Respecting patient confidentiality, some slides -- including patient photos -- have been removed from this presentation.

Office of the Clinical Director, NICHD

Infantile Neuronal Ceroid Lipofuscinosis (INCL):

Bench to Bedside

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History of NCL /Batten Disease

- Most common group of progressive neurodegenerative disorders in children
- Actually first described by Otto-Christian Stengel in Norway (published in 1826) – however, 4 sibs with juvenile NCL in family died and were buried before Stengel could do autopsies
- Multiple eponyms: Santavuori, Haltia, Jansky, Batten, Bielschowsky, Spielmeyer, Sjogren, Kufs

First clinicopathological correlation by F.E. Batten, 1903

- Defines “familial cerebromacular degeneration”
- Later on in 1914 separates this collection of diseases from Tay-Sachs

Considered “geographically ubiquitous”

- General frequency 1 per 12,500 births
- Enriched gene frequency in Finland where infantile and juvenile forms are more prevalent
- All forms autosomal recessive except for rare instances of autosomal dominant mode of inheritance in some adults

Incidence of Different types of NCL in the US

(Shriver Center Study, Waltham, Massachusetts: January, 1984 to December, 1986).

- **LINCL** is most common (35%) with a mean age-of-onset around 3 yrs.
- **JNCL** is slightly less frequent (32%) with a mean age-of-onset of 8 yrs;
- **INCL** ranked third (23%) with a mean age-of-onset 11 months;
- **Adult** form of the disease was the least common (10%) with a mean age-of-onset 25 yrs.

NEURONAL CEROID LIPOFUSCINOSIS (NCL)

Clinical Subtype	Mutant Gene	Chromosomal Location	Gene Product
Infantile NCL (INCL)	CLN 1	1p32	Palmitoyl-Protein Thioesterase (PPT)
Classic Late Infantile (LINCL)	CLN 2	11p15	Tripeptidyl Peptidase
Juvenile NCL (JNCL)	CLN 3	16p12	438-aa Membrane Protein
Adult NCL (Kufs Disease)	CLN 4	?	?
Finish Variant (LINCL)	CLN 5	13q22	407-aa Membrane Protein
Variant LINCL	CLN 6	15q21	?
Northern Epilepsy	CLN 8 (CLN 7?)	8p23	?

Neuronal Ceroid Lipofuscinosis (NCL)
(Batten Disease)

Clinical Features:

- Retinal Blindness
- Seizures
- Psychomotor deterioration
- Progressive Encephalopathy Leading to Death

Pathological Findings:

- Marked Cortical Atrophy
- Lysosomal Accumulation of Auto-fluorescent Lipopigments in Neurons and Other Cell Types
- Storage Material is Similar in Composition to the Ageing Pigments: Ceroid & Lipofuscin

NCL subtypes defined by age of onset and ultrastructural morphology

Ultrastructural Morphology

- Infantile NCL - GROD
- Classical Late-Infantile NCL - CVB
- Juvenile NCL - FPP

INFANTILE Neuronal Ceroid Lipofuscinosis (INCL)

- Caused by Lysosomal Palmitoyl-Protein Thioesterase (PPT) Deficiency
- Most severe among all the NCLs
- No effective treatment

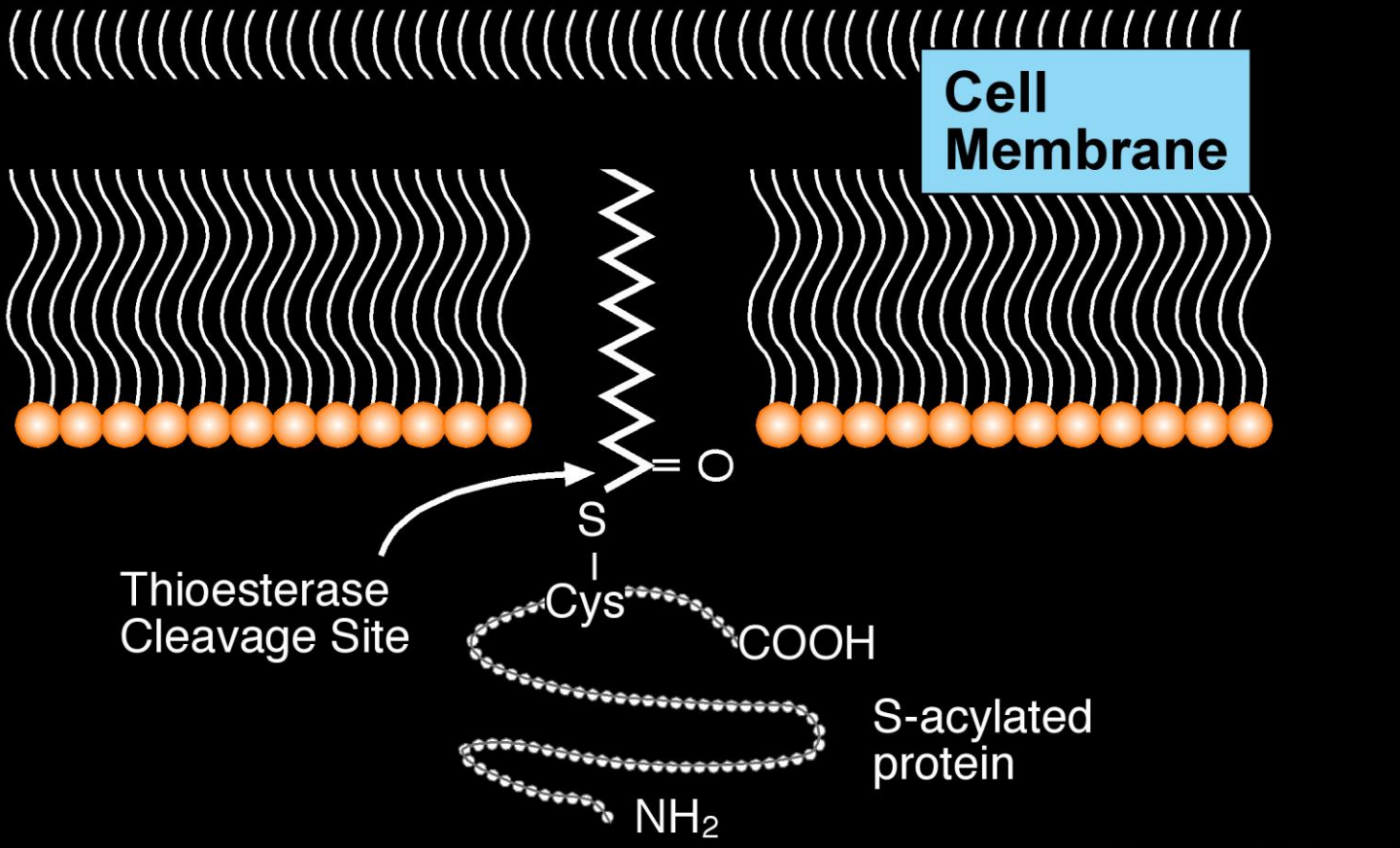
CLINICAL COUSE

- Normal At Birth
- Complete Retinal Blindness
  by 2 yrs of Age
- Ataxia & Seizures
- Flat EEG ~4 yrs of Age
- Death ~ 8-11 yrs of Age

INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (INCL)

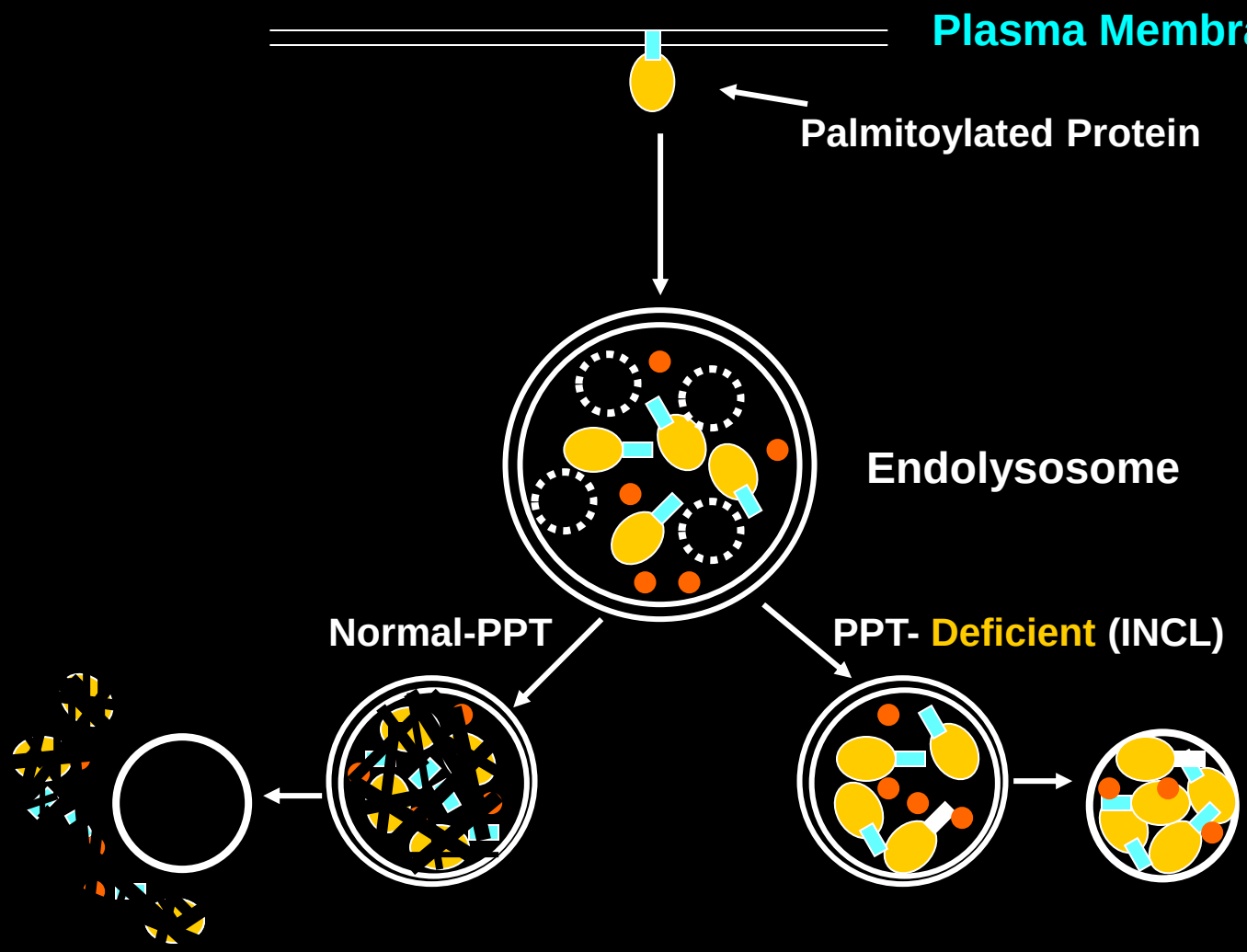
- Incidence: 1 in 100,000 births
- Due to the Lack of an Effective Treatment INCL is an Uniformly Fatal Disease

CLEAVAGE OF THIOESTER LINKAGE BY PALMITOYL-PROTEIN THIOESTERASE (PPT)



Hofmann et al 2001

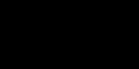
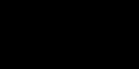
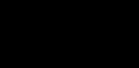
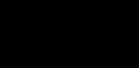
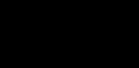
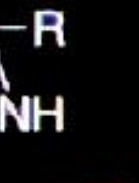
Proposed Mechanism of Pathogenesis in INCL



SINCE THIOESTER LINKAGES ARE SUSCEPTIBLE TO NUCLEOPHILIC ATTACKS

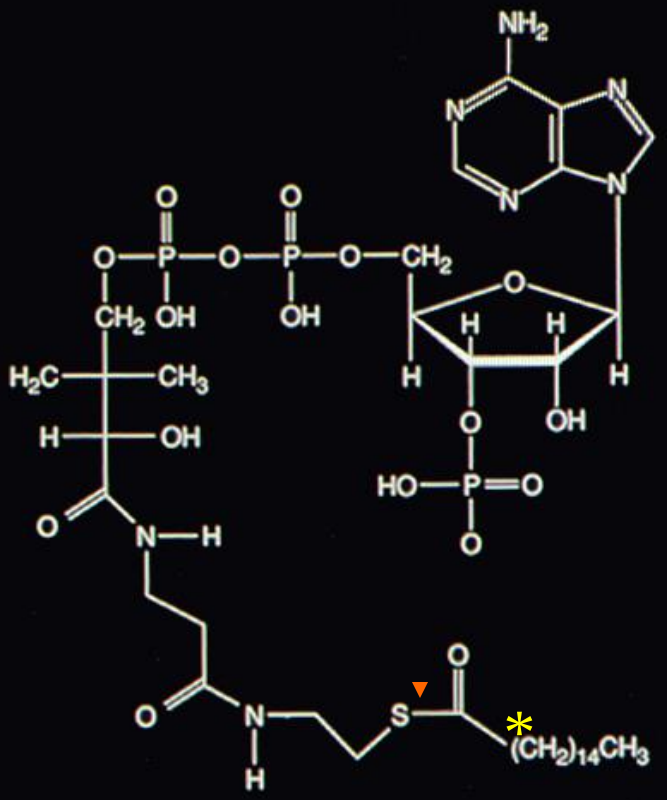
We sought to determine whether drugs with nucleophilic properties have therapeutic potential for INCL

Some functional groups that Act as nucleophiles within cells

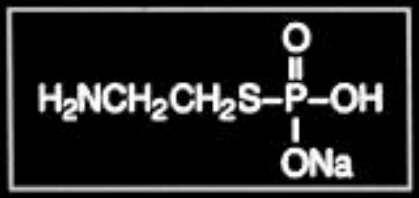
Water	
Hydroxyl (alcohol)	
Alkoxyl	
Sulfhydryl	
Sulfide	
Amino	
Carboxylate	
Imidazole	

[^{14}C]Palmitoyl-CoA:

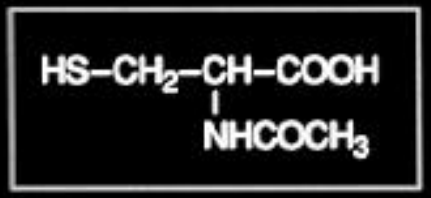
A Model Thioester Substrate of PPT



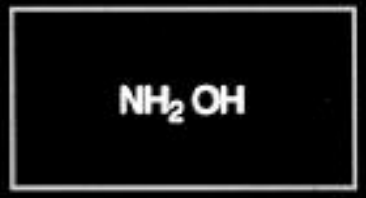
Cleavage of Thioester Linkage by Nucleophilic Drugs



Phosphocysteamine



N-Acetylcysteine



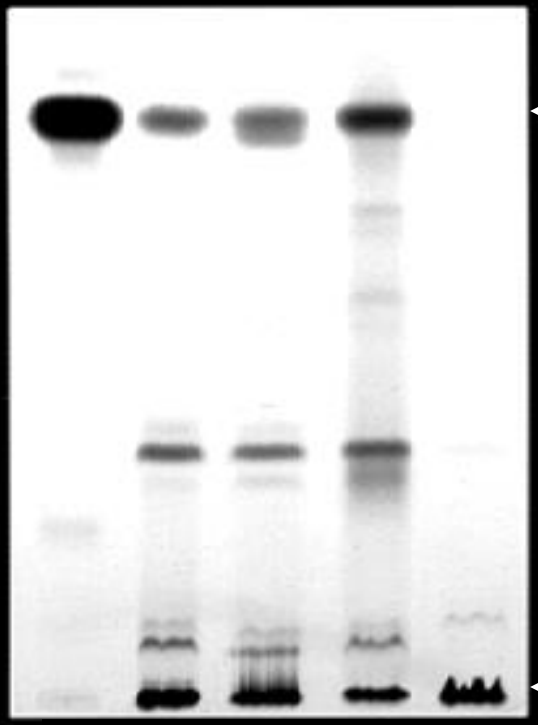
Hydroxylamine

^{14}C-Palmitoyl CoA

Drug

^{14}C-Palmitic Acid + CoA

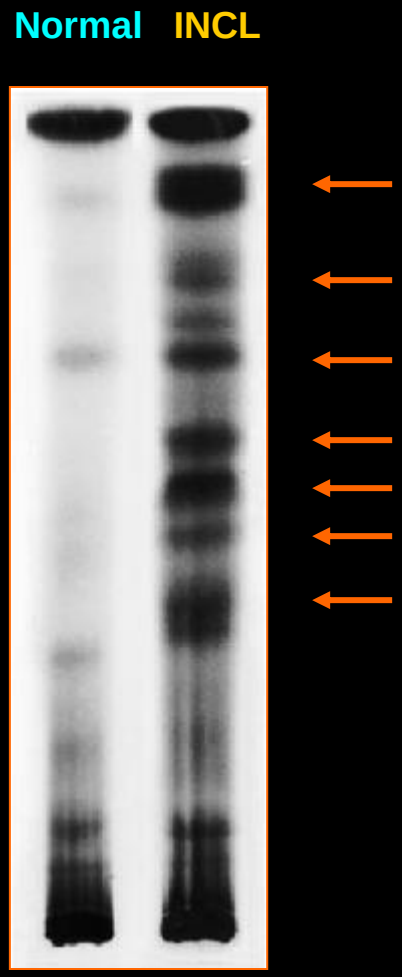
THIN LAYER CHROMATOGRAPHY



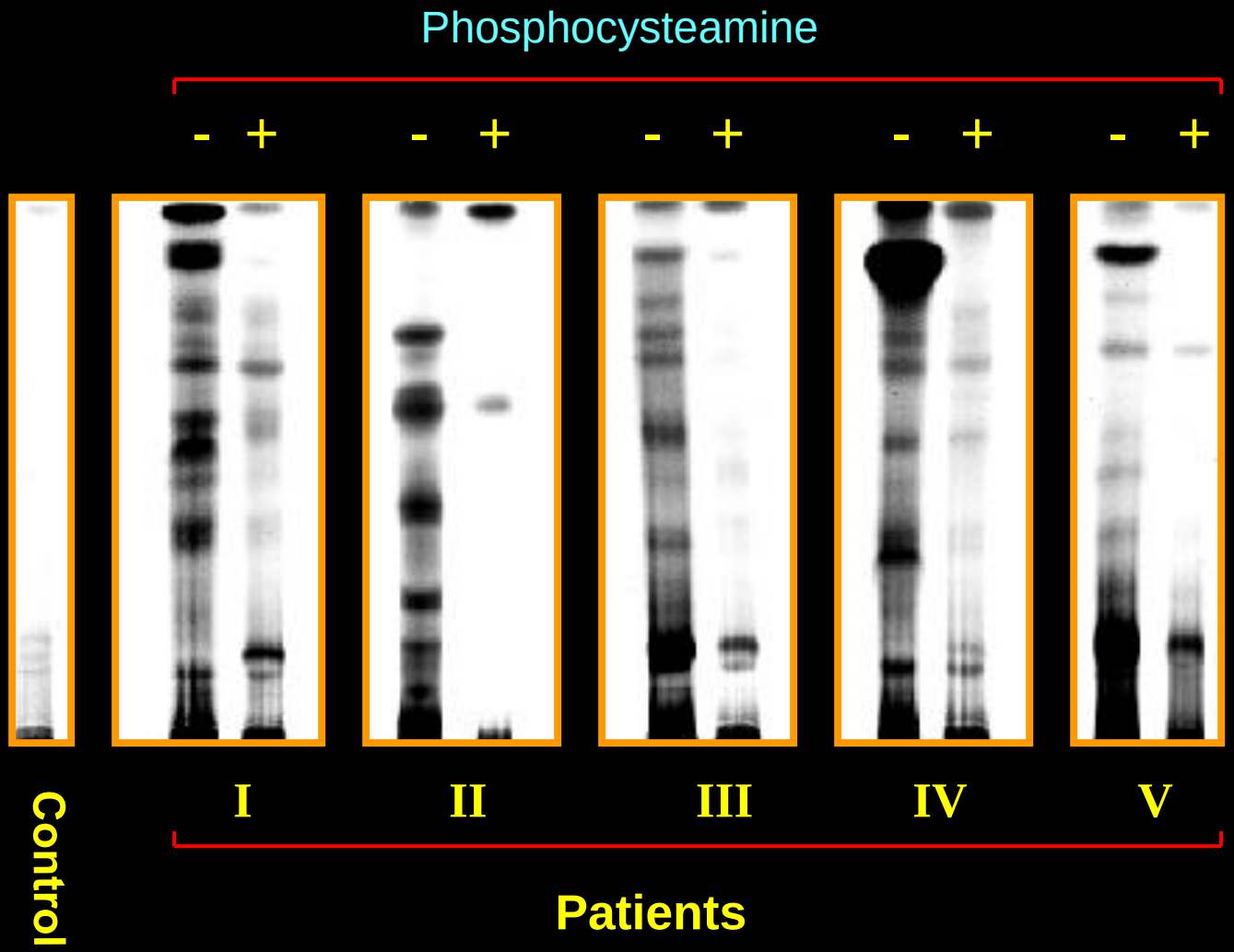
^{14}C-Palmitic Acid

^{14}C-Palmitoyl CoA

[^{35}S]Cysteine-labeled Thioesters in Normal & INCL Cells



Phosphocysteamine Mediates Degradation of Lipid-Thioesters From Acylated Proteins in INCL Cells



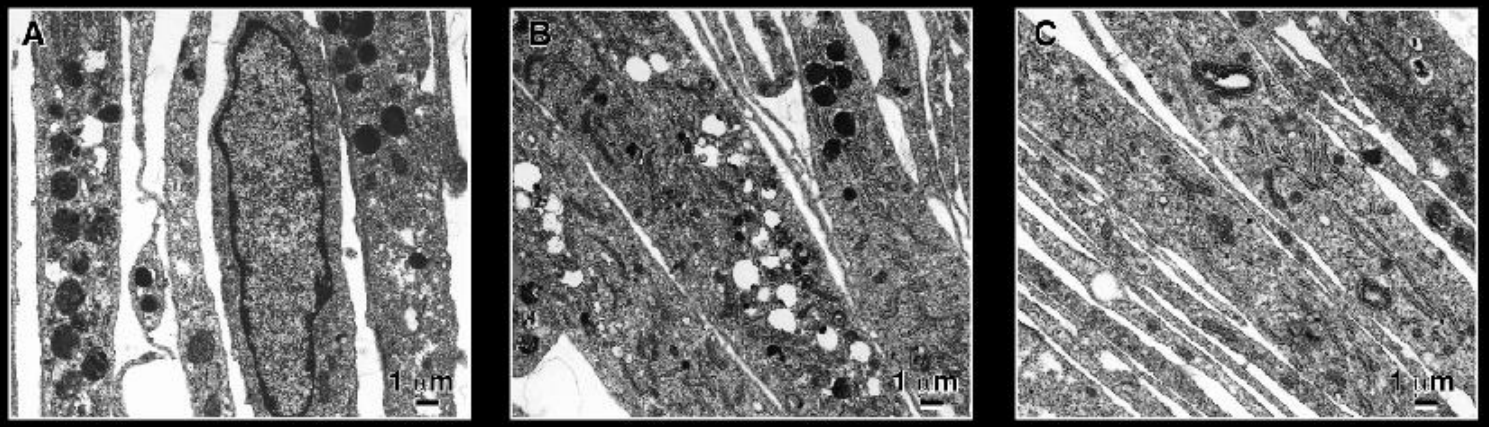
Phosphocysteamine Mediates Depletion of Lysosomal Ceroids in INCL Cells

Fibroblasts:

Untreated

1 wk

3 wks

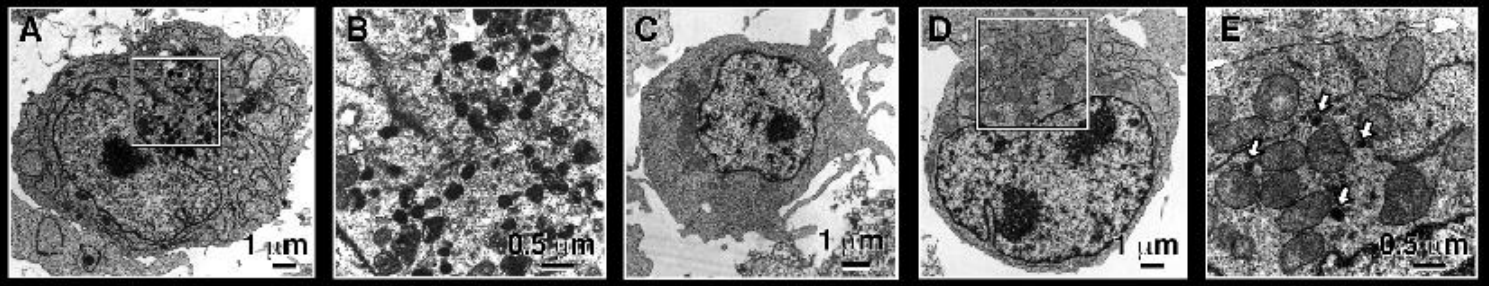


Lymphoblasts:

Untreated

1 wk

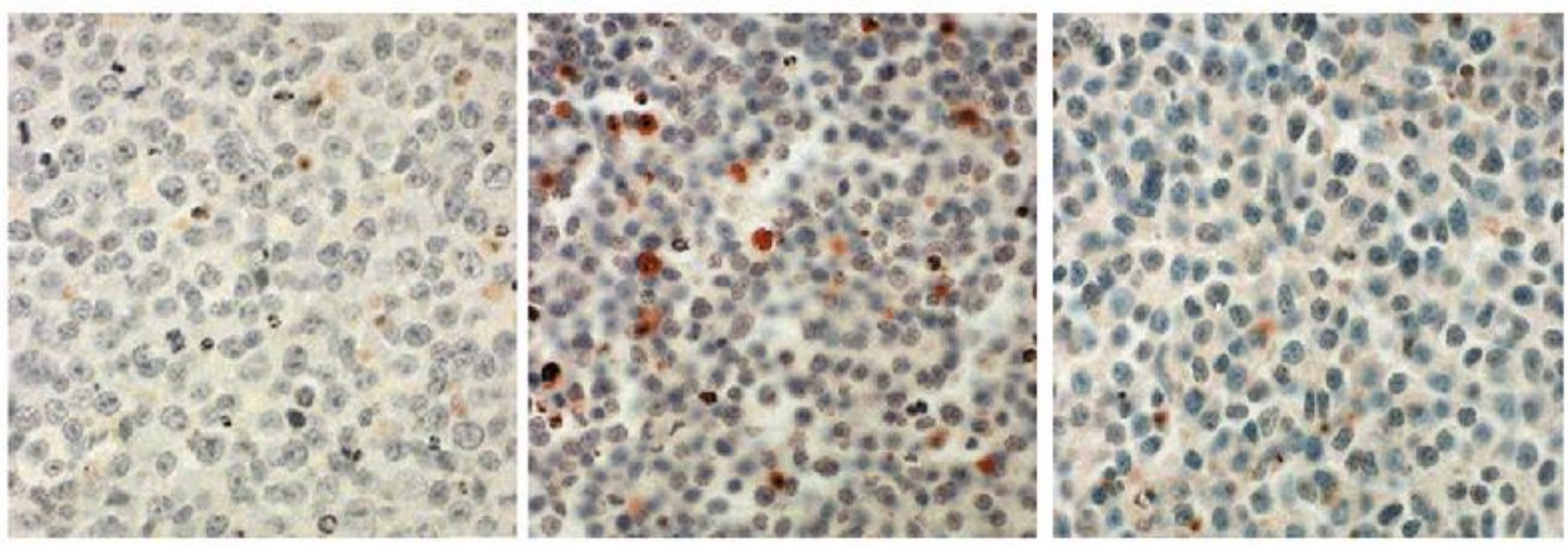
3 wks



P-Cysteamine Inhibits Apoptosis in INCL Lymphoblasts

[TUNEL Assay]

Normal Control	INCL Untreated	INCL Treated



CYSTAGON
(Cysteamine bitartrate)

- Safely used for more than 20 yrs for the treatment of Cystinosis
- Readily gets into lysosomes
- Crosses the brain-blood barrier

BENCH-TO-BEDSIDE PROTOCOL

PILOT STUDY:

To Determine Whether Cystagon is an Effective Treatment for INCL

Duration of the Protocol: 4 years

Total Number of Patients: 5

Age limit: 6 months to 3 years

Evaluation Criteria: ERG, EEG, MRI, and EM of WBCs

Number of Patients Admitted to date: 2

NATURAL HISTORY OF THE MOST SEVERE INCLs

Age	PPT Mutations L10X, R151X, R164X, E184K W296X,R122W, c.169insA	Disease Course
0--11 months	----------	Normal
11--15 months	----------	Mild Visual Deficit Abnormal ERG Mild Irritability
15M--2 Years	----------	Retinal blindness Grossly Abnormal ERG & EEG; Moderate Cortical Atrophy
2.5--4Years	----------	Total Retinal Blindness Marked Brain Atrophy Frequent Seizures Isoelectric EEG
4--11 Years	----------	Vegetative State and Death

Pedigree

- S.P.’s mother is Finnish and her father is from Estonia - ? consanguinity, in that Estonia and Finland were once united and root language is the same from these two regions
- Prenatal diagnosis of S.P.’s brother done by Dr. Santavuori’s lab in Finland and fetus was determined to be unaffected

CLINICAL OBSERVATIONS

- Markedly Less Irritable
- No Seizures
- Increased Alertness
- Efforts to Move Head and to Rollover
- Increased Pupillary Response to Light
- Increased Ability to Focus on Objects

SUMMARY

- INCL is the most severe form of NCL
- Caused by PPT-deficiency
- Phosphocysteamine appears to mediate depletion of lysosomal ceroids in INCL cells
- Preliminary results of a pilot study with Cystagon show stabilization or slight improvement in some parameters in an INCL patient

ACKNOWLEDGEMENTS

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From the Bench-to-Bedside

PILOT STUDY:

Drug: Cystagon (Cysteamine bitartrate)

Duration of the Study: 4 years

Total No. Patients to be Treated: 5

PPT-Mutations: **L10X, R151X, R164X, E184K W296X, R122W, c.169insA**

Age Limit: 6 months to 3 years

Evaluation : ERG, VEP, MRI & EM

No. Patients Admitted to Date: 2

Developmental Expression of PPT in Brain & Retina (Immuno-localization)