Researcher

Dr Stephen Palmer

Biography

Dr Stephen Palmer is a Program Director at Novogen Ltd and Conjoint Senior Lecturer at UNSW Australia. He is a former member of the Cellular and Genetic Medicine Unit in the School of Medical Sciences with research interests in understanding the role of the gene *GTF2IRD1* in brain development and human behaviour and its contribution towards the characteristic features of Williams-Beuren syndrome.

Stephen Palmer completed his PhD at University... view more

Location

Faculty of Medicine

Contact

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PhD 1992 *The Cellular Basis of Sex Determination in Mammals*

UCL

ICR The Institute of Cancer Research

Chester Beatty Labs London 1992-1996
Genetics of the XY pseudoautosomal region

FIAT LUX Walter+Eliza Hall Institute of Medical Research

1997-2000 Heart Development

Victor Chang Cardiac Research Institute

CHILDREN'S MEDICAL RESEARCH INSTITUTE

2002-2008 Skeletal Muscle Fibre type development and switching

2009-2014: Genetics and Cellular Pathology of Williams-Beuren Syndrome

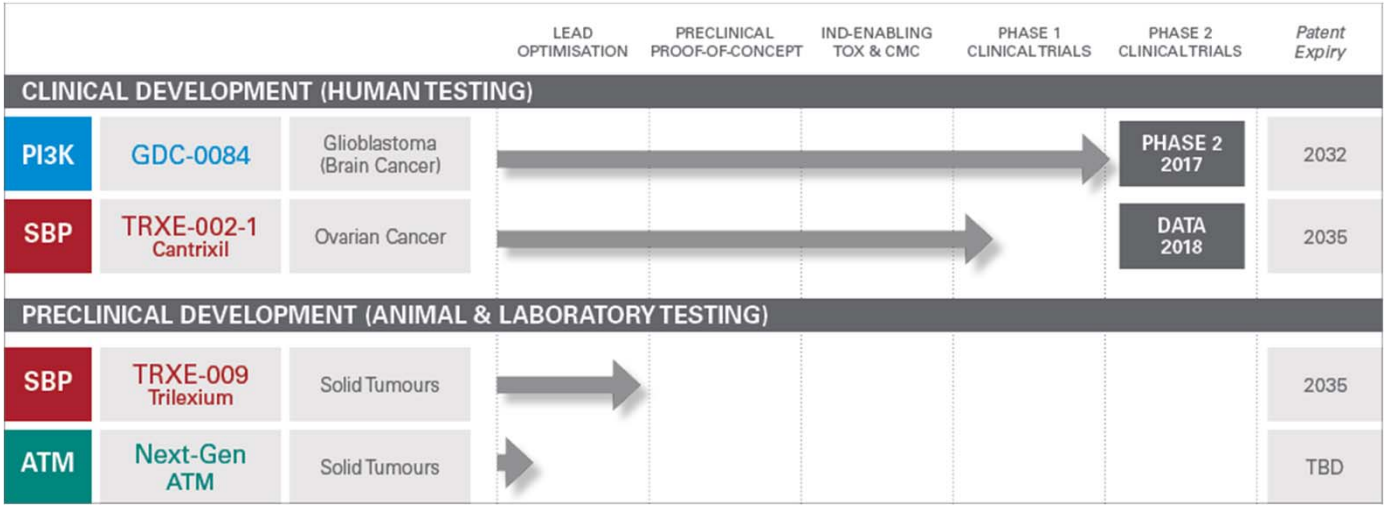
UNSW AUSTRALIA

NOVOGEN

2014 Degenerative Diseases Program Director
2017 Director of Preclinical Research

Novogen Ltd


Novogen (ASX: NRT, NASDAQ: NVGN) is an oncology-focused biotechnology company, developing innovative anti-cancer drugs. Headquartered in Sydney, Australia, Novogen collaborates with leading scientists, clinicians, and investors around the world.


LEAD OPTIMISATION
PRECLINICAL PROOF-OF-CONCEPT
IND-ENABLING TOX & CMC
PHASE 1 CLINICAL TRIALS
PHASE 2 CLINICAL TRIALS
Patent Expiry
CLINICAL DEVELOPMENT (HUMAN TESTING)
PI3K
GDC-0084
Glioblastoma (Brain Cancer)
PHASE 2 2017
2032
SBP
TRXE-002-1 Cantrixil
Ovarian Cancer
DATA 2018
2035
PRECLINICAL DEVELOPMENT (ANIMAL & LABORATORY TESTING)
SBP
TRXE-009 Trilexium
Solid Tumours
2035
ATM
Next-Gen ATM
Solid Tumours
TBD

Lysosomal Storage Disorders

The Donell Family. Megan is the founder of the Sanfilippo Children's Foundation.

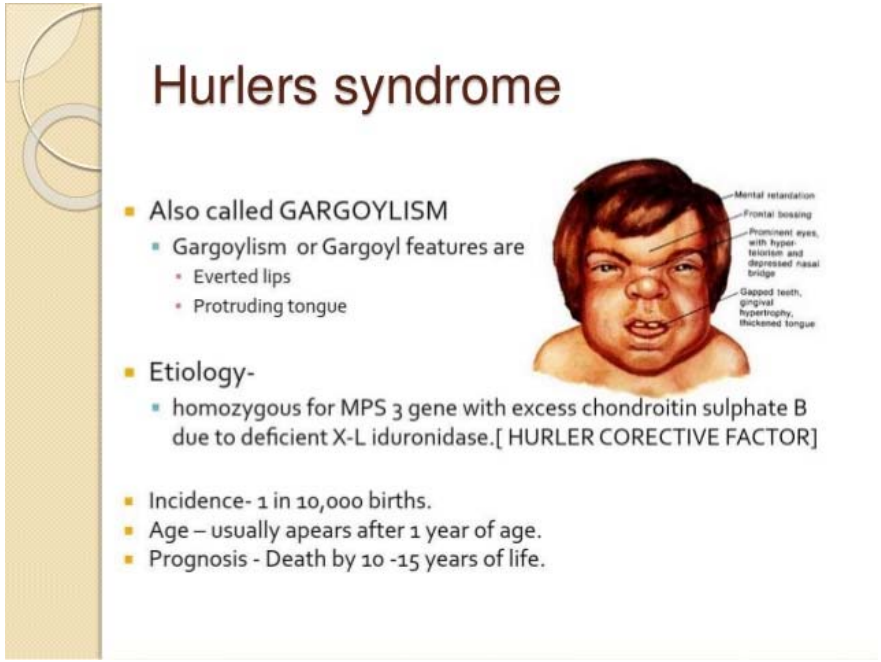
Genetics, Biology and Therapy

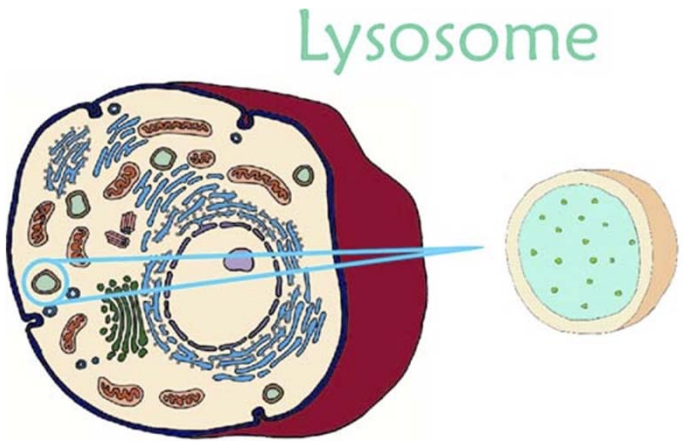
Lysosomal Storage Disorders (LSDs)

- **Incidence**: individually rare (e.g. MPSIII Sanfilippo syndrome approximately 1 in 100,000) but collectively, LSDs occur at approximately 1 in 7,000 live births

- **Genetics:** > 50 monogenic disorders – mostly autosomal recessive or X-linked

- **Presentation**: Multiple organ systems - craniofacial abnormalities, cardiomyopathy, upper airway obstruction, hepatosplenomegaly, skeletal dysplasia, ***neuropathology,*** reduced lifespan (e.g. Sanfilippo syndrome (MPSIII) life expectancy 12-20 lose motor function by age 10).





Summary of the Lysosomal Storage Disorders

LSD	DEFECTIVE ENZYME	NEUROLOGICAL FEATURES
SPHINGOLIPIDOSES • **GM1 and GM2 gangliosidosis** • **Niemann-Pick disease (NPC)** • **Gaucher disease** • **Others**	**Lysosomal hydrolases** (i.e. *Hexosaminase A* in GM2; *Sphingomyelinase* in NPC; *Glucocerebrosidase* in Gaucher disease)	Progressive neurological regression, seizures, spasticity,
MUCOPOLYSACCHARIDOSES • **MPS-III** • **Others**	**Glycosaminoglycan cleaving enzymes**	Mental retardation, behavioural disturbances and hyperactivity
GLYCOPROTEINOSES • **Mucolipidosis** • **Others**	**Glycoprotein cleaving enzymes** (*N-acetylglucosamine-1-phospotransferase* in Mucolipidosis-I)	Mental impairment, speech impairment, spasticity, neuroaxonal dystrophy
NEURONAL CEROID LIPOFUSCINOSIS • **Batten disease** • **Others**	**Lysosomal proteins (e.g. proteases)** (i.e. *CLN3 in Batten)*	Visual failure, epilepsy, decline in motor and cognitive skills
MULTIPLE SULFATASE DEFICIENCY	**Sulfatase modifier**	Rapid neurological deterioration

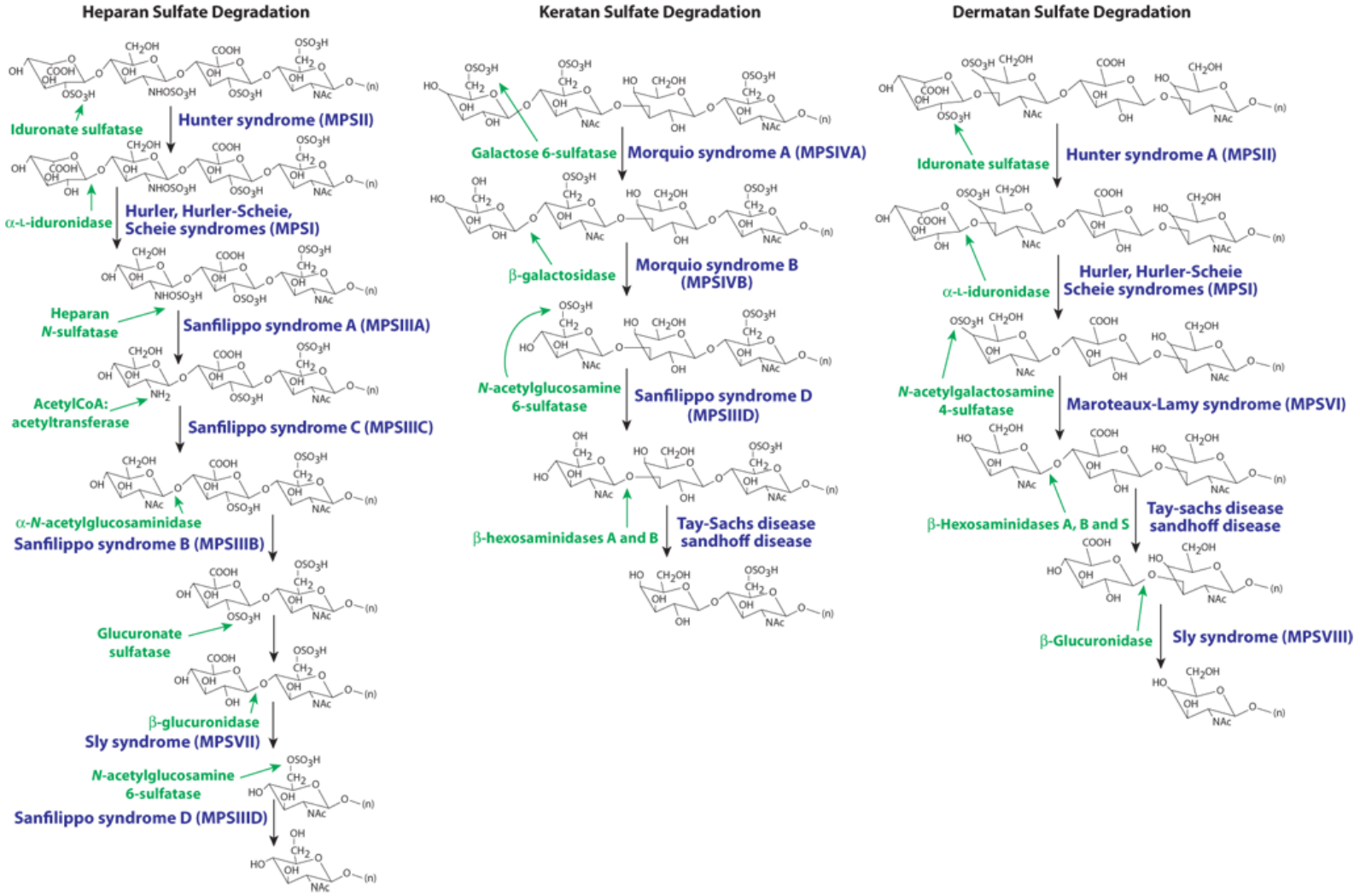
Genetics and biochemistry of the mucopolysaccharidoses

Main mucopolysaccharidoses

Type[1]	Common name Other names	OMIM	Gene	Locus	Deficient enzyme	Accumulated products	Symptoms	Incidence
MPS IH	Hurler syndrome	607014	*IDUA*	4p16.3	α-L-iduronidase	Heparan sulfate Dermatan sulfate	Intellectual disability, micrognathia, coarse facial features, macroglossia, retinal degeneration, corneal clouding, cardiomyopathy, hepatosplenomegaly	1:100,000[2]
MPS IH/S	Hurler–Scheie syndrome	607015						
MPS IS	Scheie syndrome *Formerly:* Mucopolysaccharidosis type V	607016						
MPS II	Hunter syndrome	309900	*IDS*	Xq28	Iduronate sulfatase	Heparan sulfate Dermatan sulfate	Intellectual disability (similar, but milder, symptoms to MPS I). This type exceptionally has X-linked recessive inheritance	1:250,000[3]
MPS IIIA	Sanfilippo syndrome A Sulfamidase deficiency	252900	*SGSH*	17q25.3	Heparan sulfamidase	Heparan sulfate	Developmental delay, severe hyperactivity, spasticity, motor dysfunction, death by the second decade	1:280,000[4] – 1:50,000[5]
MPS IIIB	Sanfilippo syndrome B NAGLU deficiency	252920	*NAGLU*	17q21.2	N-acetylglucosaminidase			
MPS IIIC	Sanfilippo syndrome C	252930	*HGSNAT*	8p11.21	Heparan-α-glucosaminide N-acetyltransferase			
MPS IIID	Sanfilippo syndrome D	252940	*GNS*	12q14.3	N-acetylglucosamine 6-sulfatase			
MPS IVA	Morquio syndrome A	253000	*GALNS*	16q24.3	Galactose-6-sulfate sulfatase	Keratan sulfate Chondroitin 6-sulfate	Severe skeletal dysplasia, short stature, motor dysfunction	1 in 75,000[4]
MPS IVB	Morquio syndrome B	253010	*GLB1*	3p22.3	β-galactosidase	Keratan sulfate		
MPS V	*See MPS IS (Scheie syndrome) above*							
MPS VI	Maroteaux–Lamy syndrome ARSB deficiency	253200	*ARSB*	5q14.1	N-acetylgalactosamine-4-sulfatase	Dermatan sulfate	Severe skeletal dysplasia, short stature, motor dysfunction, kyphosis, heart defects	
MPS VII	Sly syndrome GUSB deficiency	253220	*GUSB*	7q11.21	β-glucuronidase	Heparan sulfate Dermatan sulfate Chondroitin 4,6-sulfate	Hepatomegaly, skeletal dysplasia, short stature, corneal clouding, developmental delay	<1:250,000[6]
MPS IX	Natowicz syndrome Hyaluronidase deficiency	601492	*HYAL1*	3p21.31	Hyaluronidase	Hyaluronic acid	Nodular soft-tissue masses around joints, episodes of painful swelling of the masses, short-term pain, mild facial changes, short stature, normal joint movement, normal intelligence	

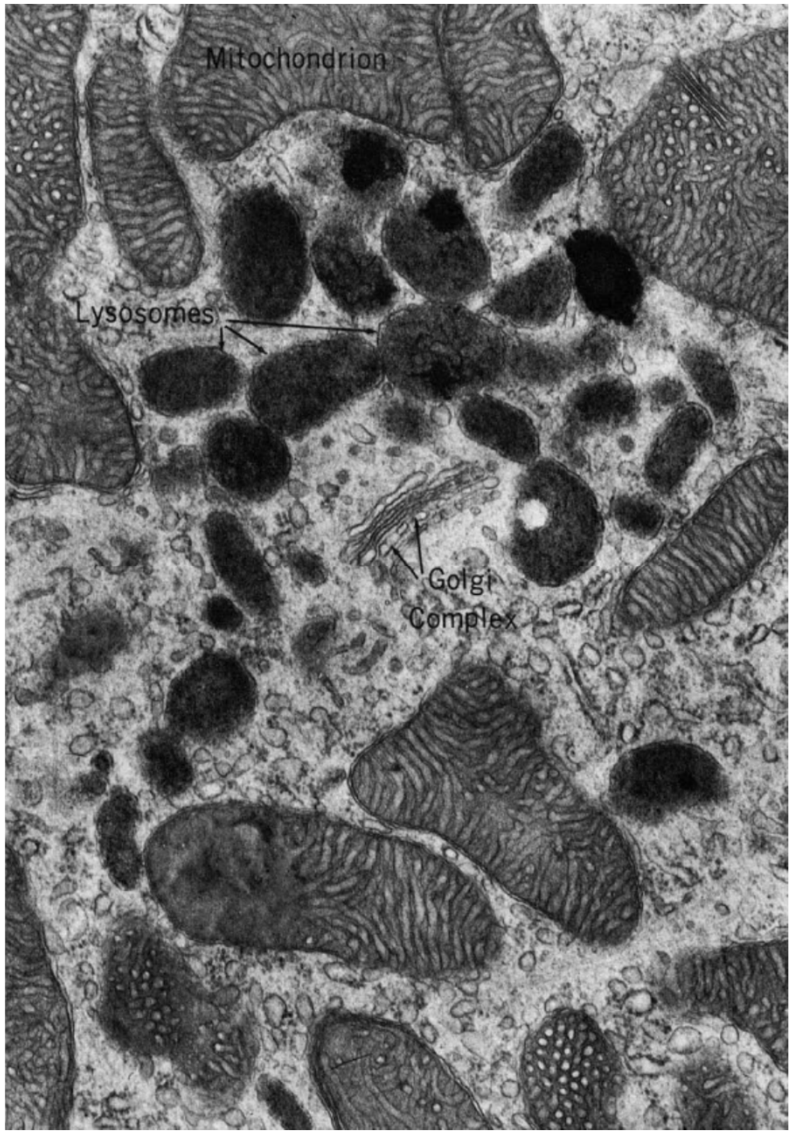
Pathways of Glycosaminoglycan (GAG) Degradation

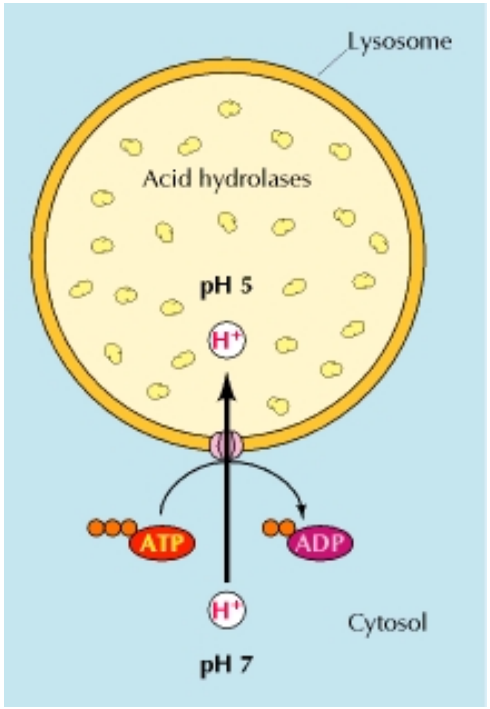
HS, KS and DS, structural carbohydrates found in the cornea, cartilage, bone, skin, extracellular matrix and at the plasma membrane



Source: Michael W. King: Integrative Medical Biochemistry Examination and Board Review, www.accesspharmacy.com

Function of the Lysosome





- subcellular electron dense organelle
- filled with >50 hydrolytic enzymes: will break down all biological macromolecules
- low pH (~5.0), membrane bound
- Considered the 'gut' or garbage disposal unit of cell
- Material for degradation trafficked to lysosome via macrophagy, endocytosis or autophagy
- Lysosomal enzymes trafficked to lysosome via M6P receptor pathway

Function of the Lysosome

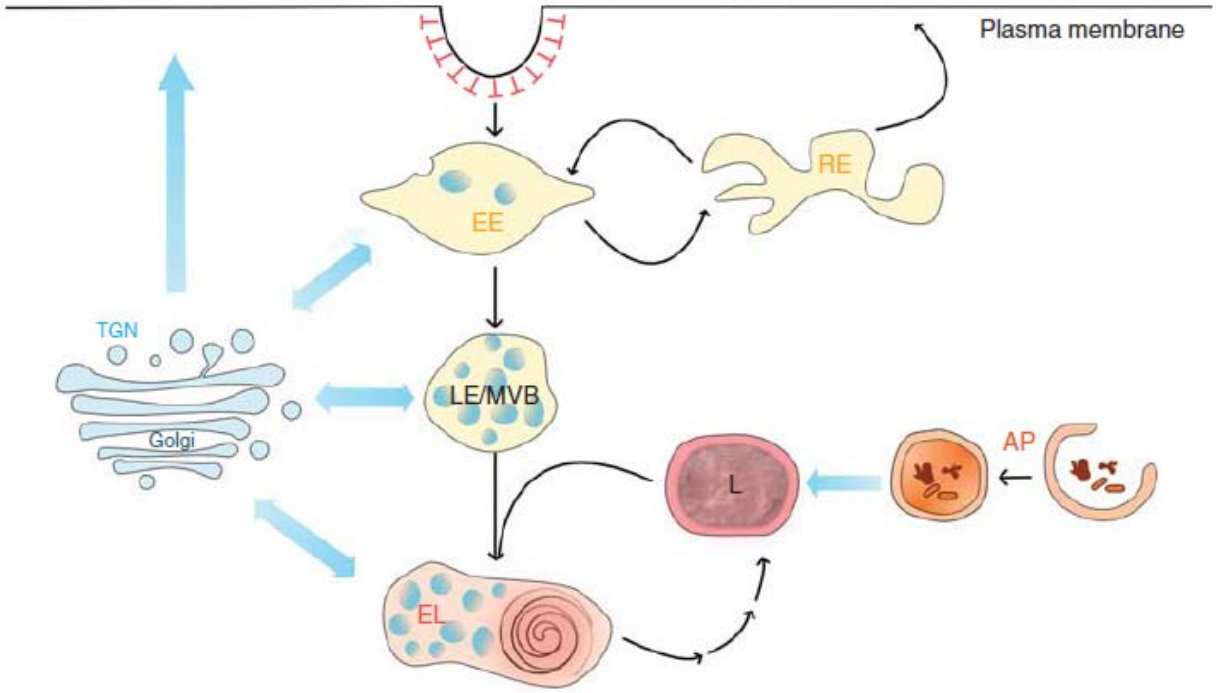
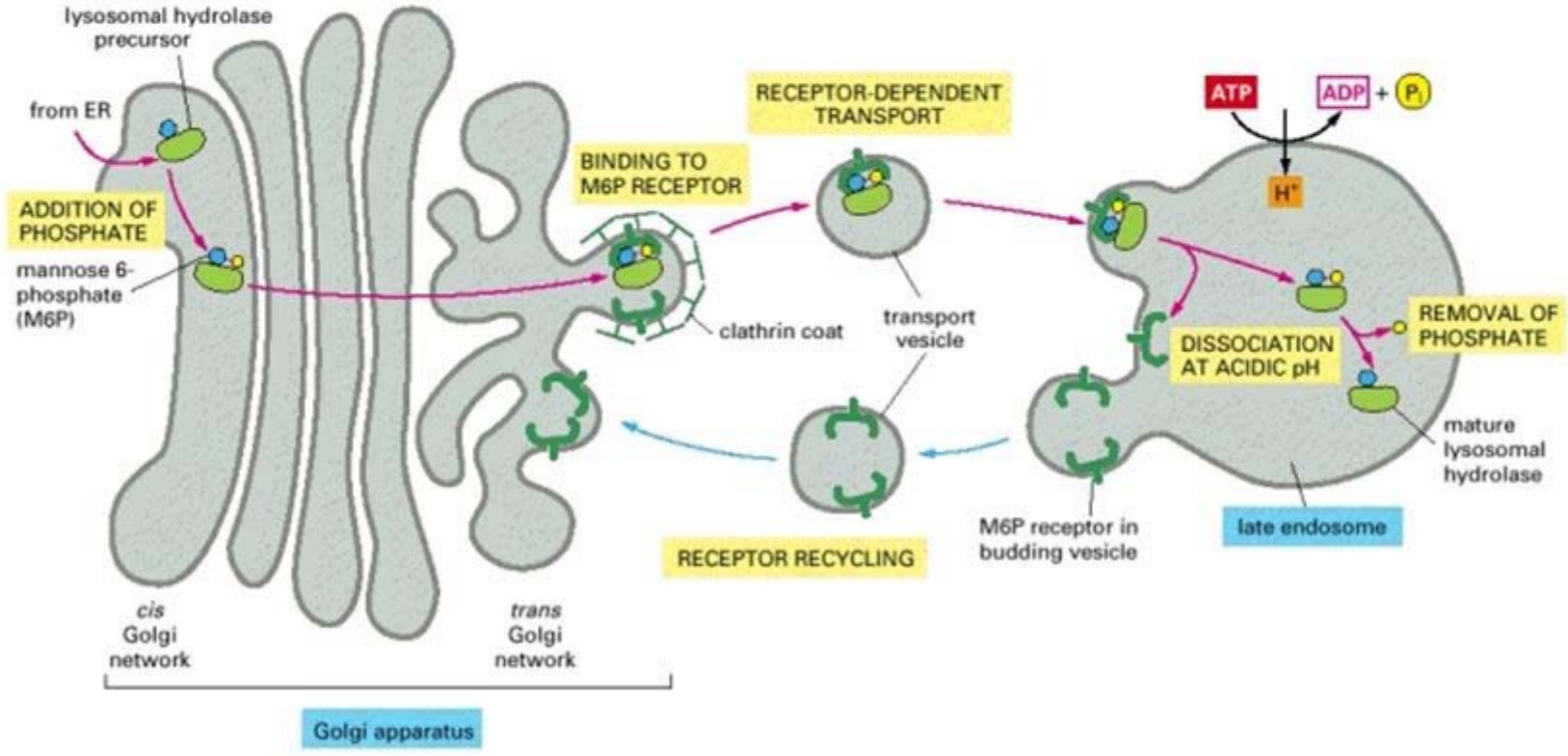


Figure 1. Delivery to lysosomes. Lysosomes (L) are terminal compartments of the endocytic and autophagic pathways (AP). Newly synthesized lysosomal proteins are delivered to them from the *trans*-Golgi network (TGN) via early endosomes (EE), recycling endosomes (RE), and late endosomes/multivesicular bodies (LE/MVB). Following lysosome fusion with late endosomes to form an endolysosome (EL), lysosomes are re-formed by a maturation process.

The Biogenesis of Lysosomes and Lysosome-Related Organelles
Luzio et al. 2014 Cold Spring Harbour Perspectives in Biology 6:a016840

Transport of newly sythesized lysosomal hydrolases to lysosomes

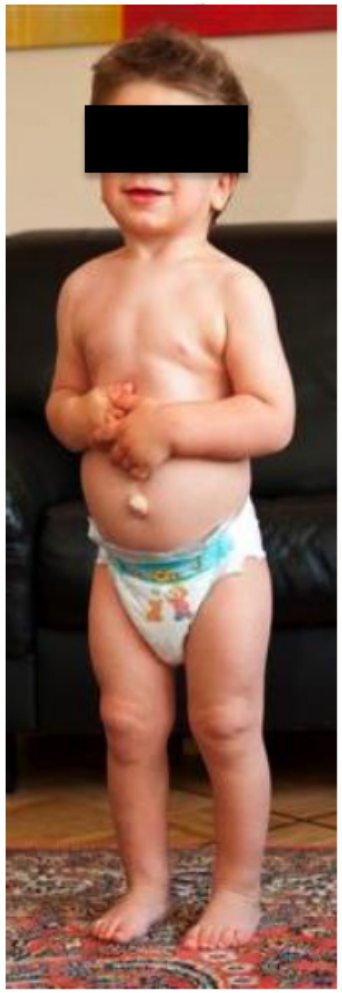
- **Lysosomal proteins** are synthesized in the RER and transported to the Golgi complex, just like secreted proteins. However, enzymes in the Golgi recognize and tag lysosome-bound proteins by phosphorylating the mannose residues.
- The **mannose-6-phosphate groups** are recognized by the mannose-6-phosphate receptors (MPR) at the trans Golgi network and hence the recognition signals for packaging.
- At late endosome / lysosome, the protein will dissociate from the receptors and the receptors can then be recycled.



MPS: A Multisystemic Disease

Respiratory:
Upper airway obstruction
Obstructive sleep apnea/snoring
Restrictive lung disease
Frequent infections
Restrictive airway disease
Rhinorrhoea

Skeletal:
Degenerative hip dysplasia
Kyphosis *or*
Kyphoscoliosis
Gibbus
Joint contractures
Genu valgum deformities



Cardiac:
Cardiomyopathy
Dysplastic valves
Heart murmur

Gastrointestinal:
Hepatosplenomegaly
Umbilical & inguinal hernia
Swallowing problems
Diarrhoea
Drooling

Peripheral nervous system:
Peripheral nerve entrapment (eg, carpal tunnel syndrome)

MPS: A Multisystemic Disease

CNS:

Hydrocephalus
atlanto-axial instability
Cervical cord compression
Myelopathy
Seizures
(Severe behaviour problems)
Sleep disturbance
Mental retardation
Developmental delay

Appearance:

Coarse face
Short stature, short neck

Eyes:

Glaucoma
Retinal dystrophy
Corneal clouding

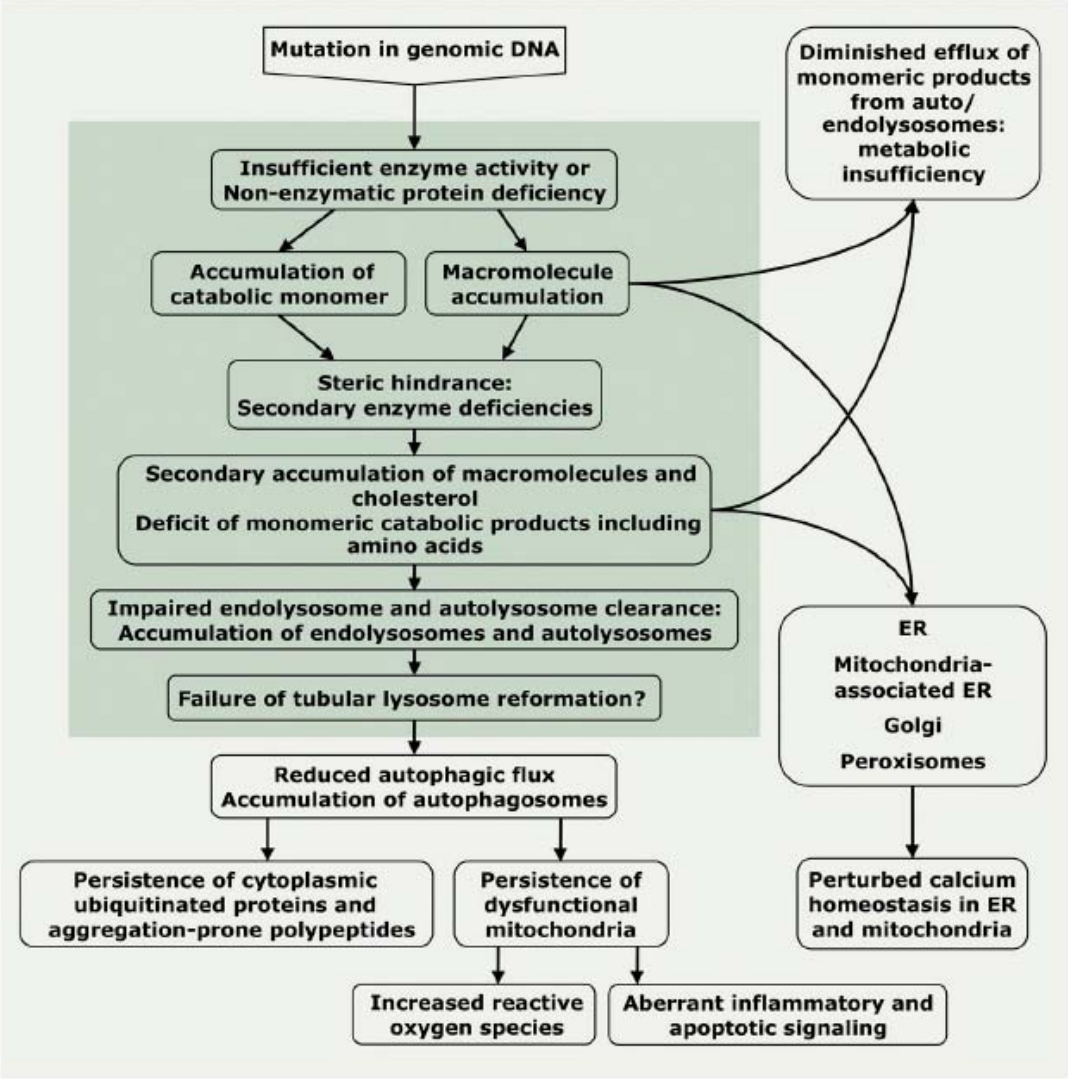
Ears:

Recurrent otitis media
Hearing loss

Dental:

Caries
Dental abscesses
cysts

Model of Cell Pathology in LSDs



Hypothetical cascade of events

- Lysosomal events are in the shaded region
- Events in the cytoplasm, autophagosomes, ER, Golgi, peroxisomes and mitochondria are outside the shaded region
- Processes have been observed in a number of LSDs but do not necessarily apply to all.

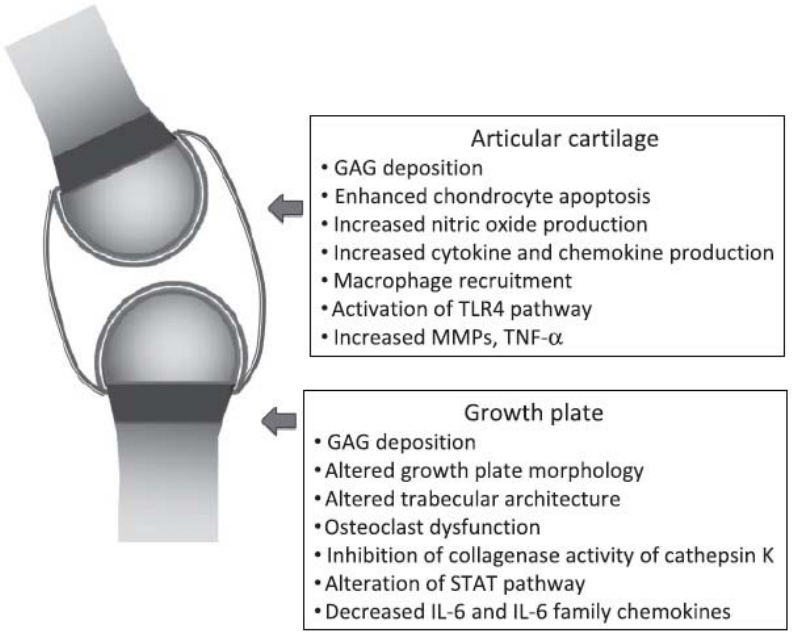
Lysosomal Storage Disorders: The Cellular Impact of Lysosomal Dysfunction.
Platt et al. (2012) J. Cell Biol. 199:723

The Pathology of Lysosomal Storage Disease

Mechanisms of Bone and Joint Disease in MPS

Stiff joints contractures and poor mobility

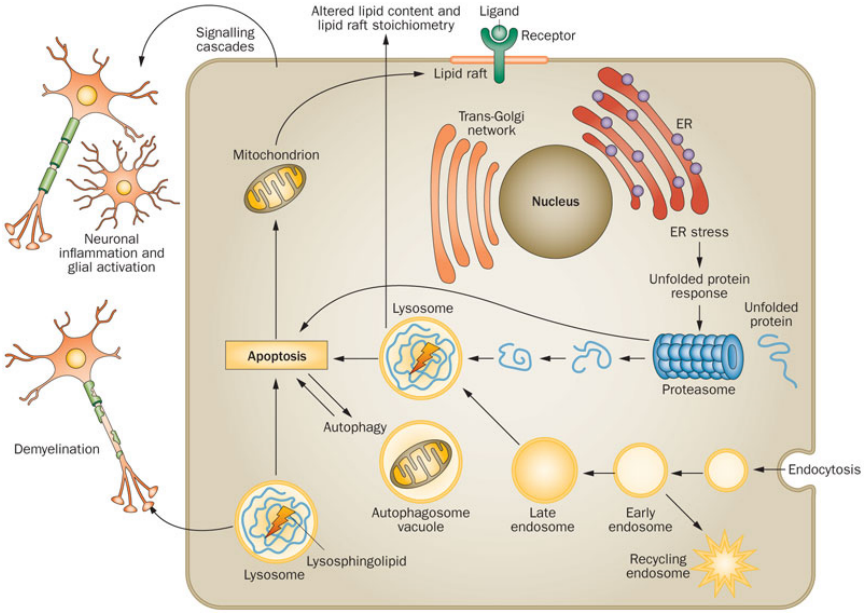
Assumption: tissue disease is caused by excessive accumulation of an inert storage material
Observation: Disease is progressive but most tissues do not show progressive accumulation



A Complex Pathogenic Cascade

- GAGs biologically active molecules as parent molecule and complex with proteins as proteoglycans
- Articular chondrocytes (dermatan sulphate production) apoptosis
- Dermatan sulphate similar to lipopolysaccharide (LPS) – the endotoxin found on gram-negative bacteria
- Elevated LPS binding protein, TLR4, CD14 and CXCR4
- Activation of TNF-α, IL-1β and macrophage inflammatory protein
- Disordered growth plate chondrocyte organisation
- Defects in endochondrial ossification
- Inhibition of the collagenase cathepsin K due to localised accumulation of heparan and dermatan sulphate
- Reduced number of cells in the proliferative zone

Neurons of the CNS and PNS are particularly susceptible to LSDs – neuropathology in two thirds of LSDs



Cellular pathology

- Accumulation of secondary substrates: gangliosides GM1 (monosialotetrahexosylganglioside), GM2 and cholesterol.
- Activation of signal transduction pathways: e.g. MAPK/ERK and Akt)
- Disturbances of lipid trafficking: some suggest shortage of simple lipid and carbohydrate substrates due to lack of recycling)
- ER stress and unfolded protein response: detection of unfolded protein in the lumen of the ER – halts translation, prolonged can lead to apoptosis
- Neuroinflammation - Gliosis: proliferation or hypertrophy of astrocytes, microglia activation – cytokine release – infiltration of macrophages
- Demyelination: Oligodendrocyte dysfunction
- Dysregulated autophagy – inefficient mitochondrial breakdown
- Apoptosis

Other Common Features of LSD Pathophysiology

and proposed causal mechanisms

Corneal clouding/opacification

GAG accumulation in corneal epithelium – disruption of the optically important arrangement of collagen fibrils. GAG accumulation in anterior chamber causes ocular hypertension and glaucoma

Respiratory disease

Upper and lower airway obstruction, cranial and spinal abnormalities (flat nasal bridge, short neck, high epiglottis, abnormal mandible, abnormal cervical vertebrae) GAG deposition in mouth nose and throat. Gingival hyperplasia, mucosal oedema, mucoid secretion.

GI Tract disease and hepatosplenomegaly

Impaired function of the neurons in the myenteric plexus, poor motility – diarrhea. Hepatosplenomegaly common in Gaucher's where glucocerebroside accumulates in spleen, liver, lung and bone marrow.

Hearing defects otitis media

GAG accumulation in the nasopharynx affecting eustachian tube function leading to chronic otitis media. MPSI&II show sensorineural hearing loss which may be due to GAG accumulation in the cochlea or cochlear nerve.

Dental abnormalities

Impairment of BMP signalling in tooth buds during development

Heart Valve and Cardiac Problems

Common in MPS I,II and VI. Valve leaflet thickening, valvular regurgitation, short chordae tendineae, calcification of the mitral annular region. May lead to left ventricular dysfunction including dilatation or hypertrophy.

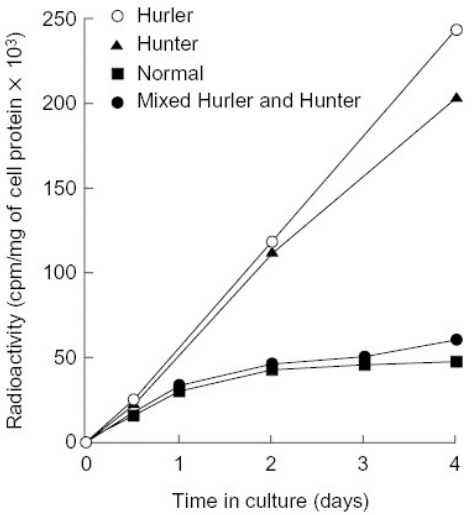
Treatment options

- *Enzyme replacement therapy (ERT) – several approved*
  - Fabry — Fabrazyme [Genzyme]
  - Gaucher type I — Cerezyme [Genzyme]
  - MPSI Hurler — Aldurazyme [Genzyme]
  - MPS II Hunter — Elaprase [Shire]
  - MPS IVA Morquino — Vimizim [BioMarin]
  - MPS VI Maroteaux-Lamy — Naglazyme [BioMarin]
  - Pompe — Myozyme, Lumizyme, [Genzyme]
- *Allogeneic Hematopoetic Stem Cell Transplantation*
  - Bone Marrow Transplant
  - Umbilical Cord Blood
- *Gene therapy – several in clinical trial stages*
  - Ex vivo HSC transduction
  - In vivo – intracranial or intrahepatic injection
- *Substrate reduction therapy (SRT) – several approved*
  - Gaucher and neurological Niemann-Pick type C — Miglustat [Oxford GlycoSciences]
  - Fabry — Migalastat [Amicus]
  - Gaucher — Eligustat [Genzyme]
- *Gene editing using CRISPR-Cas9 modification of endogenous allele*
  - ?

Enzyme Replacement Therapy - Discovery

First suggested by De Duve 1964 – "*it may be well to keep in mind that any substance taken up intracellularly in an endocytic process is likely to end up within lysosomes … opens up possibilities for replacement therapy*"

The Neufeld '*cross-correction*' experiment (1968): Fibroblasts from MPS I (Hurler) and MPS II (Hunter) mixed together in the same dish corrected the ^{35}S-mucoploysaccharide accumulation. The '**Corrective Factors**' were hypothesised to be enzymes secreted by one cell and taken up by the other



Discovery of the '***uptake signal*** **(1972):** Studies on inclusion-cell disease (I-cell disease) showed that while the fibroblast lysosomes had multiple enzyme deficiencies, enzymes in the surrounding culture medium were not corrective, suggesting they were not taken up into the lysosomes as expected

Kaplan et al. 1977 showed that the best inhibitor of β-glucuronidase uptake was mannose-6-phosphate (by competitive inhibition).

Addition of **mannose-6-phosphate** to enzymes permitted cellular uptake and turned out to be the same signalling system used by cells for targeting nascent hydrolases to lysosomes from the golgi.

Enzyme Replacement Therapy – First Success

Gaucher's Disease Type I (non neuropathic): Thought to be a good candidate because development is relatively normal, minimal neurological impairment and the main problems of liver and spleen enlargement and bone lesions might be reversible if caught early.

Brian Berman: Diagnosed with Gaucher's in 1983 (Age 3). In 1984 entered the first ERT clinical trial run by Roscoe Brady. Brian was the only child enrolled. Used glucocerebrosidase isolated from human placental tissue and modified with the uptake signal.

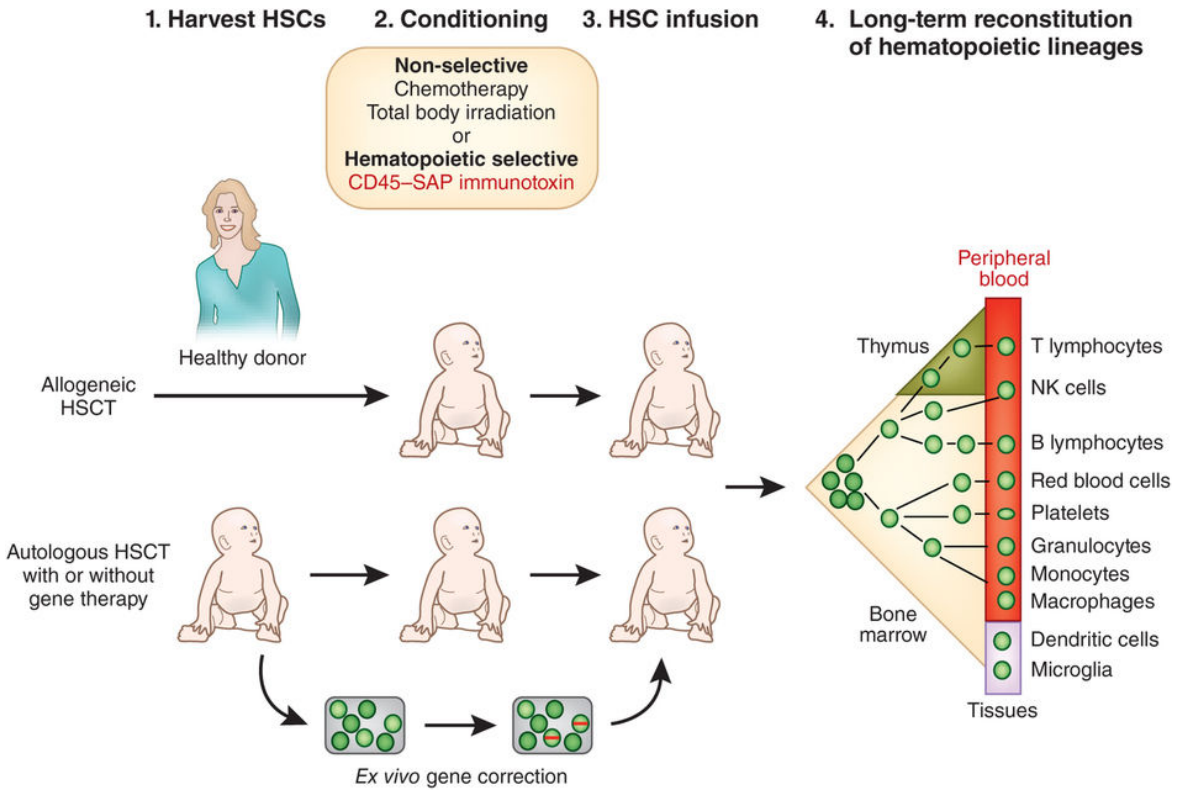
Brian Berman Today: Has joint problems and compromised immune system but otherwise healthy. Glucocerebrosidase now made using recombinant DNA technology in cell lines and modified for human use. Sold as Cerezyme delivered IV 2.5U/kg every 2 weeks. Costs $200,000 per annum per patient.

- **Current ERT treatments cannot rectify neuropathology because the proteins are unable to cross the blood-brain barrier.**

HSC Therapy for LSDs

Allogeneic Hematopoetic Stem Cell Transplant: Not gene therapy but similar principal of cross-correction from cells that secrete the normal enzyme. Thousands performed. Very successful in some types

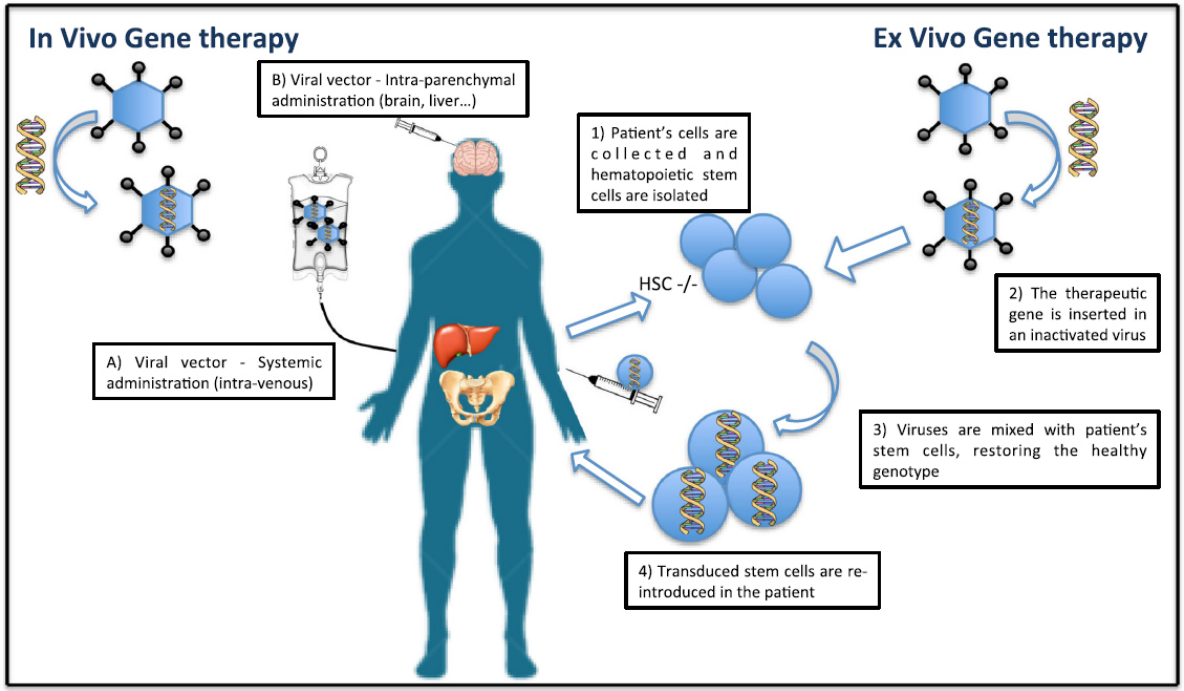
Aldenhoven et al. (2015): 56 MPS I (Hurler) patients treated with bone marrow transplants in early childhood. Considerable residual disease burden. CNS preservation is dependent on how early provided. Overall survival (95.2%) event-free survival (90.3%). Neurological outcomes poor in those patients that were already showing signs in infancy.

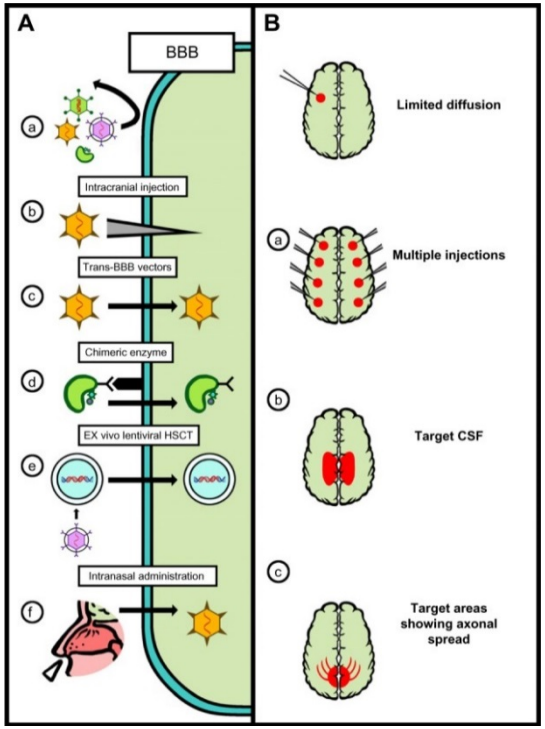


Gene Therapy

Ex vivo gene therapy using Autologous HSCT: Lentivirus or Adeno-associated virus vectors. 2010 (Milan) Non-randomised open-label trial Lentiviral transduction of HSC for the treatment of metachromatic leukodystrophy (lack of arylsulfatase – ARSA – [sphingolipidosis]). 2016, Results published on 9 patients: stable engraftment and ARSA production including in CSF. Gross Motor Function Measurement scores similar to normal, IQ in 8/9 within normal range.

In vivo gene therapy: adaptive immune response to viral proteins or the transgene are a major problem. Liver-directed gene therapy limits response and seems to induce tolerance. Intracranial injections for neurological LSDs, infantile neuronal ceroid lipofuscinosis (INCL) and MPSIIIA. INCL trial unsuccessful due to lack of immunosuppression. MPSIIIA trial showing reduced neurological decline so far.



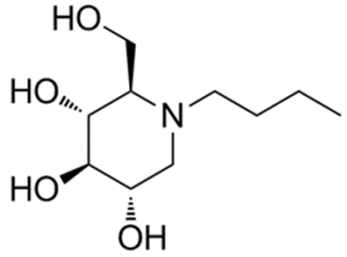


Substrate Reduction Therapy (SRT)

- Partial inhibition of substrate biosynthesis reducing impact of disease
- Orally bioavailable small molecule drug
- Penetration to all cells in the body including restricted access sites – bone and brain
- No adaptive immune response complications
- Very cheap to manufacture

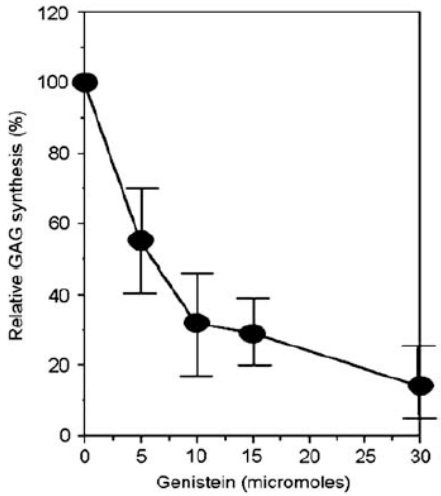
Development of Miglustat for Niemann-Pick and Gaucher's Disease

- Glycosphingolipids (GSLs) are synthesised in the Golgi apparatus by the addition of monosaccharides to ceramide using glycosyltransferase enzymes
- Once the GSL has finished its job it is normally recycled in the lysosome
- SRT would involve a drug to block the enzyme **ceramide glucosyltransferase**
- **N-alkylated imino sugars** mimic the monosaccharides used in biosynthesis and bind to the enzyme
- **N-butyl-deoxygalactonojirimycin (*N*B-DGJ):** was found to be effective and had good drug-like properties
- After animal based studies, clinical trials began in 1998 in Type I Gaucher's Disease
- Approved in 2002 by EMA and 2003 by FDA for mild-moderate GD1 treatment where ERT was not recommended
- Subsequent work by the same group led to the development of the improved **Eliglustat** (FDA approved 2014, EMA 2015)

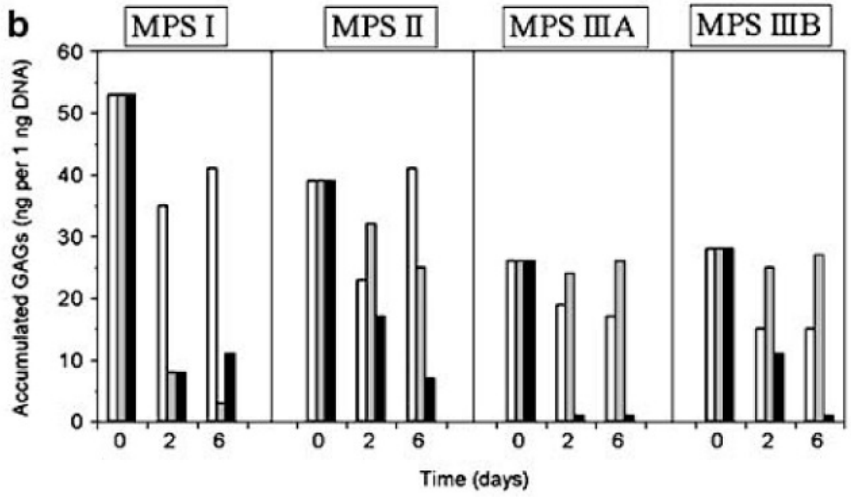


(*N*B-DGJ): Miglustat (Zavesca) Oxford GlycoSciences marketed by Actelion

Genistein – potential novel SRT agent for the neuropathic mucopolysaccharidoses



GAG synthesis inhibited in fibroblasts derived from MPS I patient with Increasing concentrations of genistein



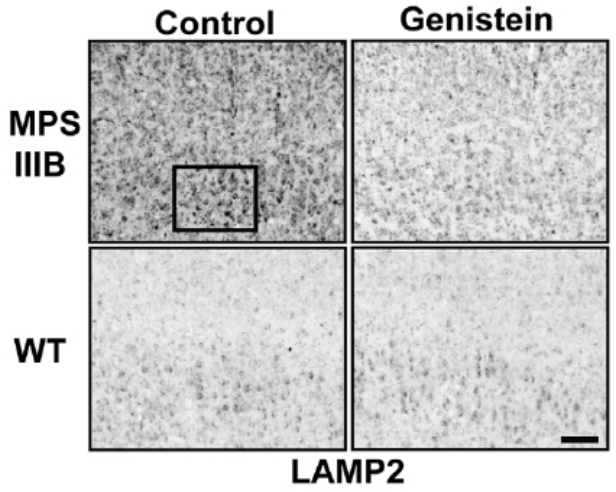
GAG accumulation is reduced in 4 types of MPS by treatment with 10μM genistein. *White* - untreated, *grey* – treated with Aldurazyme, the ERT for MPS I, *black* - 10μM genistein.

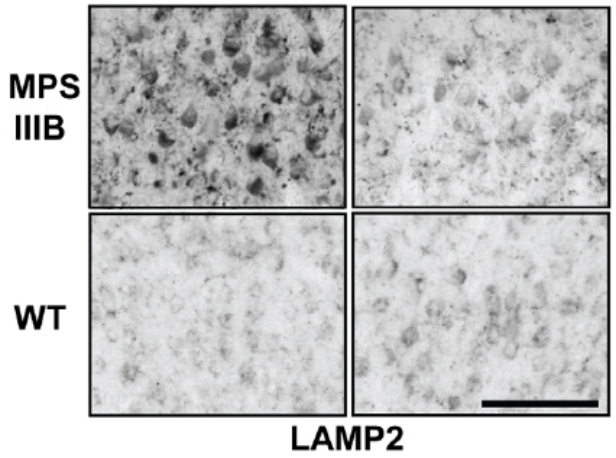
UNIWERSYTET GDAŃSKI

Prof. Grzegorz Wegrzyn

Impact of oral genistein on MPSIIIB mouse brain tissue 11 months

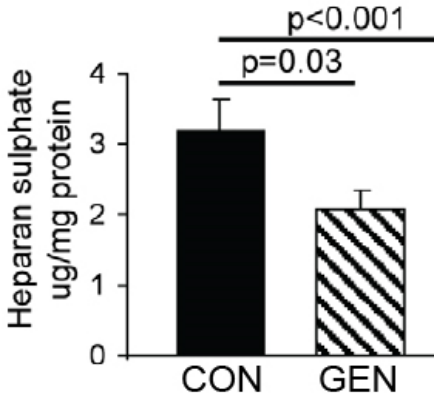
LAMP = lysosomal associated membrane protein





Genistein aglycone (160mg/kg/day)

- Improved hair morphology
- Reduced plasma and urine GAG levels
- Improved behaviour
- Reduced neuroinflammation
- Reduced heparan sulphate in the brain



Dr Brian Bigger

Questions

1. What are the 2 main types of genetic inheritance found in LSDs?

2. Describe the key features of hydrolytic enzyme delivery to the lysosome

3. Why does replacement of the missing enzyme in LSD patients by ERT or gene therapy often cause an adaptive immune response against the normal human protein?

4. What was the critical finding in Neufeld's 'cross-correction' experiment