the other types, the protein product of the major mutated gene NPC1 is a transporter in the endosomal-lysosomal system, which moves large water-insoluble molecules (e.g. cholesterol) through the cell. The protein coded by the NPC2 gene more closely resembles an enzyme structurally but seems to act in cooperation with the NPC1 protein in transporting molecules in the cell.

The disruption of this transport systems results in the accumulation of cholesterol and glycolipids in lysosomes.

In NPC large amounts of free or unesterified cholesterol accumulate in lysosomes, and leads to relative deficiency of this molecule in multiple membranes and for steroid synthesis.



Drugs used in NPC

There is no known cure for NPC, neither is any approved disease modifying treatment, only supportive care.

- Trappsol (HPBCD) FDA orphan drug status
- Adrabetadex (VTS-270) FDA orphan drug status
- Arimoclomol FDA orphan drug status
- Zavesca (Miglustat) FDA orphan drug status withdrawn
- Allopregnanolone FDA orphan drug status withdrawn
- Also, there are several on-going clinival trials with other compounds, e.g. vorinostat, lithium carbonate, etc.

Source: https://clinicaltrials.gov/ct2/results?cond=Niemann-Pick+Disease%2C+Type+C

Discovery

PNAS

Cyclodextrin overcomes deficient lysosome-to-endoplasmic reticulum transport of cholesterol in Niemann-Pick type C cells

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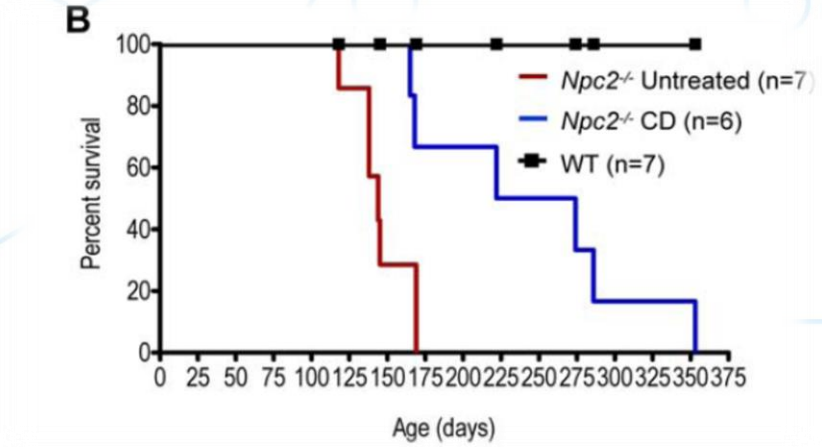
Contributed by Joseph L. Goldstein, September 23, 2009 (sent for review September 15, 2009)



„The Nobel Prize in Physiology or Medicine 1985 was awarded jointly to Michael S. Brown and Joseph L. Goldstein "for their discoveries concerning the regulation of cholesterol metabolism"

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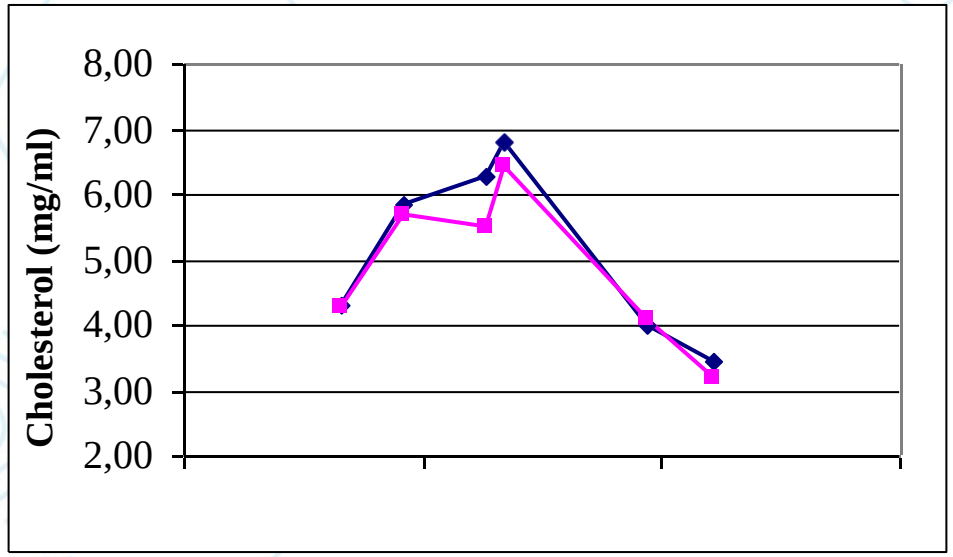
Is cholesterol the therapeutic target in this treatment?

Aqueous solubility of Cholesterol in the presence of 10% HPBCD of different degree of substitution



Cyclodextrins with zero cholesterol affinity (HPGCD, SBECD) were also shown effective in various studies to treat NPC, even in animal models

HPGCD Outperforms HPBCD as a Potential Treatment for NPC Disease (Matsuo, I. Stem Cells, 2014)

Malanga, M., Szemán, J., Fenyvesi, É., Puskás, I., Csabai K., Gyémánt Gy., Fenyvesi, F., Szente, L. "BACK TO THE FUTURE": A NEW LOOK AT HYDROXYPROPYL BETA-CYCLODEXTRINS
Journal of Pharmaceutical Sciences, Volume 105, Issue 9, 2921–2931 (2016)

IP background for cyclodextrins

ASDERA LLC.

WO2019067145A1 (under investigation) Use of cyclodextrins in diseases and disorders involving phospholipid dysregulation

The patent describes the possibel usage of alfa- and hydroxypropyl-alfa-cyclodextrins in several neurodegenerative diseases.

Vtesse Inc

US10300086B2 (patent expired) Hydroxypropyl beta-cyclodextrin compositions and methods

The patent describes the usage of hydroxypropyl-beta-cyclodextrin (HPBCD) in NPC

Japan Maize Prod [JP]; Univ Kumamoto Nat Univ Corp [JP] – National University Corporation Kuammaoto Univerity, Nihon Shokuhin

Kakao Co., Ltd.

EP3078379A1 (patent lapsed) Drug ForThe Treatment
Accumulation Disorders, and Screening Method For Same
of Cholesterol
The patent describes the usage of hydroxypropyl-gamma-cyclodextrin (HPGCD) in NPC.

Ongoing research with cyclodextrins

Indications	Marketed	Pre-Registration	Filing rejected/Withdrawn	Phase III	Phase II	Phase I	Phase 0	IND/CTA Filed	Pre Clinical	Discovery
NPC, Type C	2	0	0	2	2	3	1	1	14	5

Ongoing clinical trials for NPC treatment with cyclodextrins:

- Mallinckrodt (VTS-270) – HPBCD – injected to CNS directly (Phase II)
- Cyclo Therapeutics (Trappsol) – HPBCD – iv administration (Phase III)

Orphan drug status in the US and EU

New companies in the field:

- Oraxion-beta-CD covalent polymer prodrug (ORX-301), preclinical

Ongoing research with cyclodextrins

Research groups have different approaches on creating more potent derivatives and explanations for MoA:

- Cyclodextrin-based polyrotaxanes and polymers to improve BBB penetration and prolong circulation
  (Purdue Univ, Tokyo Medical Univ, Aten Porus Life Sci Istanbul Technical University)

- Create „targeted" cyclodextrins that can be recognized by receptors and improve delivery
  (Kumamoto Univ)

- understand structure-activity relationships for a rational design of the most effective CD derivatives
  (NIH, Albert Einstein College, CycloLab)

CycloLab's approach

HPBCD development is an accidental discovery, not a systematic drug development

HPBCD is a right, reasonable, but not ideal choice

Advantages of HPBCD:

- safe, non-toxic parenteral excipient, 20 years of experience with its use
- available in pharmaceutical grade, at a reasonable price
- Regulatory tox. Dossier (by Janssen, J&J)Listed in EP, USP pharmacopoeia

Disadvantages of HPBCD:

- developed as an excipient, not an API
- a composite isomeric mixture (not a single well-defined chemical entity)
- the active compound (fraction) is not known
- loose, permissive quality requirements set by USP (DS= 2.7-10.5)

CycloLab's approach

Current focus is on commercially available HPBCD, without knowing if this is the right candidate to develop

- Chosen due to existing safety data and „hanging low"
- HPGCD, SBECD, HPACD, supramolecule CDs others shown efficient

Minor efforts on understanding the mechanism of action and improving product performance

- CD designed for the real target (cholesterol? phospholipids? affecting gene expression? Differences on different cell types/ages?)
- CD crossing blood brain barrier

Minor efforts on understanding the root cause of side effects and improving product safety

Regulatory revision – is the CD API or excipient?

CycloLab's approach

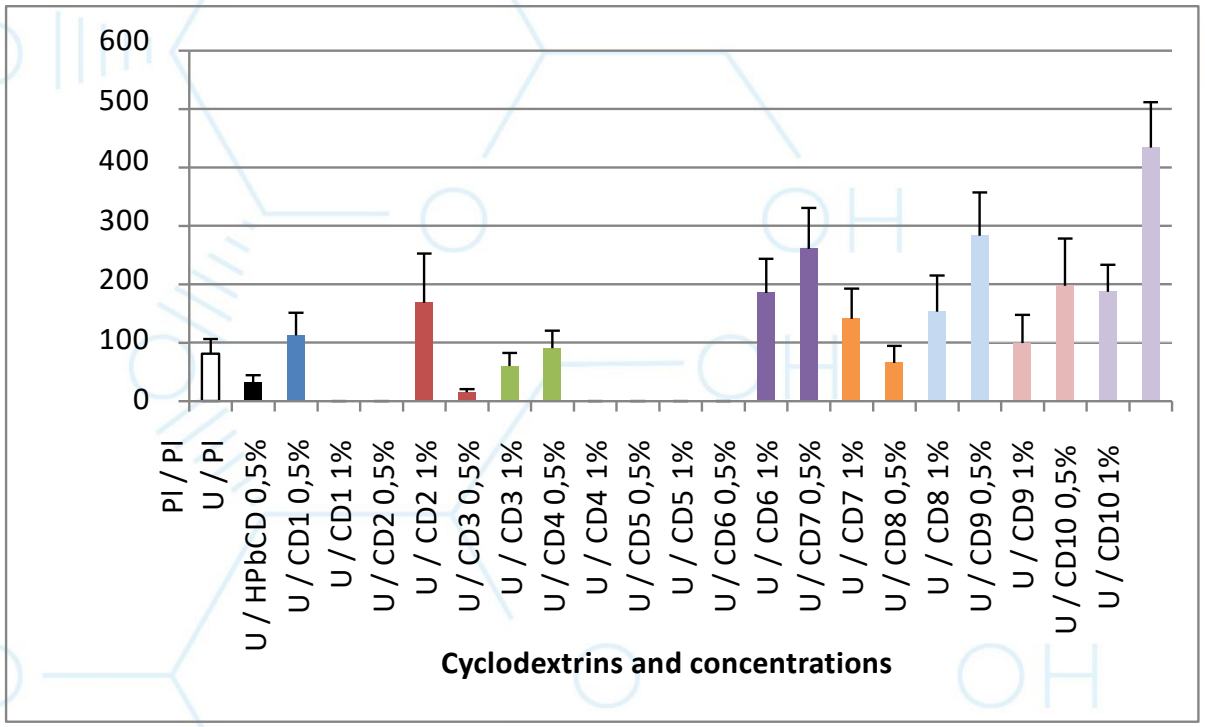
As the mechanism of action is not understood, there is no reliable in vitro model to screen cyclodextrins. Cyclolab decided to evaluate a wide range of CDs in zebrafish model where cholesterol accummulation was triggered.

Characteristics of zebrafish

- Small, robust freshwater fish
- Easy to maintain, high fecundity (200 eggs / week)
- Most organs fully functional between 3 – 5 dpf.
- Larvae are transparent
- Genome fully sequenced
- Genome, genetic pathways and development highly conserved between zebrafish and humans
- Easy genetic manipulation
- Large behavioral repertoire

CycloLab's results

Several compounds tested to understand SAR and activity against reference (HPBCD)



PI/PI: wild type larvae (negative control)

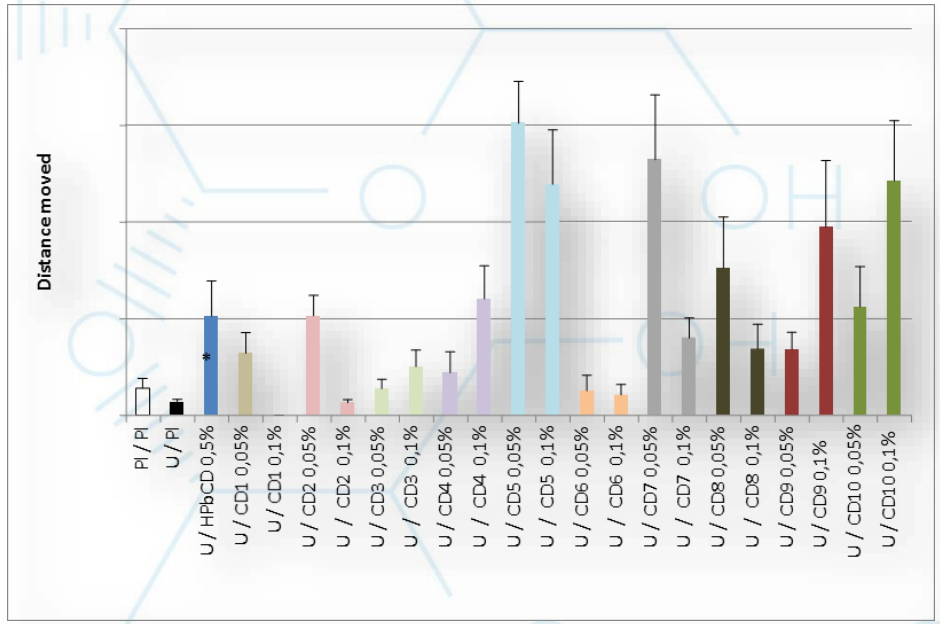
U/PI: triggered cholesterol accumulation (positive control)

U/HPBCD: alternative treatment

Several candidates were clearly more or equally promising than HPBCD

CycloLab's results

Selected compounds evaluated at an order of magnitude lower concentration compared to HPBCD. Further derivatives added based on SARs concluded from the 1st set.



PI/PI: wild type larvae (negative control)

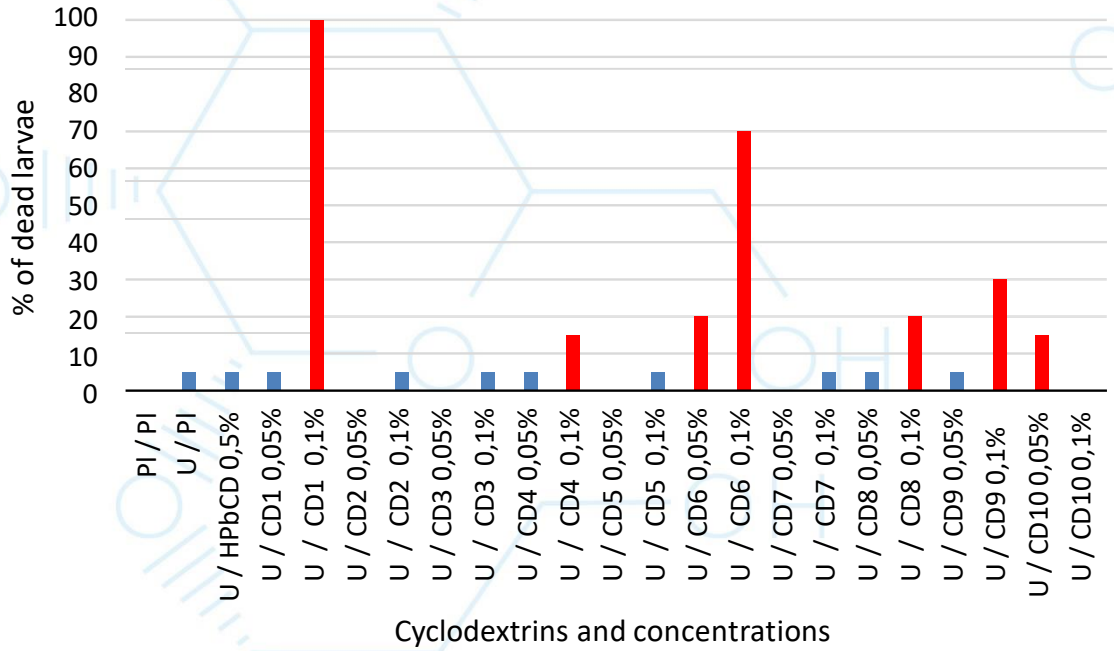
U/PI: triggered cholesterol accumulation (positive control)

U/HPBCD: alternative treatment at 0.5% concentration level

Several candidates were clearly more promising than HPBCD even at 10-fold lower concentration

CycloLab's results

CDs also tested for toxicity against reference (HPBCD)

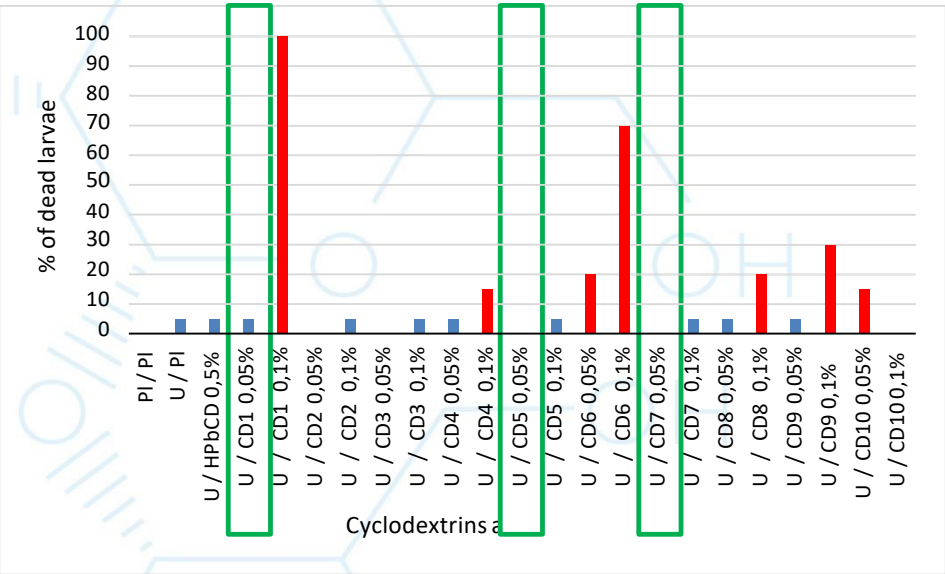


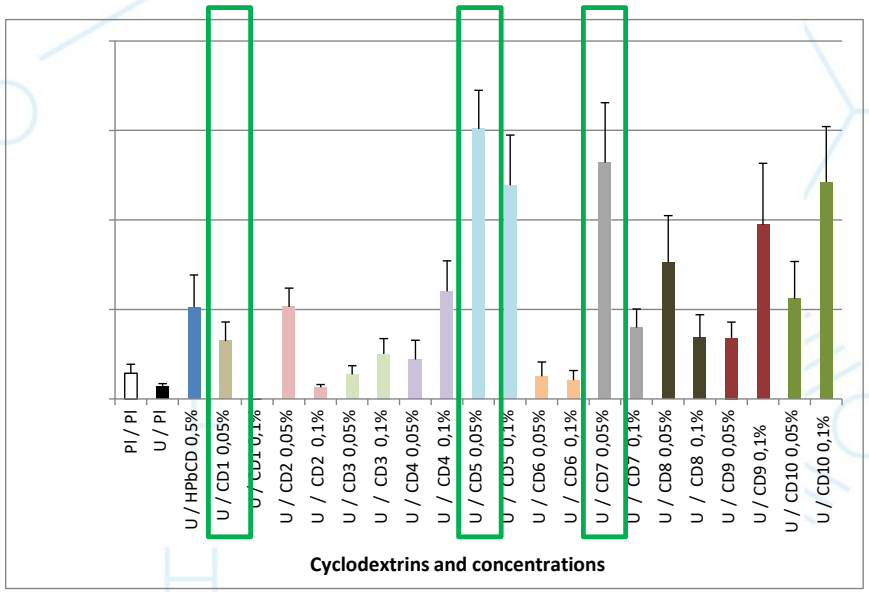
PI/PI: wild type larvae (negative control)
U/PI: triggered cholesterol accummulation (positive control)
U/HPBCD: alternative treatment

Several candidates were more toxic compared to HPBCD, whereas others were equally safe or safer

CycloLab's results

Cyclodextrins were shortlisted for cholesterol mobilization studies



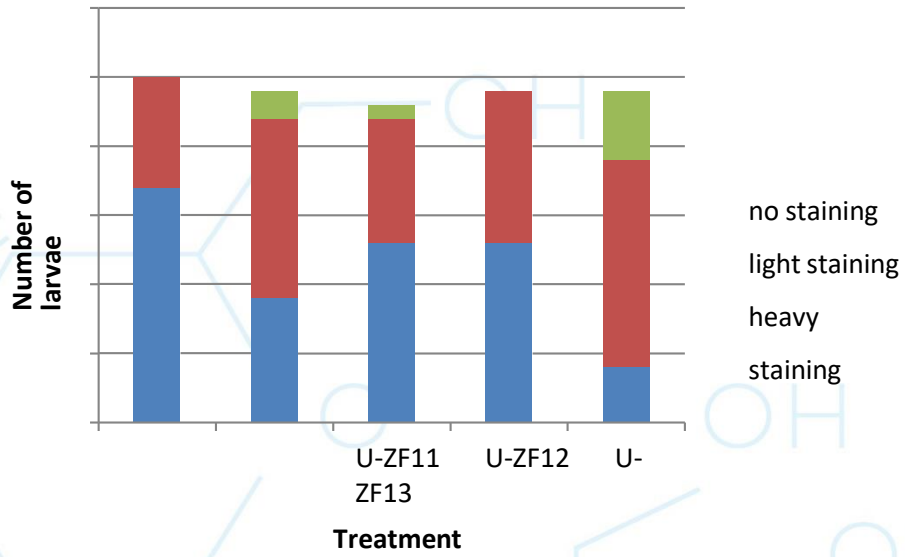


For the proof of concept study, 3 candidates at a single concentration were selected and compared to HPBCD

CycloLab's results

- Filippin staining – cholesterol mobilization study
- CDs tested at 10x lower concentration compared to HPBCD
- Larvae classified as no staining, light staining and heavy staining, corresponding to no (green), mild (red) and intense (blue) cholesterol accumulation

One candidate had no effect, one was comparable to HPBCD and U-ZF13 had significantly higher activity in mobilizing cholesterol7



Conclusions

- A wide variety of cyclodextrins were tested on animal models to reverse the behavioral effects of cholesterol accumulation and evaluate their toxicity
- SAR were (compared selected drawn and compounds of superior activity to HPBCD) and comparable safety profile selected

- Via filipping staining the cholesterol removal potency of certain lead compounds were confirmed
- Based on the results generated these are ideal candidates to Further optimize, evaluate in animal model and further develop to discover a new, more potent and safer Cyclodextrin to treat cholesterol-associated lysosomal diseases

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CD-based therapy in Niemann-Pick C disease

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