Sphingolipid structure and function

Targeted cures…

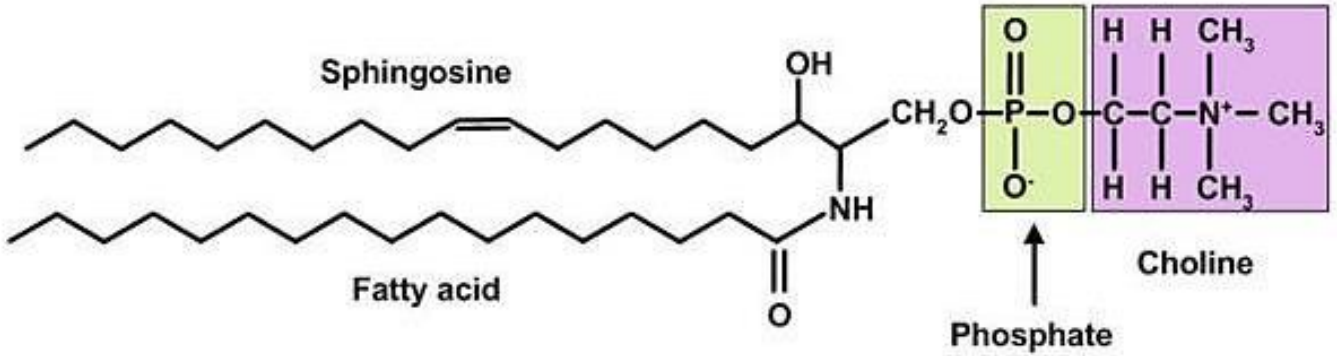
Timothy M Cox

University of Cambridge

ESGLD course, Valpré
Lyon, 14 September 2017

Sphingolipids

Thudichum (1874)



Sphingomyelin

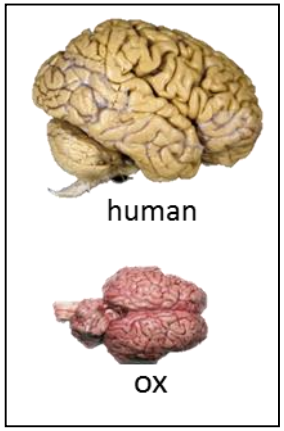
- Sphingolipids are an integral part of the plasma membrane bilayer
- Cell recognition, signal transduction, antigen display, control of proliferation and cell death (up and down), angiogenesis, lymphocyte trafficking, senescence, autophagy, cancer . . .

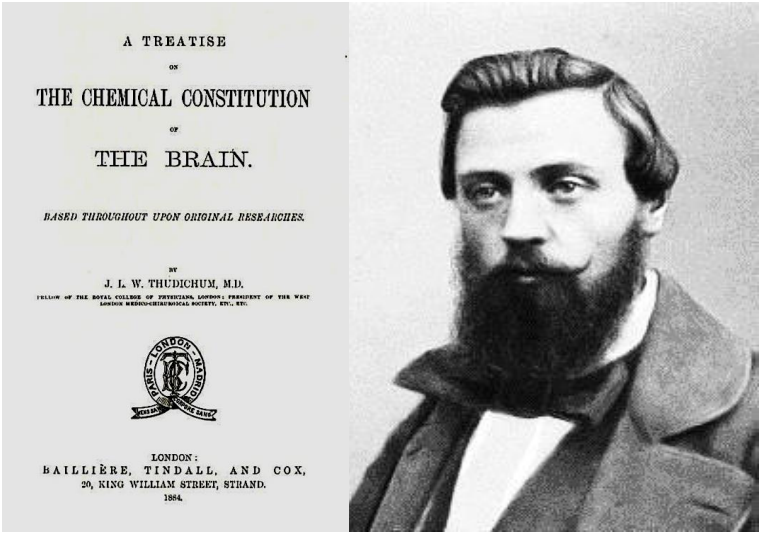
Discovery of Sphingolipids

The Chemical Constitution of the Brain

140 discrete chemicals

- Sulphatides
- Sphingosine
- Sphingomyelin
- Cerebrosides
- Psychosines



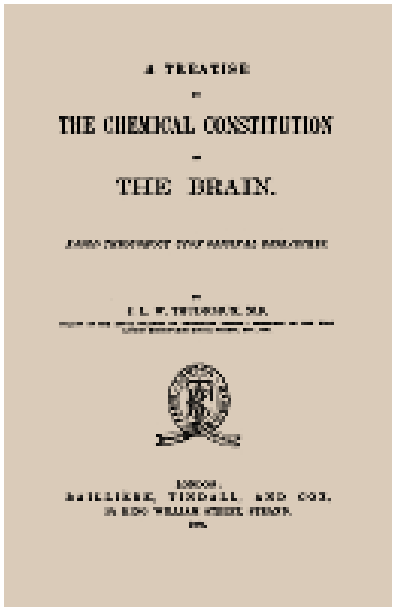

A TREATISE
ON
THE CHEMICAL CONSTITUTION
OF
THE BRAIN.
BASED THROUGHOUT UPON ORIGINAL RESEARCHES.
BY
J. L. W. THUDICHUM, M.D.
LONDON:
BAILLIÈRE, TINDALL, AND COX,
20, KING WILLIAM STREET, STRAND.
1884.

JLW Thudichum
(1829-1901)

'When the normal composition of brain shall be known to the uttermost item, then pathology can begin its search for abnormal compounds or derangements of quantities'

Great Sphinx, Chephren Pyramid, Giza, Egypt

Enigma of the Sphinx


A TREATISE
ON
THE CHEMICAL CONSTITUTION
OF
THE BRAIN.

BAILLIERE, TINDALL AND COX.

THE CHEMICAL COMPOSITION OF THE BRAIN

based throughout upon original researches

JLW Thudichum MD

Baillière, Tindall and Cox
London
20, King William Street, Strand
1884

'In almost all chemolyses of nitrogenised principles by acids or alkalis in watery or or spirituous solution, there has been formed cerebrose (*galactose*), psychosin, sphingosin and neurostearic acid (*or their breakdown products*)'

'In a state of fine powder they are extracted with pure ether in the cold. The fatty acids dissolve, while a body remains insoluble, which is of an alkaloidal nature, and to which, *in commemoration of the many enigmas which it presented to the inquirer, I have given the name of Sphingosin*'

Riddles of the Sphinges

Egyptian

I never was, am always to be

No one ever saw me, nor ever will

And yet I am the confidence of all

To live and breathe on this terrestrial ball

Tomorrow

Greek

Which creature in the morning goes on four legs

at mid-day on two

and in the evening upon three

and the more legs it has, the weaker it be?

Man

Sphingolipids

The Sphinx

A monster that came to Thebes from remotest Ethiopia

Daughter of Typhon and Echidne - or of the dog Orthrus and the Chimera

Woman's head

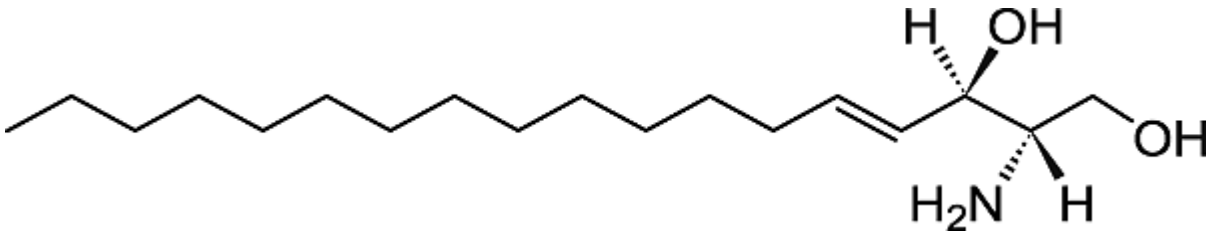
Lion's body

Serpent's tail

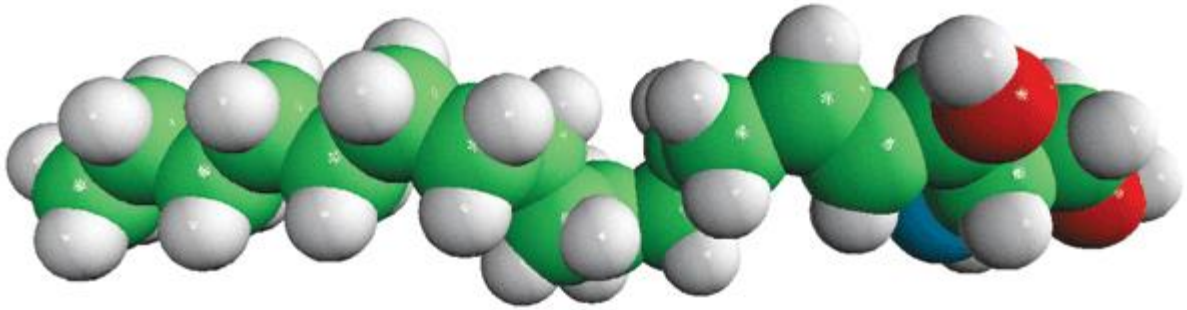
Eagle's wings

Sphingosine

D-*erythro*-sphingosine

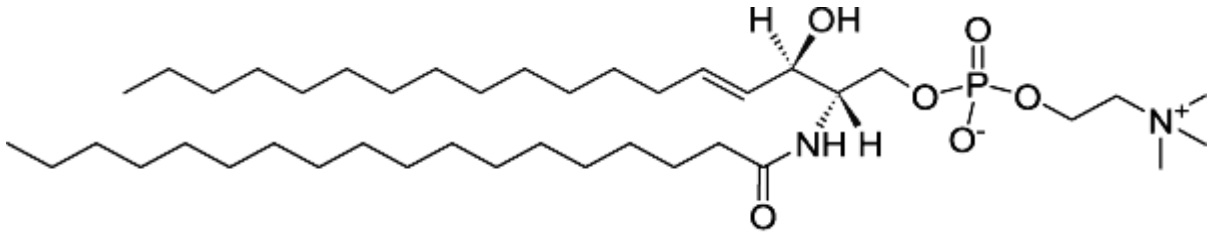


Sphingosine 2S, 3R
(2S,3R,4E)-2-aminooctadec-4-ene-1,3-diol

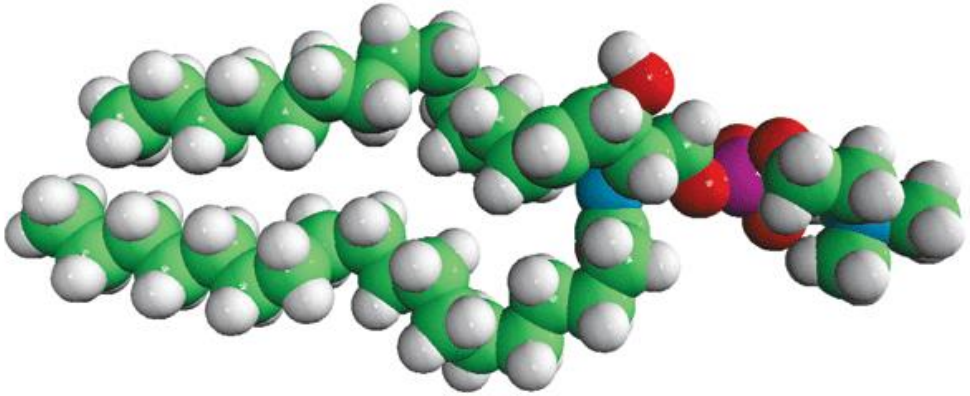


Long-chain amino alcohol – a nitrogenous cationic amphiphile with an unsaturated hydrocarbon chain

Sphingomyelin - Thudichum (1874)



N-octadecanoyl-D-erythro-sphingosylphosphorylcholine [C18]



Sphingolipids

- Lipids with a sphingosine backbone - hydrocarbon chain, amine group and two hydroxyl groups
- The amine is linked to a fatty acid; the (1) hydroxyl group may be functionalized by a phosphate, sugar or other substituent

Sphingolipids (Basic)

- The primary alcoholic function at carbon-1 of ceramide is where further moieties are attached – phosphocholine (in sphingomyelins) and saccharides (in glycosphingolipids)

- A single monosaccharide addition (glucose or galactose) gives rise to a cerebroside.
  - up to 20 sugars in the oligosaccharide chain

- When these sugars are uncharged - neutral glycosphingolipids

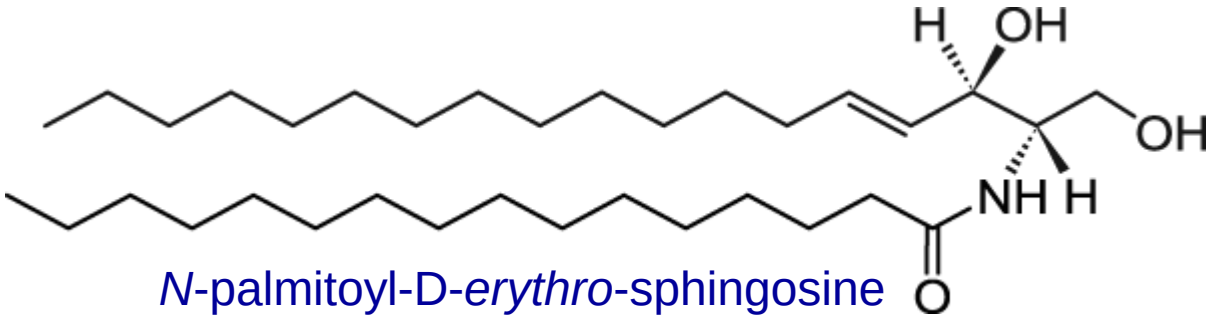
- One or more sialic acids - gangliosides - a sulphate group - sulphatides

- If phosphate is attached at the C-1 carbon - ceramide-1-phosphate

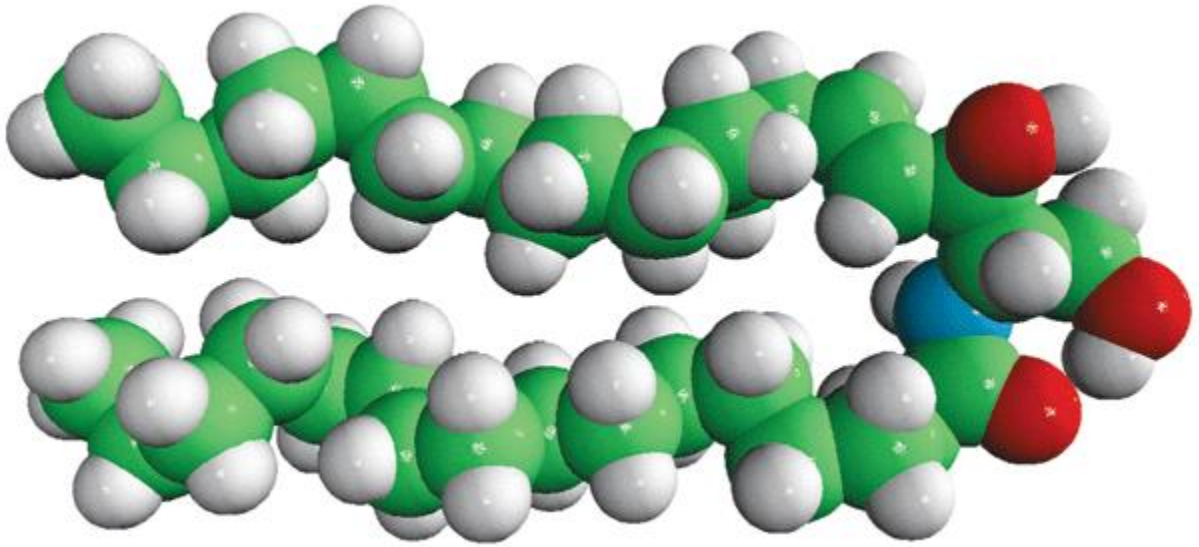
- An additional acyl group at C-1 generates 1-O-acylceramide

Ceramides

C16 Ceramide (d18:1/16:0)

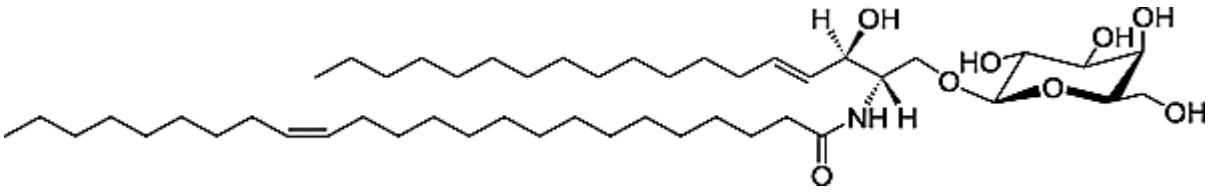


N-palmitoyl-D-*erythro*-sphingosine

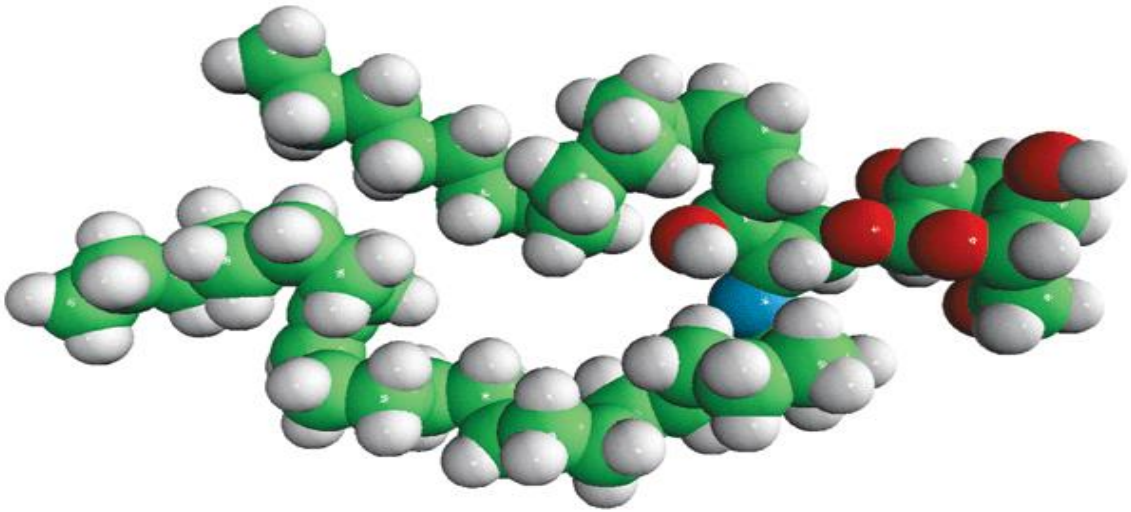


- Very hydrophobic
- Increase membrane permeability
- Segregate laterally into columns
- Induce flip-flop motion of lipids

Galactocerebroside – ‘kerasin’ Thudichum (1874)

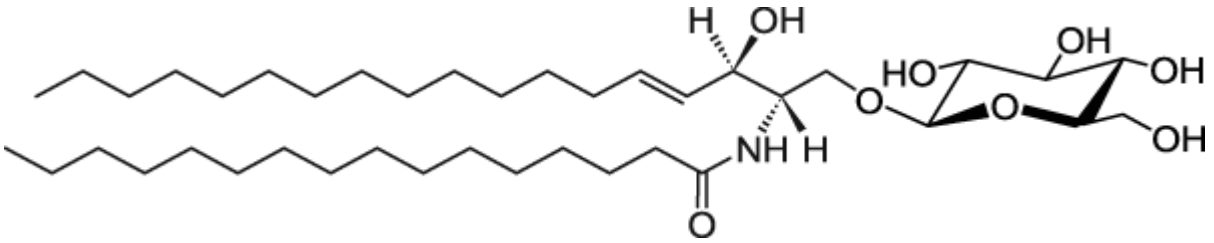


C24:1 ß-D-galactosyl ceramide

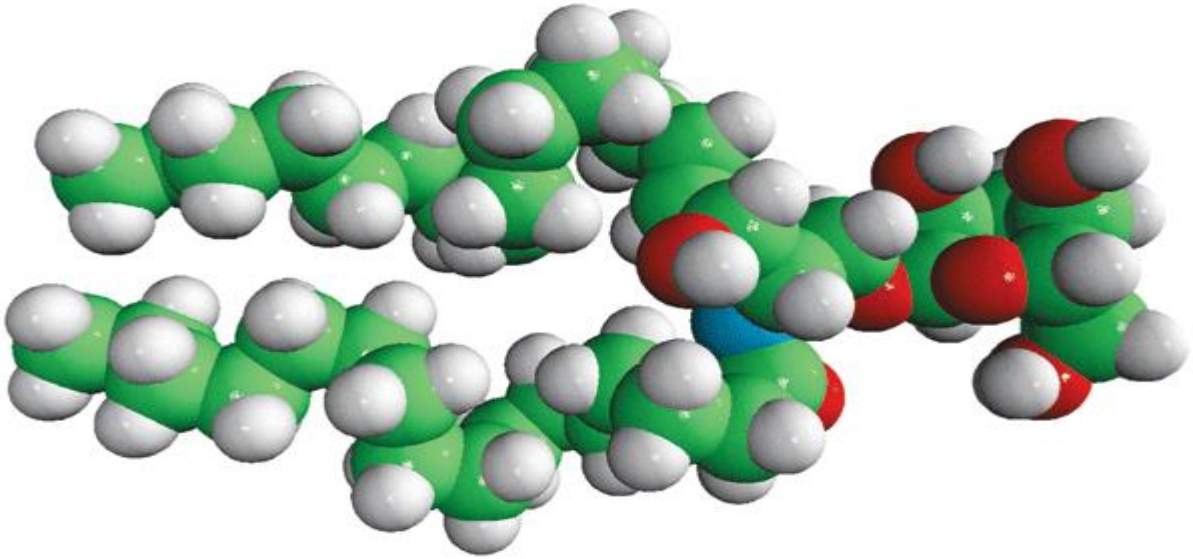


Glucocerebroside

C16 Glucosyl(ß) Ceramide (d18:1/16:0)

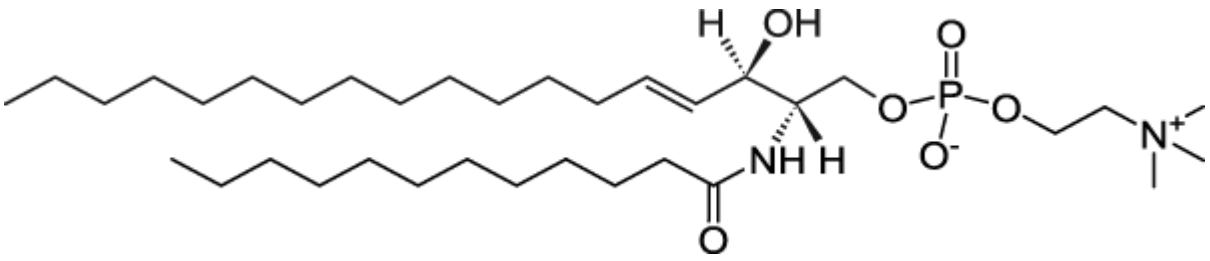


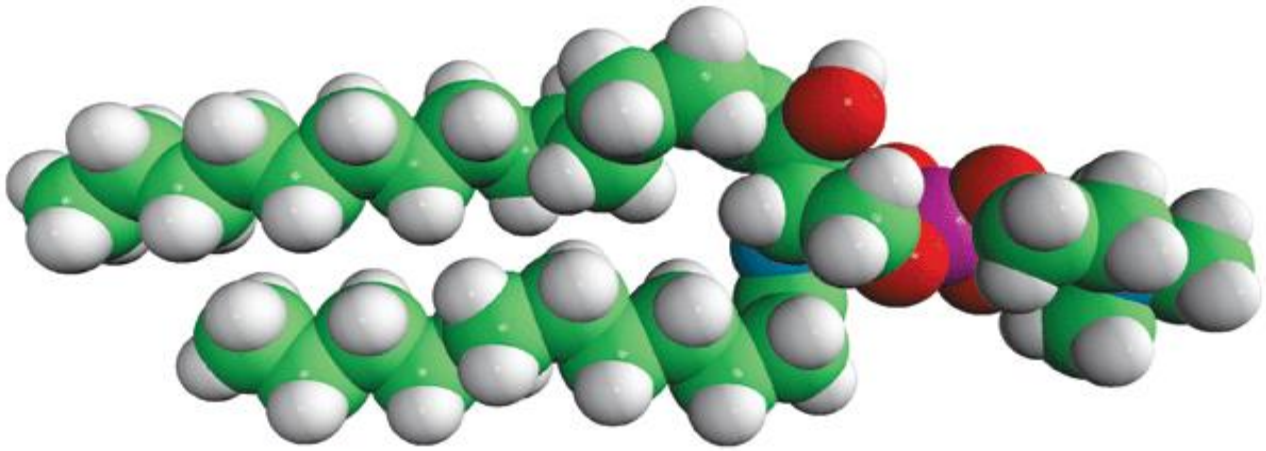
D-glucosyl-ß-1,1' N-palmitoyl-D-*erythro*-sphingosine



Sphingomyelin(s)

Sphingomyelin (d18:1/12:0)



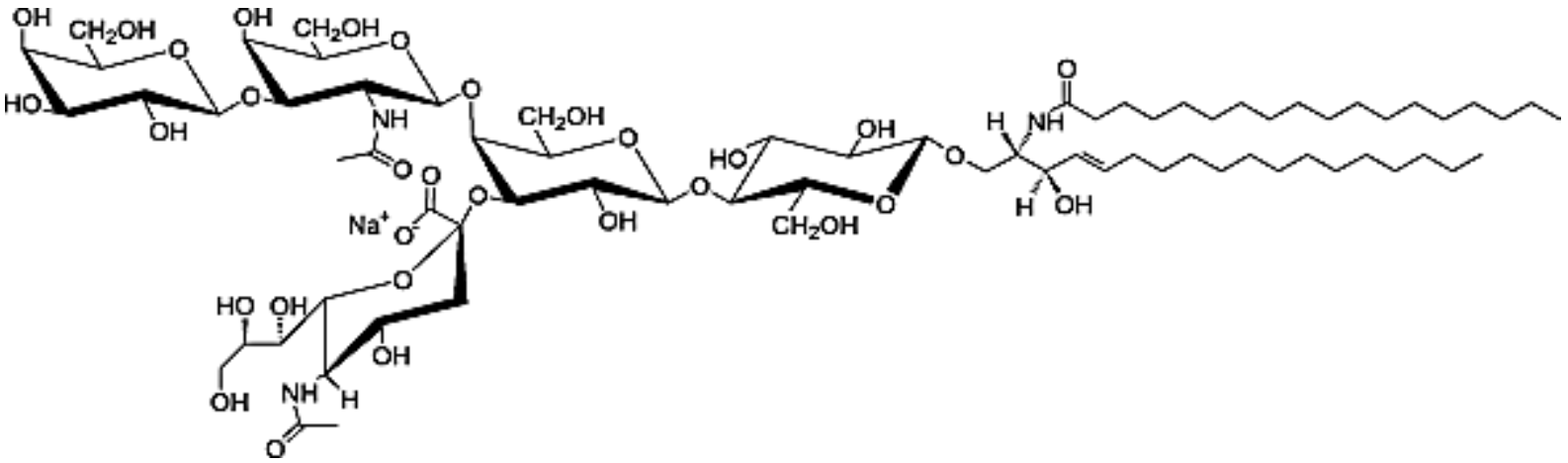


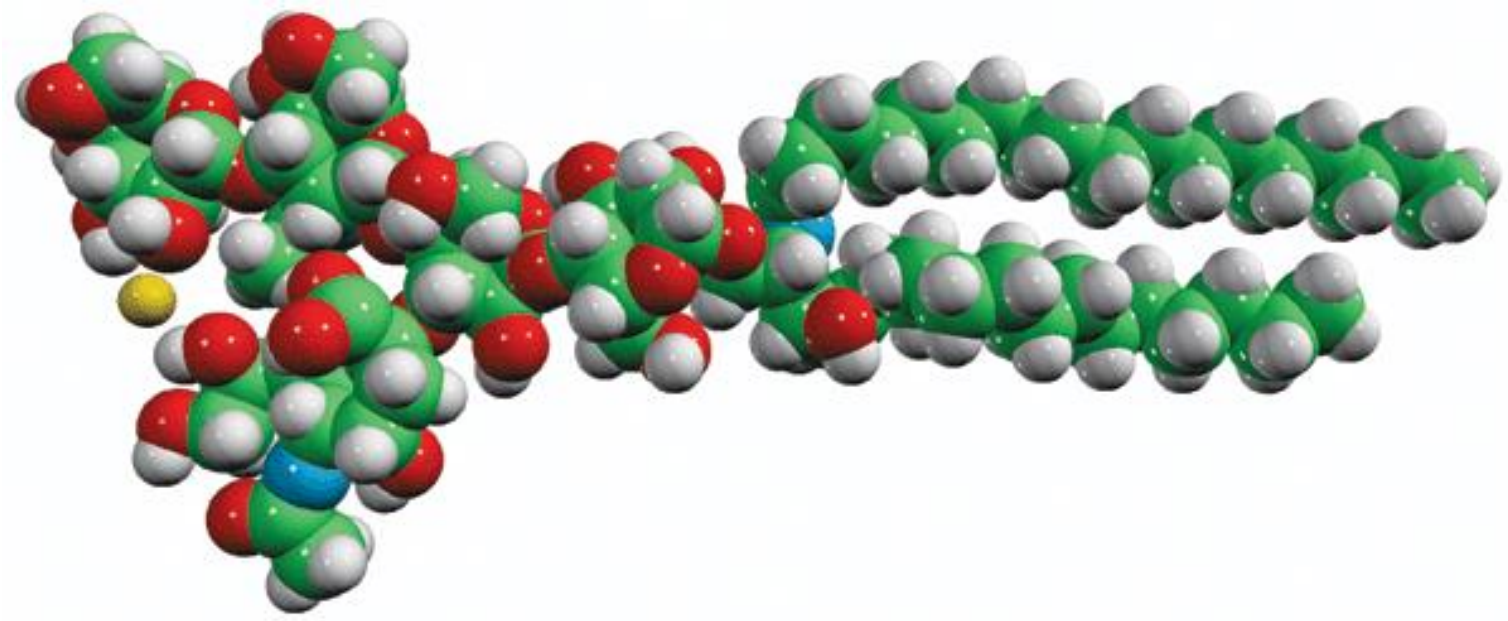
***N*-(dodecanoyl)-sphing-4-enine-1-phosphocholine**

- Intermolecular interactions via 2-amide & 3-hydroxy groups and sphingosine 4,5-*trans* Δ bond
- Sphingomyelin binds with high-affinity to cholesterol, forming tight liquid-ordered domains in in the liquid-disordered membrane phase to form lipid rafts

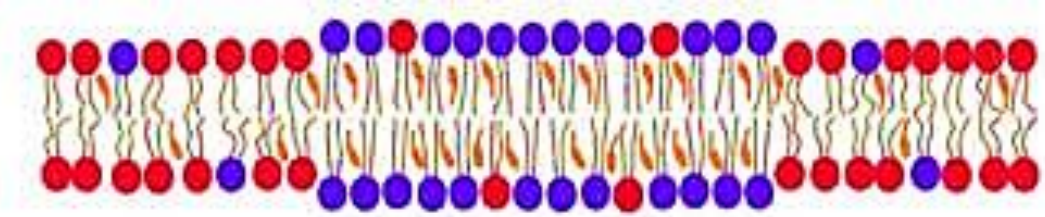
Ganglioside(s)

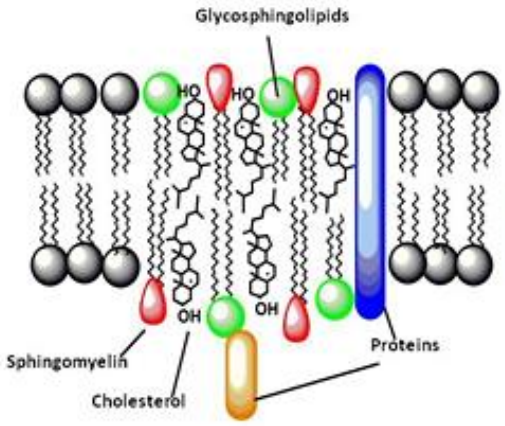
GM1 ganglioside

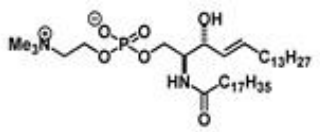




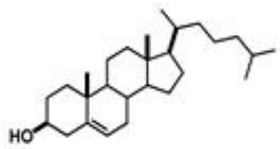
Lipid Rafts







Sphingomyelin (SM)



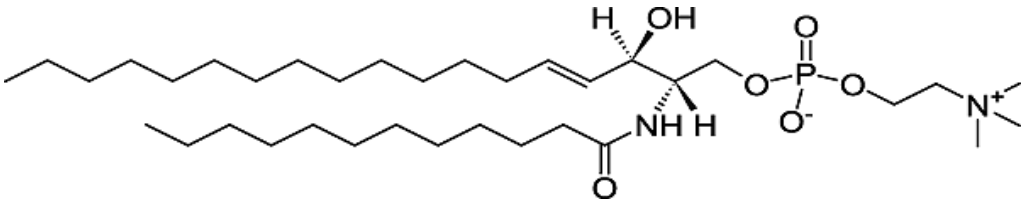
Cholesterol

- **Receptors & Channels**
- **Signalling**
- **Recognition**
- **Viral entry**

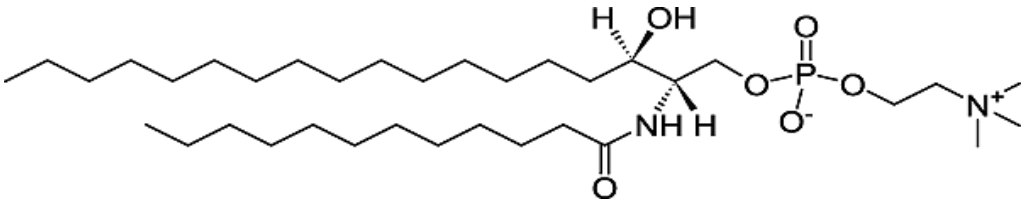
Basic physical chemistry of sphingolipids

- Ceramide moiety highly hydrophobic
- Phosphorylcholine or oligosaccharides strong amphipathic effect
- Different properties phosphorylcholine or phospatidylcholine
- Parafinic tails interdigitate with on both sides of the membrane
- Interactions increase melting point, viscosity & stability
- Greater permeability to electrolytes

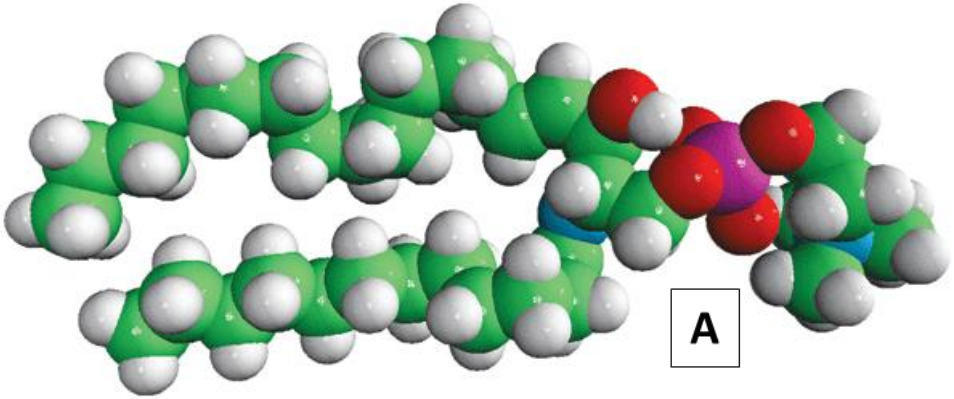
Structural Specification



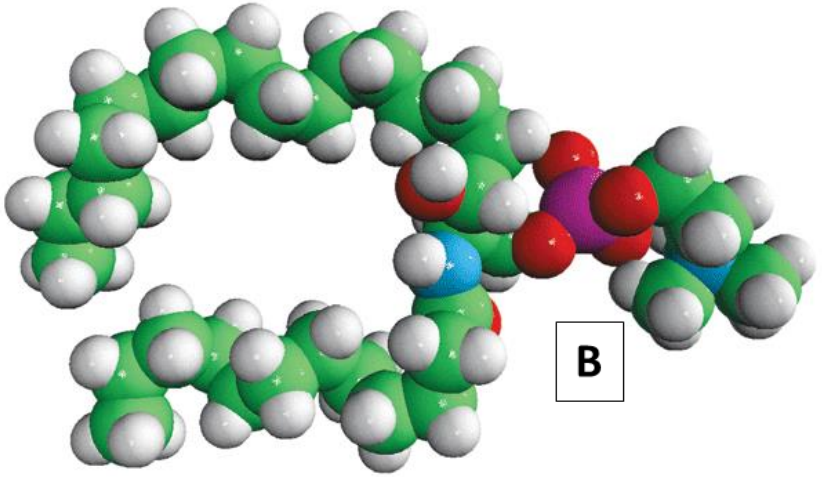
A. *N*-lauroyl-D-erythro-sphingosylphosphorylcholine



B. *N*-lauroyl-D-*erythro*-sphinganylphosphorylcholine

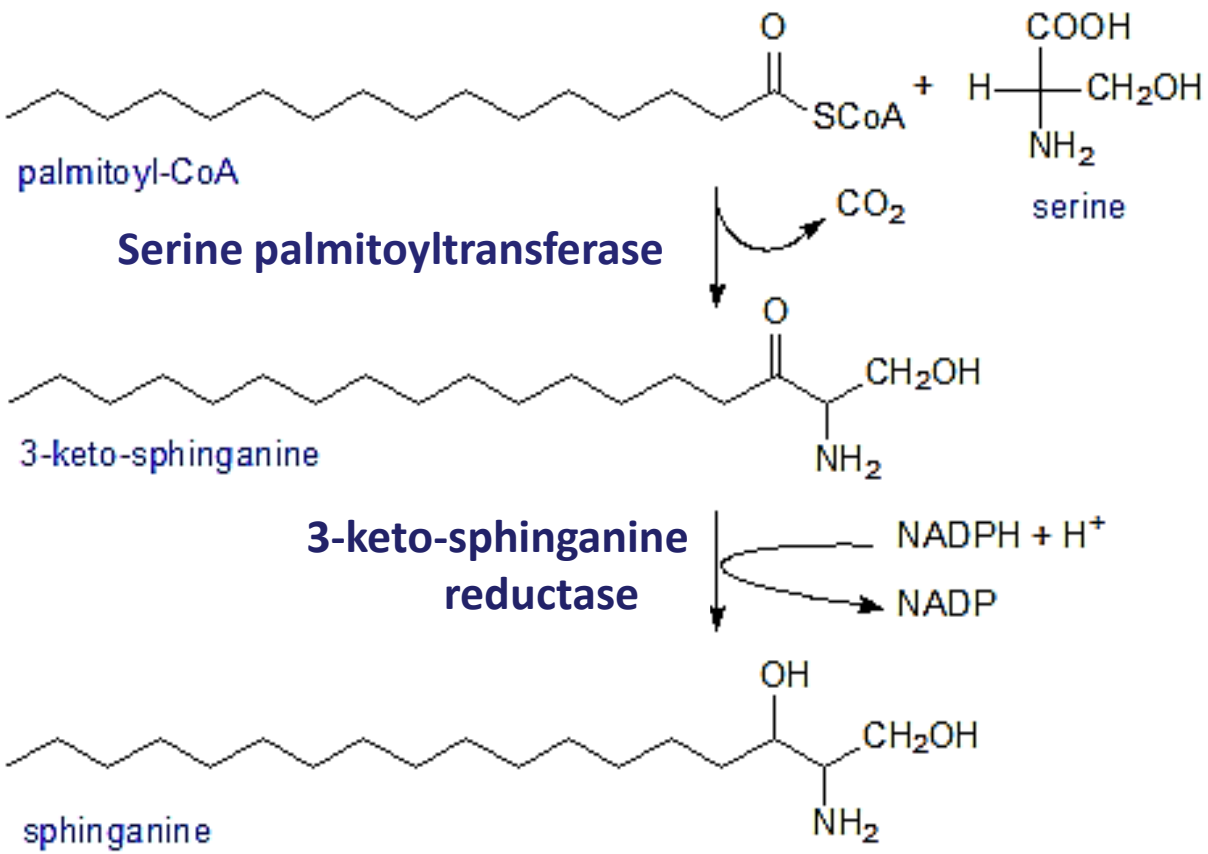


12:0 SM (d18:1/12:0)



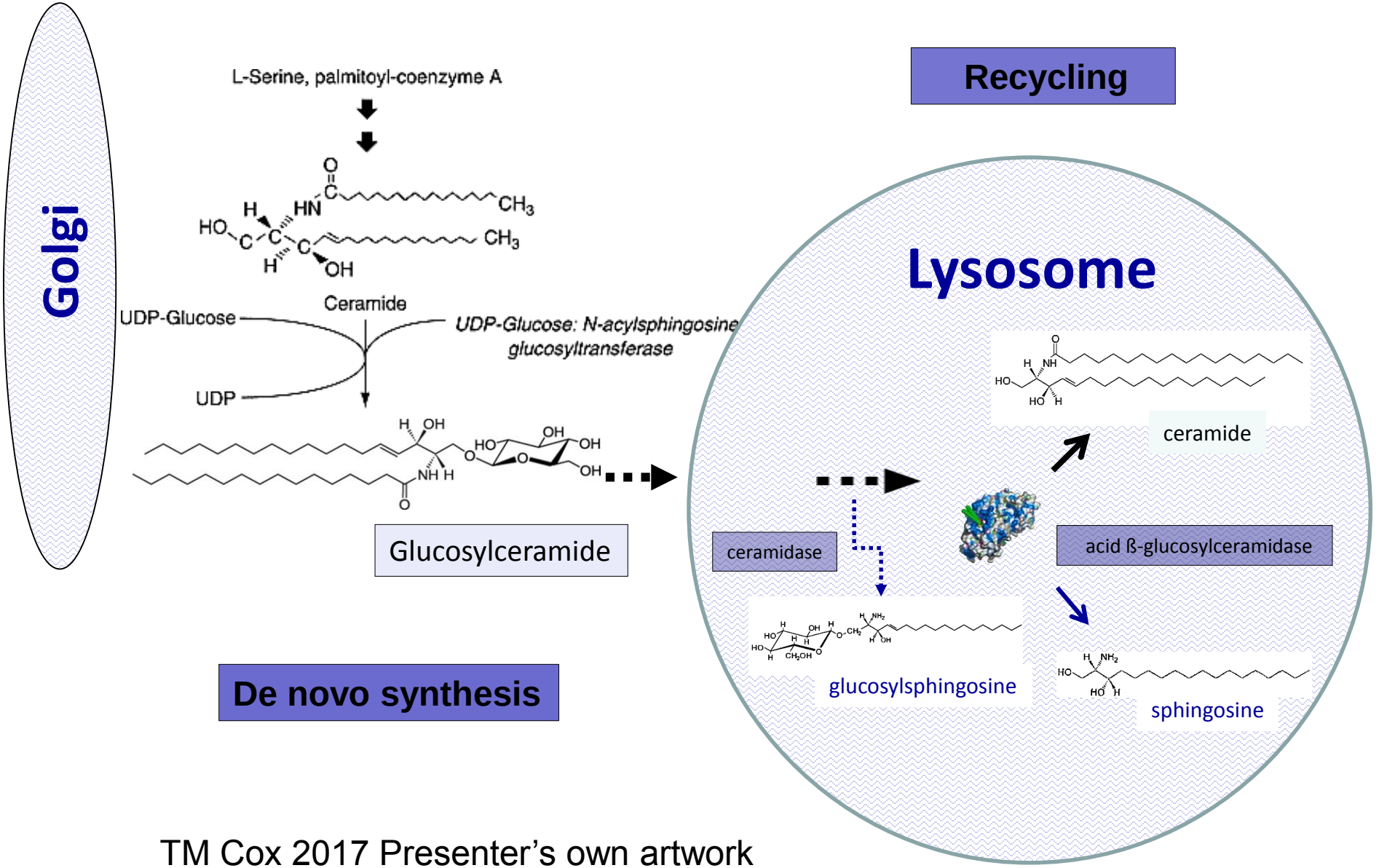
12:0 Dihydro SM (d18:0/12:0)

De novo biosynthesis of sphingolipids



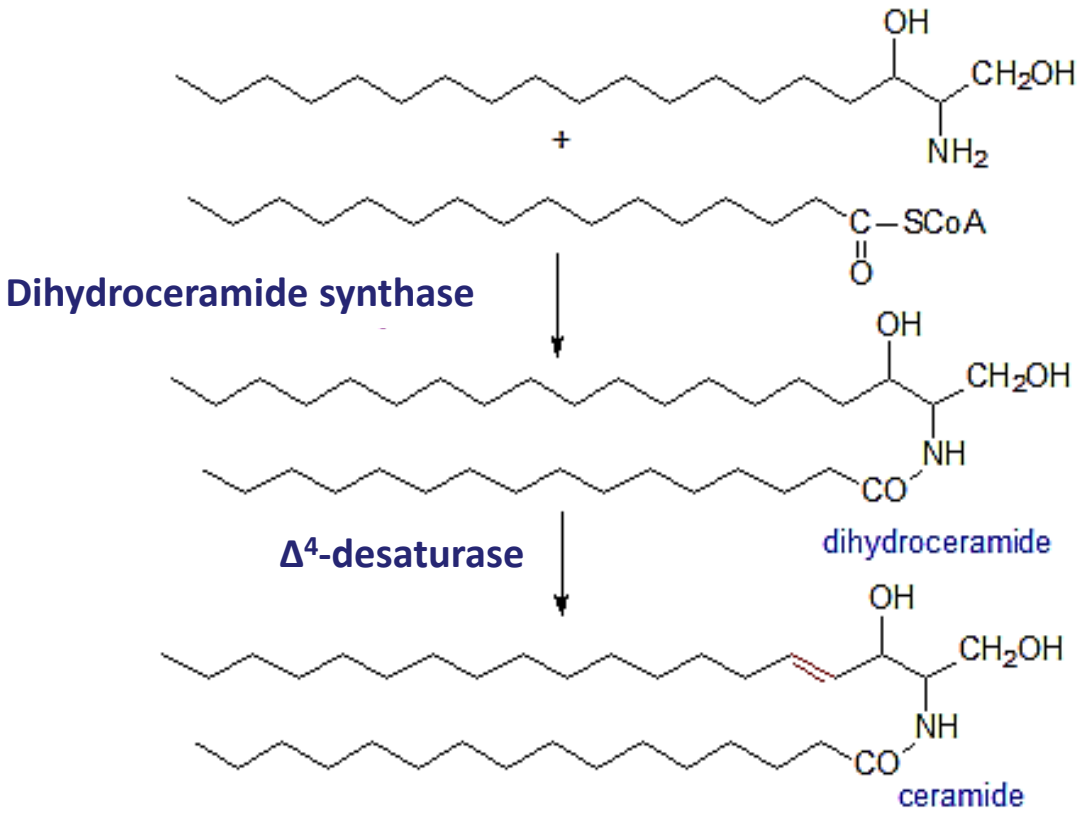
Free sphinganine is rapidly N-acylated by acyl CoA to form dihydroceramides by dihydroceramide synthases located on the cytosolic face of the endoplasmic reticulum - multiple isoforms of this ‘ceramide synthase’

De novo Synthesis and Metabolism of Glucosylceramide



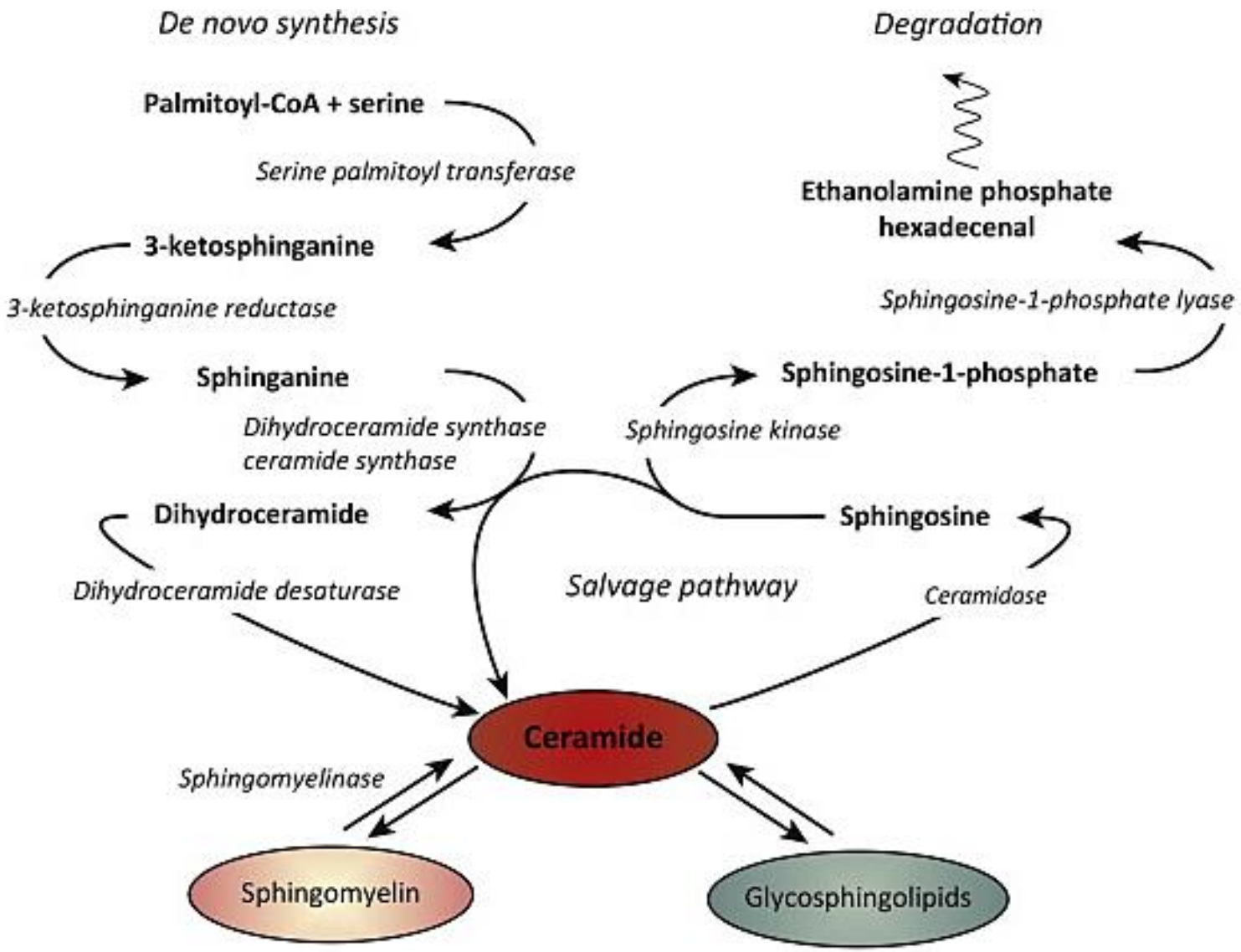
TM Cox 2017 Presenter's own artwork

Addition of long-chain bases (sphingoids or sphingoid bases)



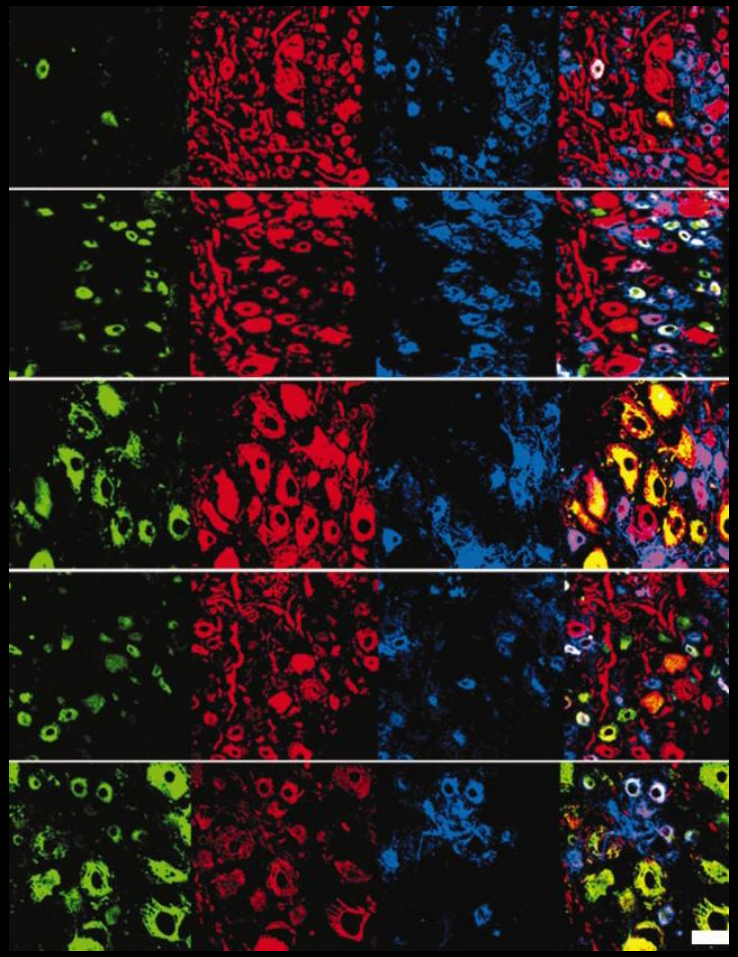
- **C18 aliphatic chain**
- **Hydroxyl groups in positions 1 and 3**
- **An amine group in position 2**
- **Double bond at position 4 in *trans* (E)**

Central Rôle of Ceramide in Sphingolipid metabolism



Gangliosides in Nervous Tissue

MAb NF IB4 Merge



GD1a-1

GD1a-2b

GD1b-1

GM1-2b

GT1-2b

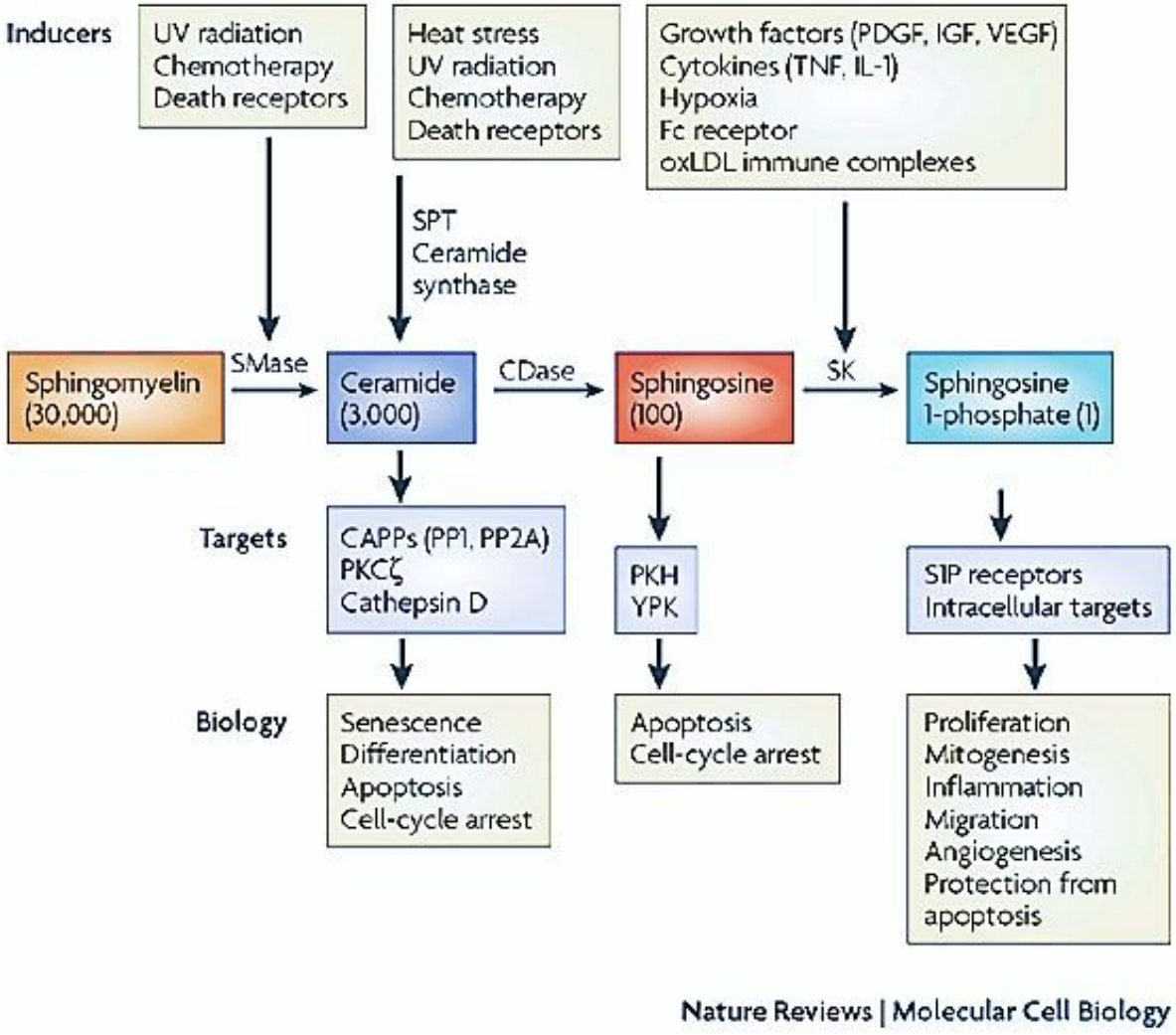
Fresh-frozen rat dorsal root ganglia triple-labelled with anti-ganglioside mAbs (green), neuro®lament (red) & IB-4 (blue). Co-localization is shown merged Bar = 20 mm.

Neuro®lament staining was used to label myelinated ®bres, most DRG cells, ventral horn cells and neuropil. IB-4 was used as a marker for Remak bundles in spinal roots and peripheral nerves and for non-peptidergic DRG neurones.

Y Gong et al (2002) *Brain*,125: 2491-2506

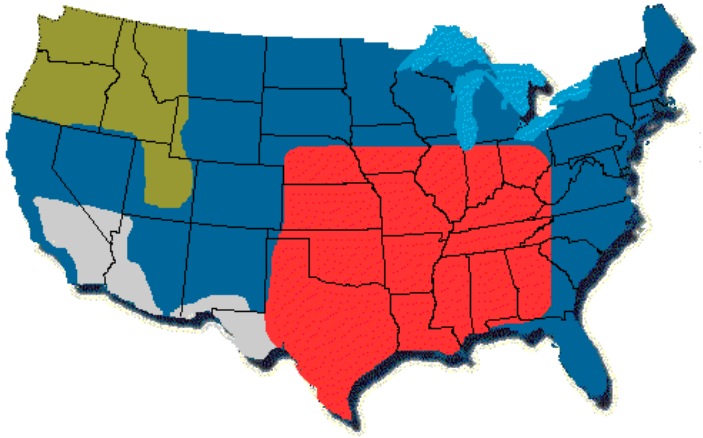
Other notable bioactive sphingolipids

Rôle of bioactive sphingoid bases



Nature Reviews | Molecular Cell Biology

Loxoscelism – a public health problem



51,865 incidents notified 2001-8*

- Most common locations of Brown Recluse Spiders
- Most common locations of other Recluse Species
- Most common locations of Hobo Spiders

YELLOW SAC SPIDERS ARE FOUND ALL OVER THE USA

*Ministério da Saúde. Brasília: Brasil.
Sistema de Informação de Agravos de Notificação SINAN
[updated 2012 Feb cited 2012 Feb].

Bite of the Spider

Day 3 Day 6 Day 10

Sphingomyelinase D in *Loxosceles* venom

Exclusively catalyses transphosphatidylation -not hydrolysis - forming cyclic ceramide phosphates from sphingomyelin substrates

Lajoie DM, Zobel-Thropp PA, Kumirov VK, Bandarian V, Binford GJ, et al. (2013) Phospholipase D Toxins of Brown Spider Venom Convert Lysophosphatidylcholine and Sphingomyelin to Cyclic Phosphates. PLoS ONE 8(8): e72372.

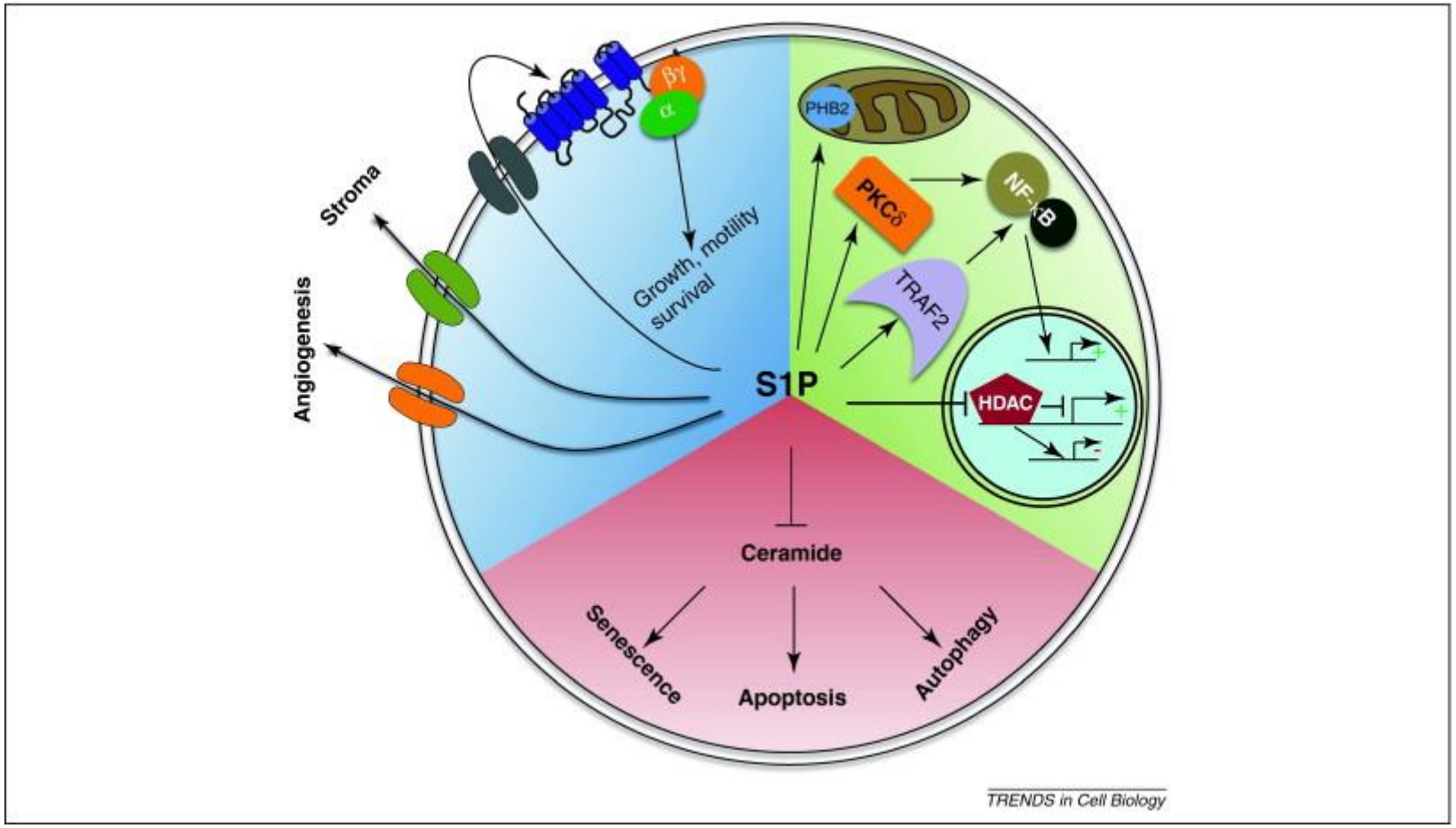
Experimental Loxoscelism

Venom from *Loxosceles* sp.

Endothelial apoptosis with thrombosis,
necrosis and persistent chronic ulceration

Pereira NB, Kalapothakis E, Vasconcelos AC,
Chatzaki M, Campos LP,Vieira F, Verçosa BLA,
Silva SS, Ferreira WM, Moro LJ (2012) - online
Venom. Anim. Toxins incl. Trop. Dis 18: 277-86.

Cell Death and Proliferation

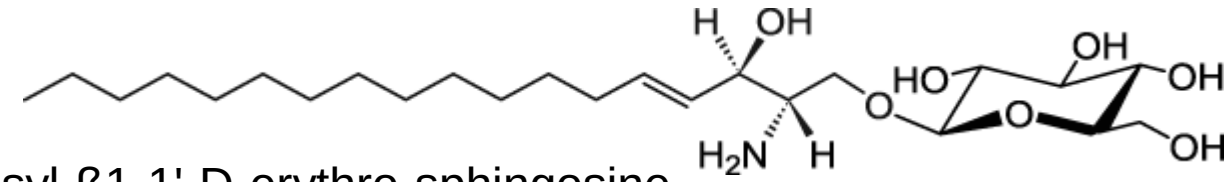


‘Psychosines’

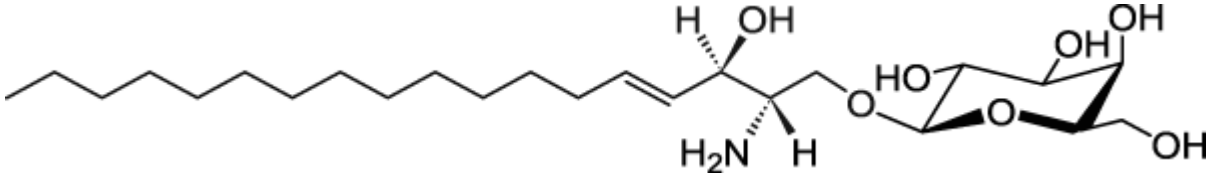
Glucosyl(ß) Sphingosine (d18:1)

Galactosyl(ß) Sphingosine (d18:1)

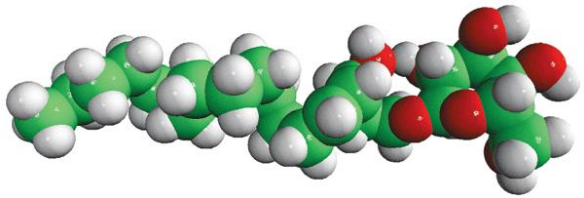
and



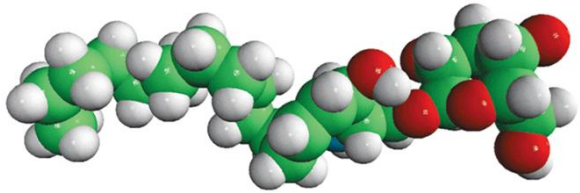
D-glucosyl-ß1-1'-D-erythro-sphingosine



D-galactosyl-ß1-1'-D-*erythro*-sphingosine

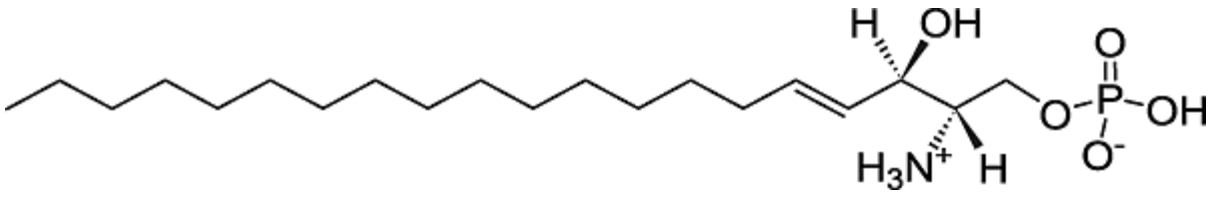


Glucosylsphingosine

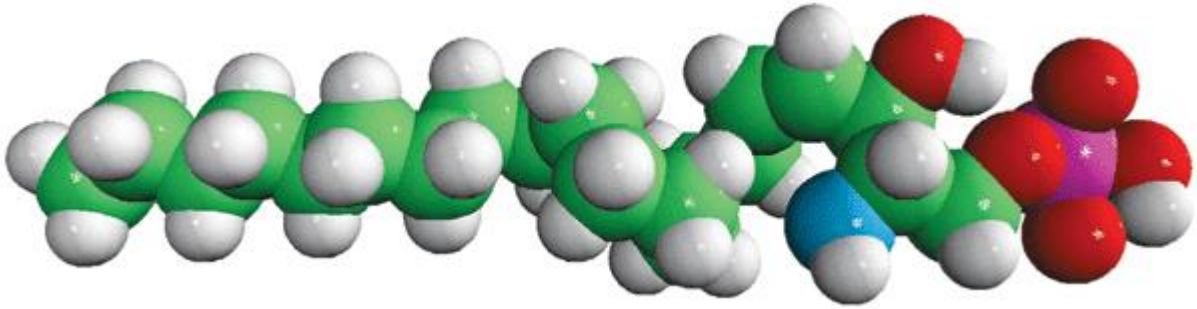


Galactosylsphingosine

Sphingosine 1-phosphate (d20:1)

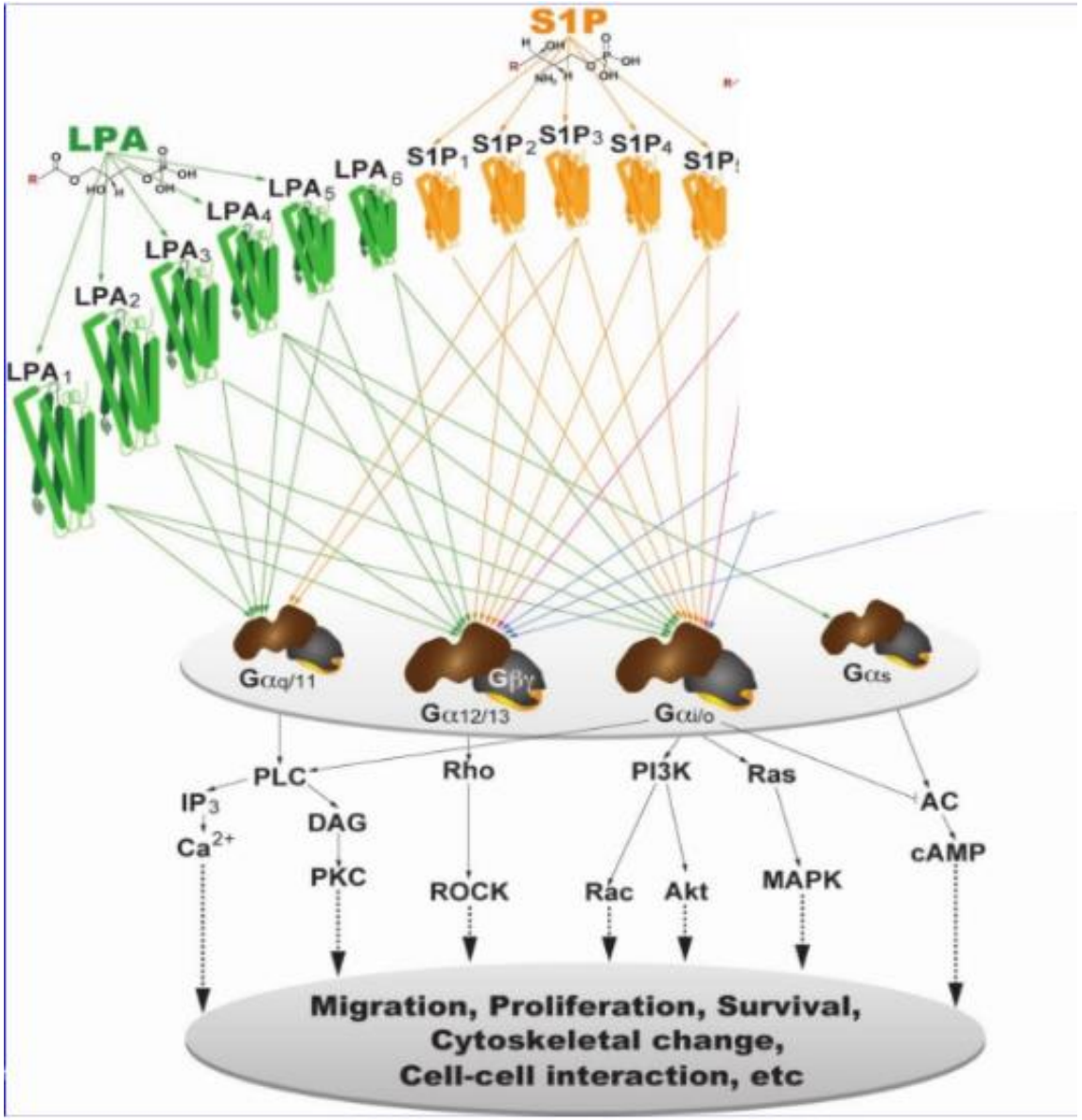


D-erythro-sphingosine-1-phosphate (C20 base)



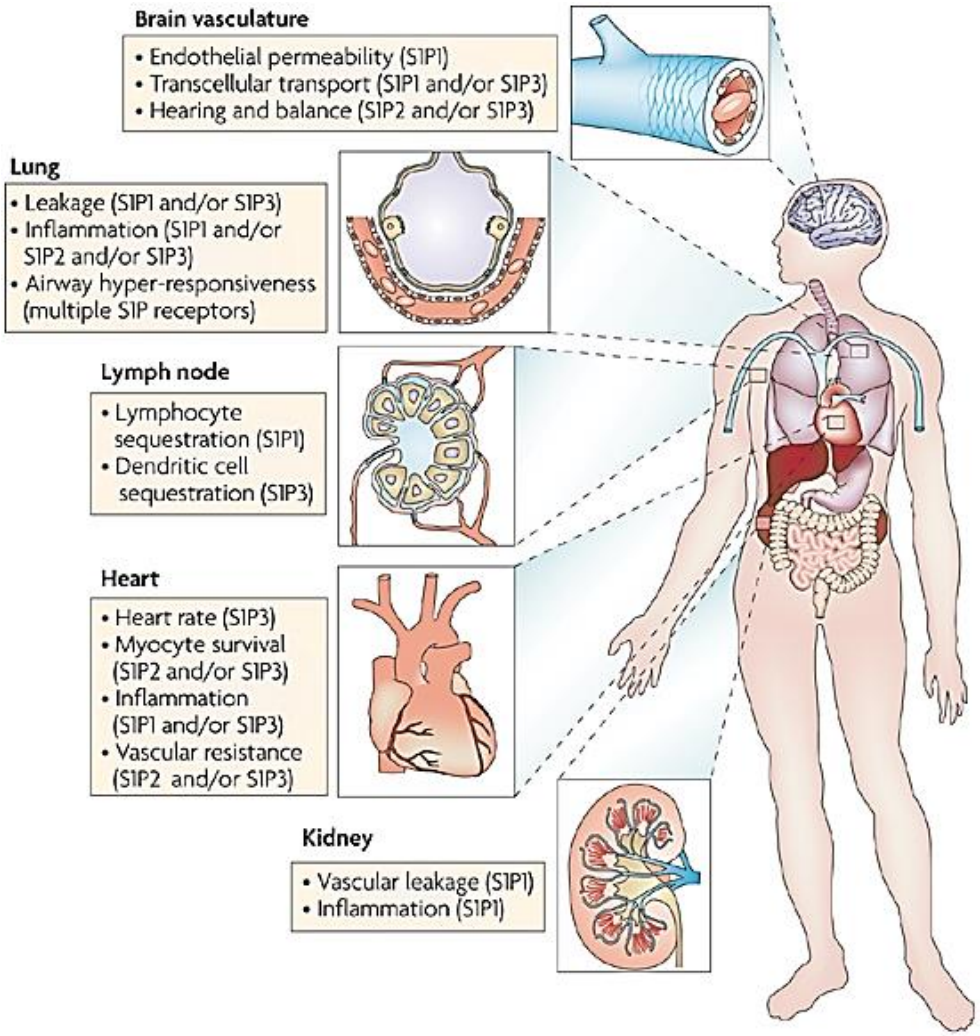
Water-soluble; stabilizes membrane structure

Binds to G-protein coupled receptors (Edg)



Choi et al (2010) Ann Reviews Pharm Toxicol; Mutoh et al, (2012) Brit J Pharmacol

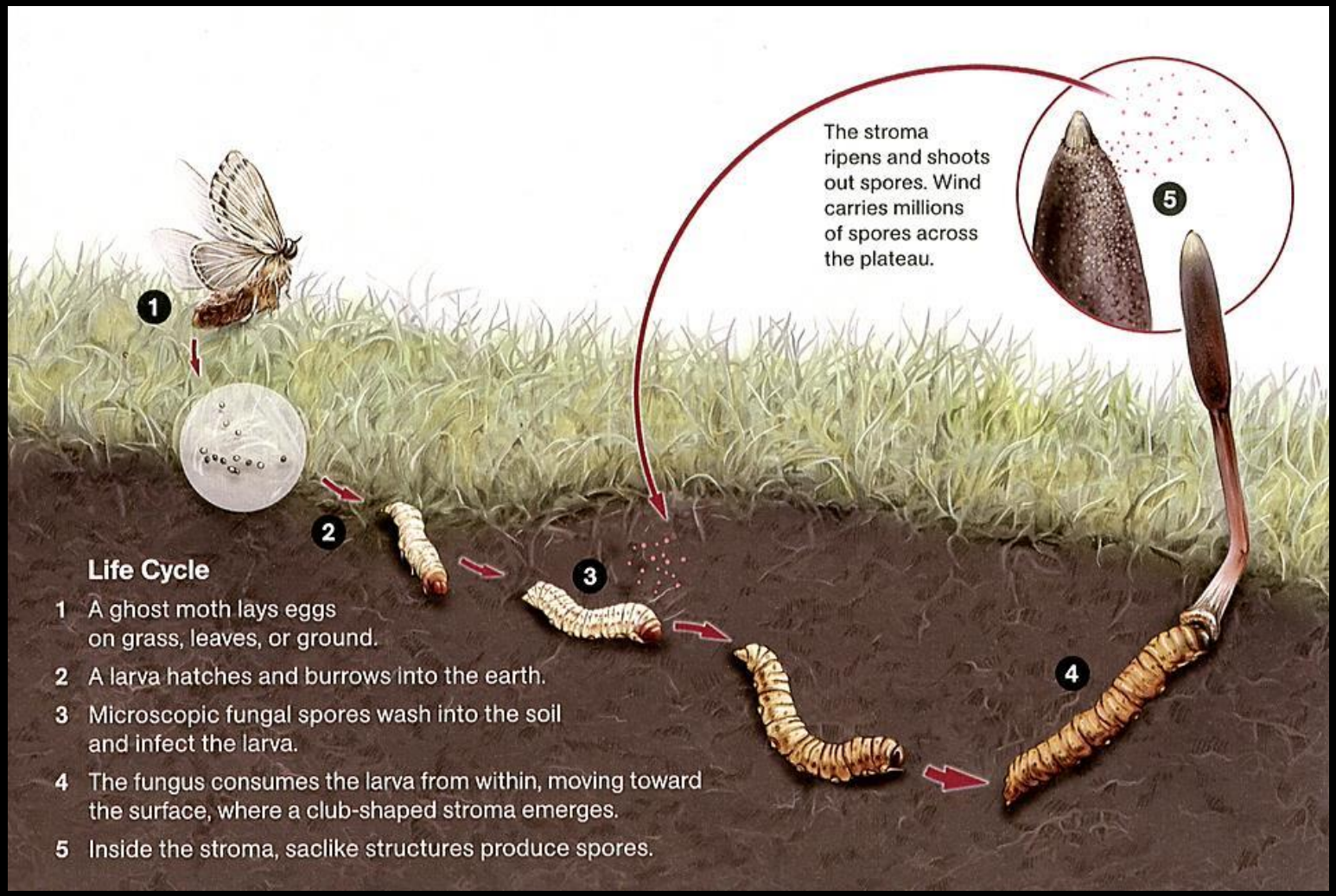
Sphingosine 1-phosphate



Nature Reviews | Drug Discovery

Ophiocordyceps sinensis

Yartsa gunbu – Summer grass, Winter worm



The National Geographic (August 2012): 118-29

‘**An Ocean of Aphrodisiacal Qualities**’

‘**Bestowing inconceivable advantages**’

Faultless Treasure….

Alleviates:

- **Back pain**
- **Impotence**
- **Jaundice**
- **Fatigue**
- **Hair loss**

Treats:

- **Tuberculosis**
- **Asthma, Bronchitis, Emphysema**
- **Hepatitis**
- **HIV-AIDS**

Chengdu - Szechuan

Zhong Shi Caterpillar Fungus Hall

The National Geographic (August 2012): 118-29

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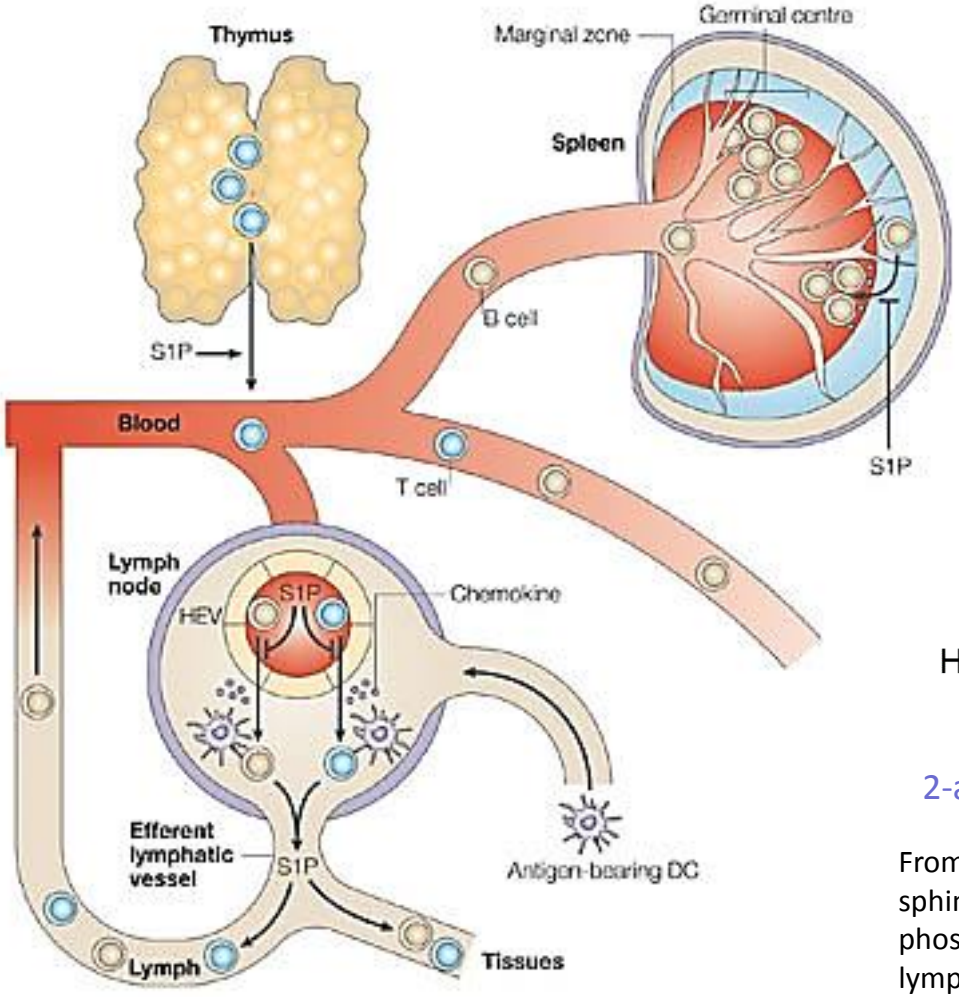
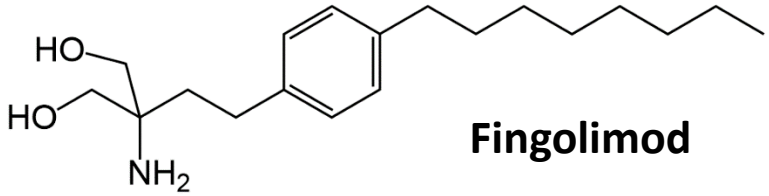
- **Hepatitis**
- **HIV-AIDS**


NOVARTIS
2D Code
NDC-0078-0607-51
GILENYA™
(fingolimod)
capsules
0.5 mg
Equivalent to 0.56 mg
fingolimod hydrochloride
28 Capsules
Rx only
This package contains a four-week supply of capsules.
Dispense with enclosed Medication Guide.
OPEN HERE

Sphingosine 1-phosphate and immunosuppression





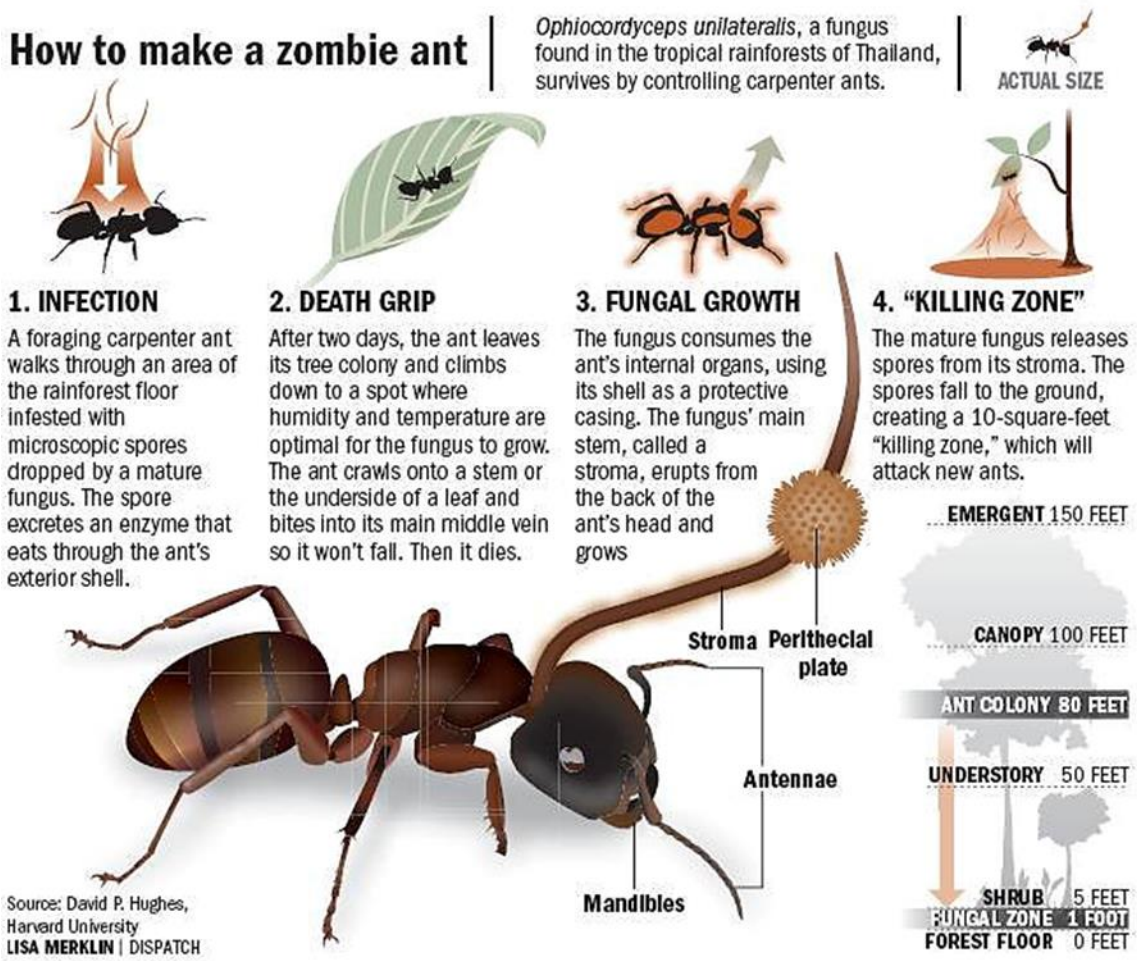
Fingolimod

2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol

From the myriocin metabolite of the fungus *Isaria sinclairii* sphingosine analogue phosphorylated by sphingosine kinases: phosphofingolimod inactivates S1PR1 and prevents egress of lymphocytes from germinal centres in lymph nodes

Ophiocordyceps unilateralis

Alfred Russell Wallace (1849)

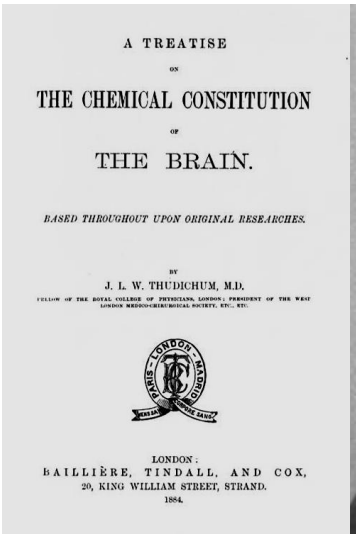


A treatise on the chemical constitution of the brain (1884)

‘The most diversified chemical laboratory of the animal body…’

St Thomas’s Hospital ~ 1870

140 brain chemicals: sulphatides,sphingosine, sphingomyelin, cerebrosides and psychosines



A TREATISE
ON
THE CHEMICAL CONSTITUTION
OF
THE BRAIN.

BASED THROUGHOUT UPON ORIGINAL RESEARCHES.

BY
J. L. W. THUDICHUM, M.D.

LONDON:
BAILLIÈRE, TINDALL, AND COX,
20, KING WILLIAM STREET, STRAND.
1884.

JLW Thudichum
(1829-1901)

‘When the normal composition of brain shall be known to the uttermost item, then pathology can begin its search for abnormal compounds or derangements of quantities’

Lysosomal disorders

Overall birth frequency per 100,000 population

The Czech Republic	12.3
The Netherlands	14.0
Australia	12.9
Italy	12.1

(see -Poupetová H, Ledvinová J, Berná L, Dvoráková L, Kozich V, Elleder M. J Inherit Metab Dis. 2010 33: 387-96)

Sphingolipidoses

When you are older….

Progressive Neurodegeneration

Infantile Tay-Sachs disease

Elsie Williams (centre) celebrates her fifth birthday with Matayah Gibbons and family friend Tracey Hartley. Photo: JOSH HEARD

Wongarbon NSW 2831, Australia

Late-onset Tay-Sachs disease

36 years

42 years

My name is Vera Pesotchinsky

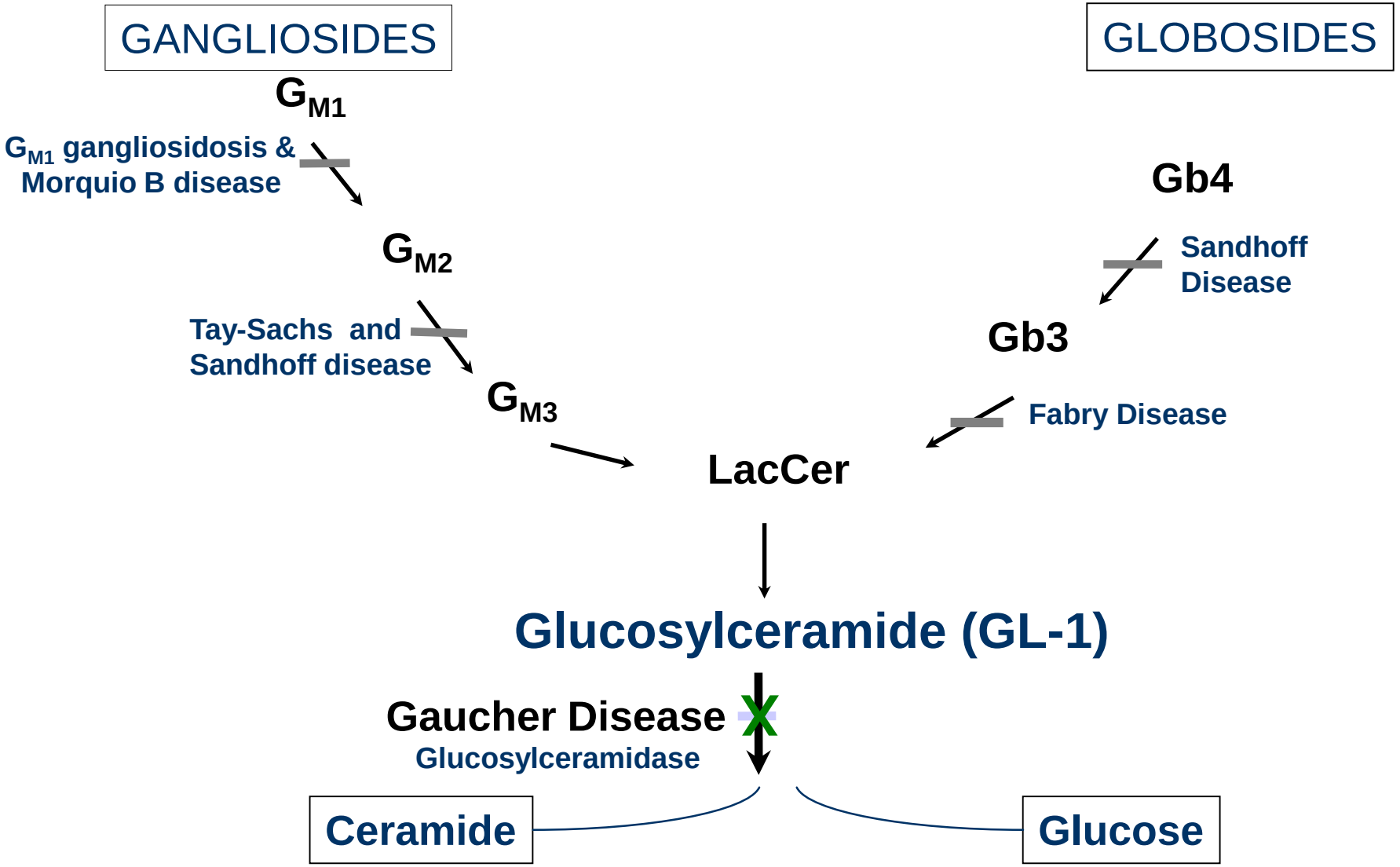
I was diagnosed with Late-onset Tay-Sachs disease in 2000 (LOTS)

- 8 years misdiagnosis
- MBA from Santa Clara Universi & BA from Wellesley College double major economics & Russian Area Studies

I was always told that my physical and speech difficulties were psychiatric

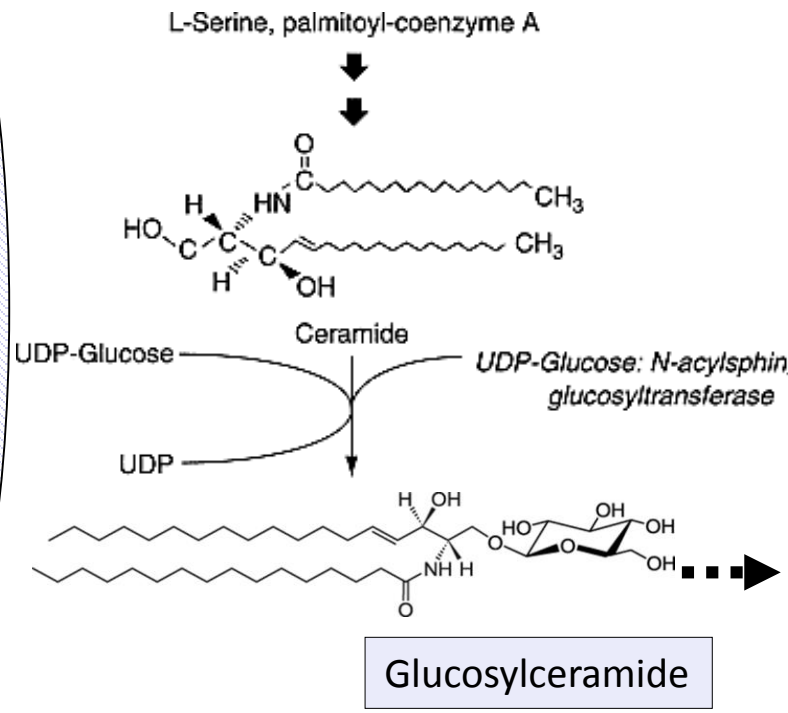
Even when I stuttered in high school & college, nobody recommended me to a neurologist - I was repeatedly sent to psychiatrists instead!

Lysosomal degradation of Glycosphingolipids



De novo Synthesis and Metabolism of Glucosylceramide

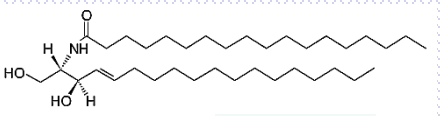
Golgi



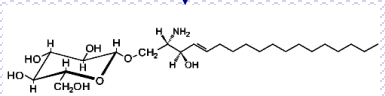
De novo synthesis

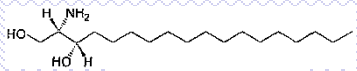
Recycling

Lysosome



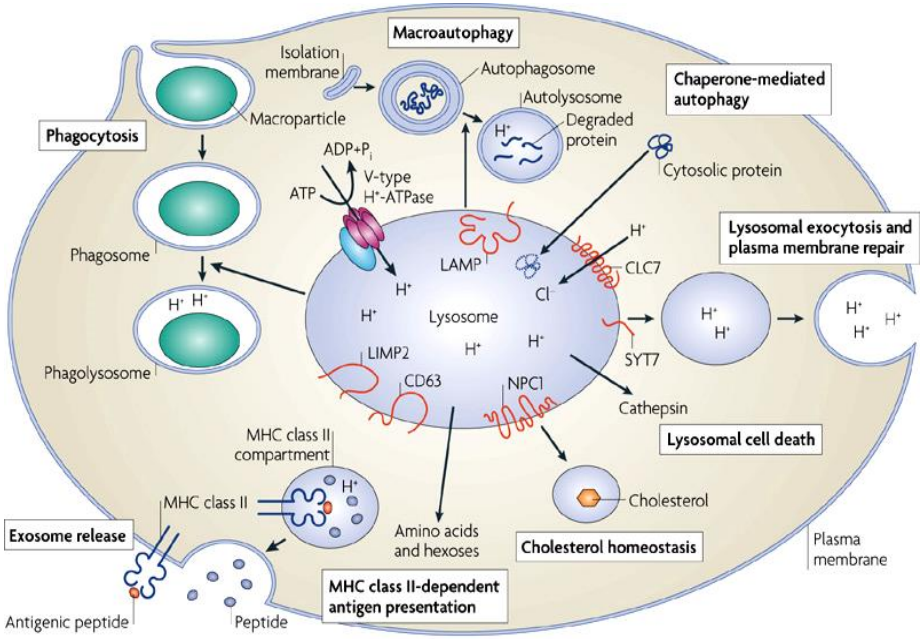
ceramidase



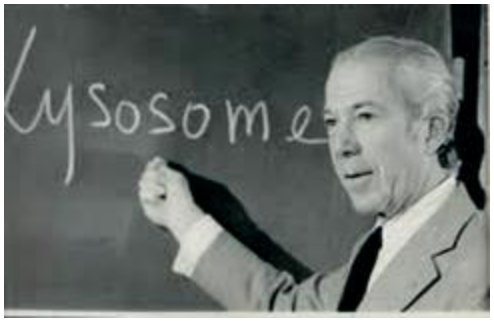


TM Cox Presenter's Artwork

The Unique Cell Biology of the Lysosome



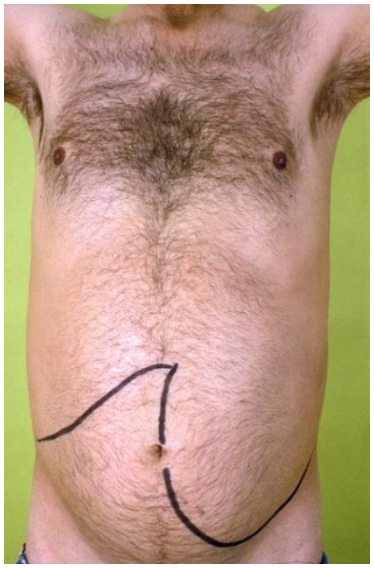
P Saftig & J Klumperman (2009)
Nat Rev Mol Cell Biol 10: 623-35

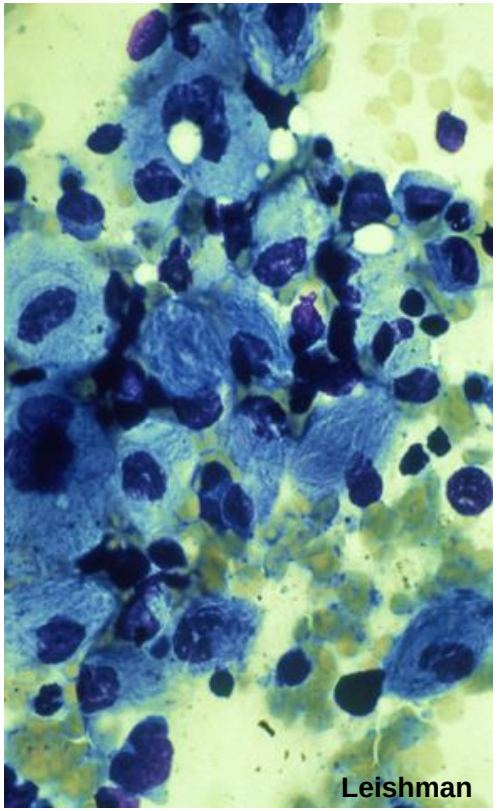


Christian de Duve
Nobel Prize 1974

Gaucher disease – a ‘single-gene’ disorder

Dr Ernest Gaucher
(1882)

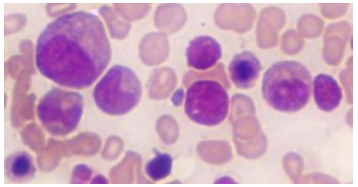




- **Autosomal recessive disease**
- **1/60,000 live births**
- **Acid ß-glucosidase deficiency**
- **Excess glycosphingolipids**

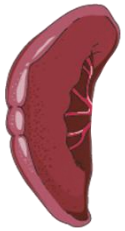
TM Cox Personal Clinical archive

Monocyte-derived Macrophages in Gaucher Disease



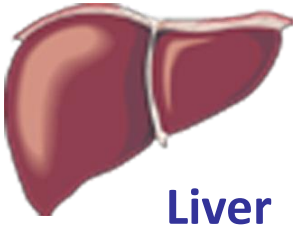
Macrophages

Spleen



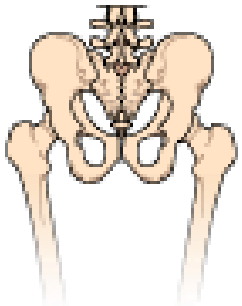
Anaemia
Leukopenia
Thrombocytopenia
Splenomegaly

Liver

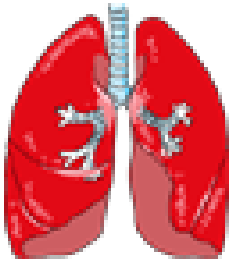


Hepatomegaly
Lobar fibrosis
Cirrhosis

Bone
Bone Marrow



Marrow failure
Skeletal disease



Lung

Pulmonary infiltration
1°pulmonary hypertension

Pain and impaired quality of life

Modified from E Beutler and GA Grabowski, *The Metabolic & Molecular Bases of Inherited Disease* 2001

Evolving therapies for Gaucher disease

Restore tissue macrophages

Organ transplantation

Haematopoietic stem cells

Other differentiated stem cells

Decrease toxic molecules

Enhance breakdown

Targeted lysosomal enzyme delivery

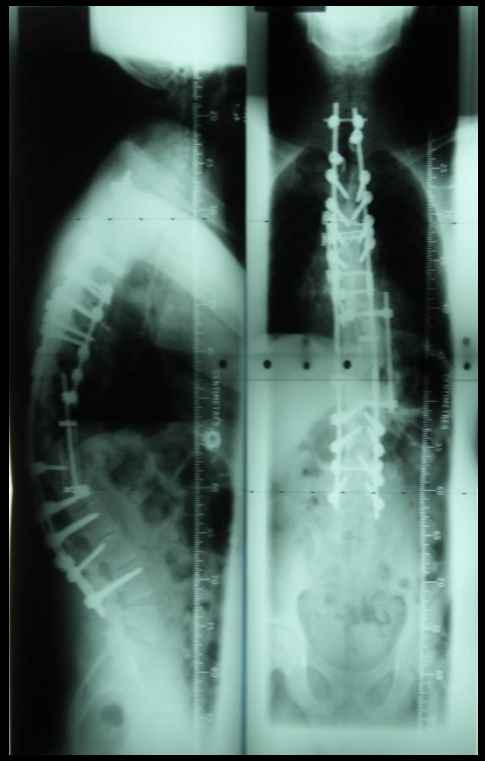
Enhance activity of mutant enzyme

[Gene transfer]

Attenuate formation

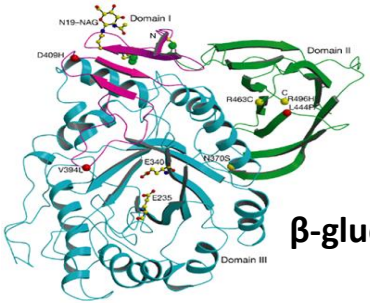
Inhibit sphingolipid biosynthesis

Late Sequelae of Skeletal Gaucher Disease

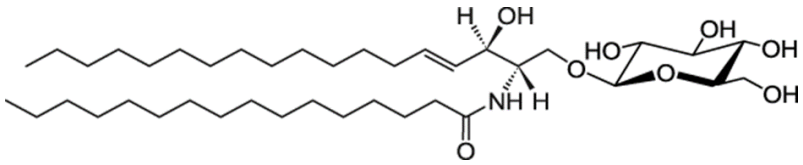


TM Cox and PB Deegan - personal clinical archive
(not for publication or distribution)

The Molecular Defect in Gaucher's Disease

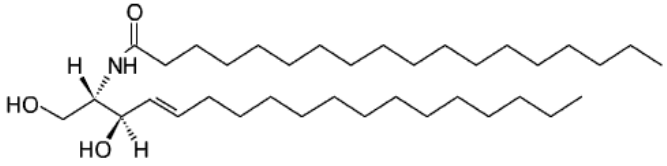


β-glucocerebrosidase



β-glucosylceramide

Sap C

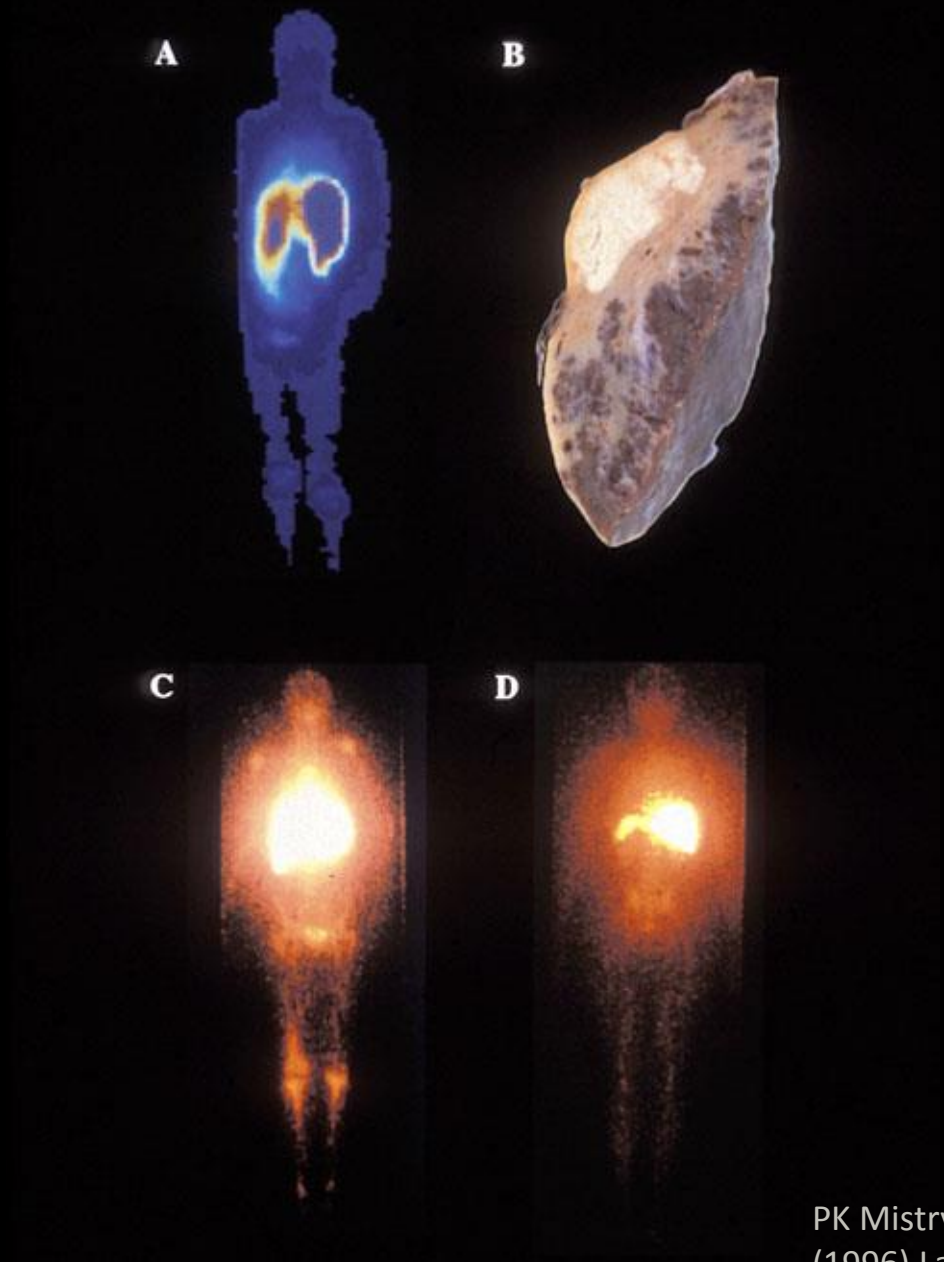


Ceramide (+ D-glucose)

Correct defect by targeting β-glucocerebrosidase to macrophages

AD Patrick, 1964
RO Brady, 1964
Dvir et al., 2004

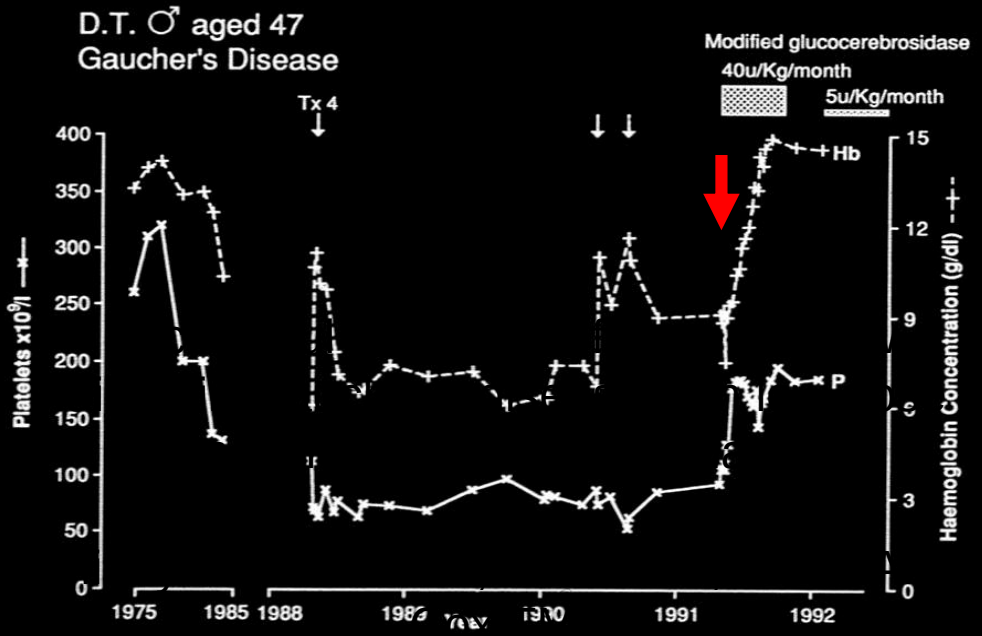
Therapeutic delivery of proteins to macrophages in Gaucher disease

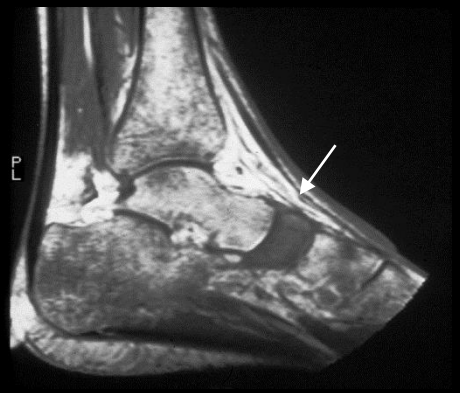


PK Mistry, EP Wraight & TM Cox
(1996) Lancet 348(9041):1555-59

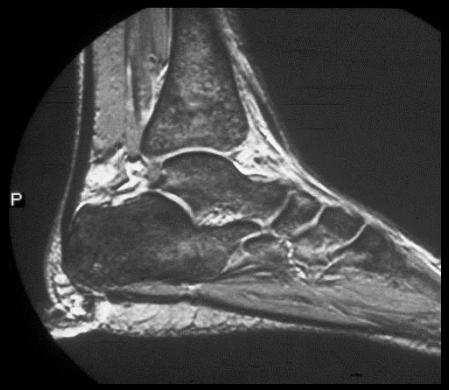
Mannose-terminated human glucocerebrosidase

Effects of enzyme therapy in Gaucher disease





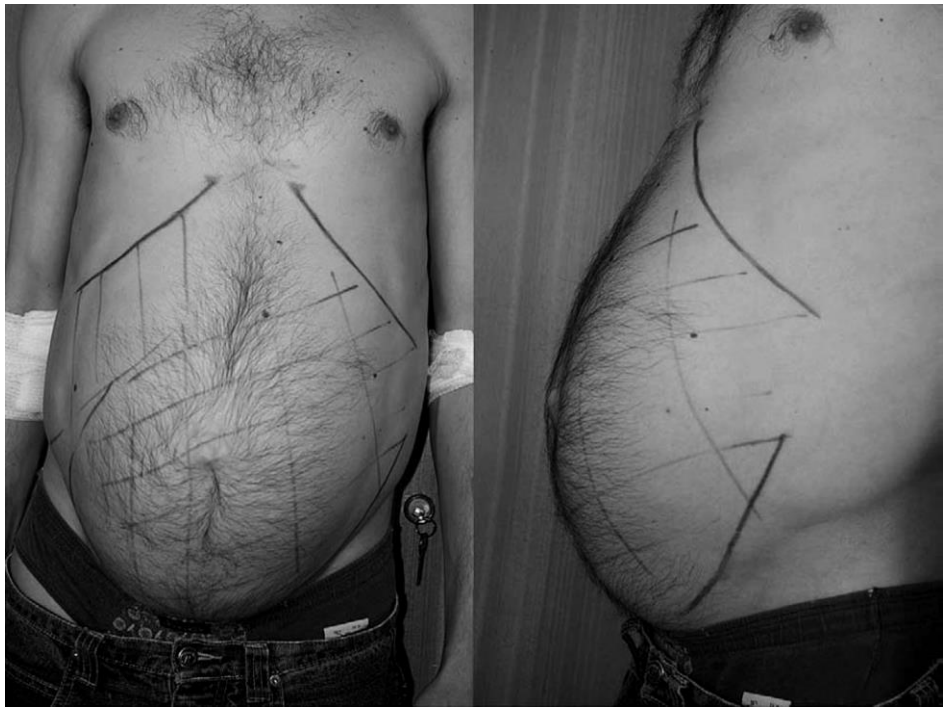
May 1991

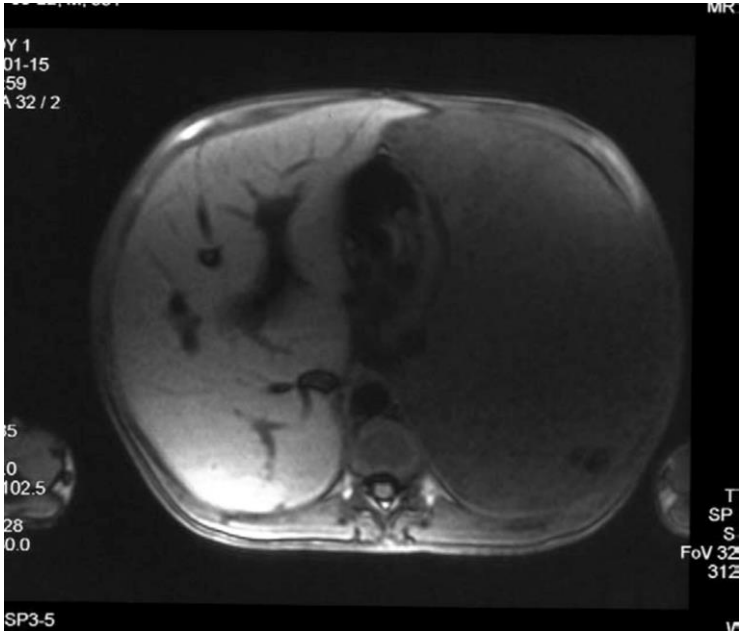


November 1991

Successful treatment of bone marrow failure in Gaucher's disease with low-dose modified glucocerebrosidase.
Mistry PK, Davies S, Corfield A, Dixon AK, Cox TM. (1992) Q J Med. 83(303):541-6

MZ Images from Poland





Tylki-Szymańska A, Czartoryska, B,Vanier M-T, Poorthuis BJMH, Groener JAE, Ługowska A, Millatc G, Vaccaro AM Jurkiewicz, E. (2007) *Clin. Genet*. **72:** 538-42.

Diagnostic Assays for Gaucher Disease

Plasma/ Serum Biomarkers

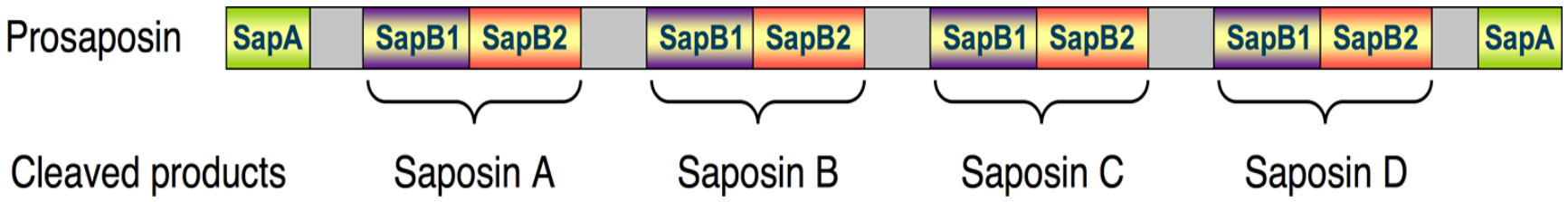
	MZ		AZ
Chitotriosidase	10,045 nmol/mL/h	(<150)	11,308
CCL-18/ PARC	1859 ng/mL	(<72)	-
Glucosylceramide	17.5 (mmol/l)	(4.3 -11.3)	

β-Glucocerebrosidase (GBA1)

Acid ß-Glucosidase (nmol/h/mg protein)

	MZ	AZ	
In leukocytes	4. 4	4.1	(2.7 - 7.4)
In skin fibroblasts	116	231	(111- 455)

Human Prosaposin: individual Saposins generated by cleavage



Saposin A Stimulates enzymatic hydrolysis of glucocerebroside, and galactocerebroside. Associated with Krabbe disease variant

Saposin B First heat-stable activator - required for sulphatide breakdown by arylsulphatase A. Activates several enzymes
Associated with atypical metachromatic leukodystrophy

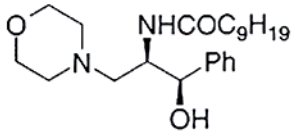
Saposin C Second saposin discovered. Stimulates glucocerebroside and galactocerebroside breakdown. Associated with rare juvenile variant of Gaucher disease

Saposin D Predicted from prosaposin sequence. Stimulates acid ceramidase

Rebalancing Sphingolipid Synthesis and Degradation

Norman Radin PhD

Therapy aims to avoid harmful excess sphingolipids by decreasing rate of synthesis in most cells – slower formation compensates for reduced rate of degradation in the lysosome



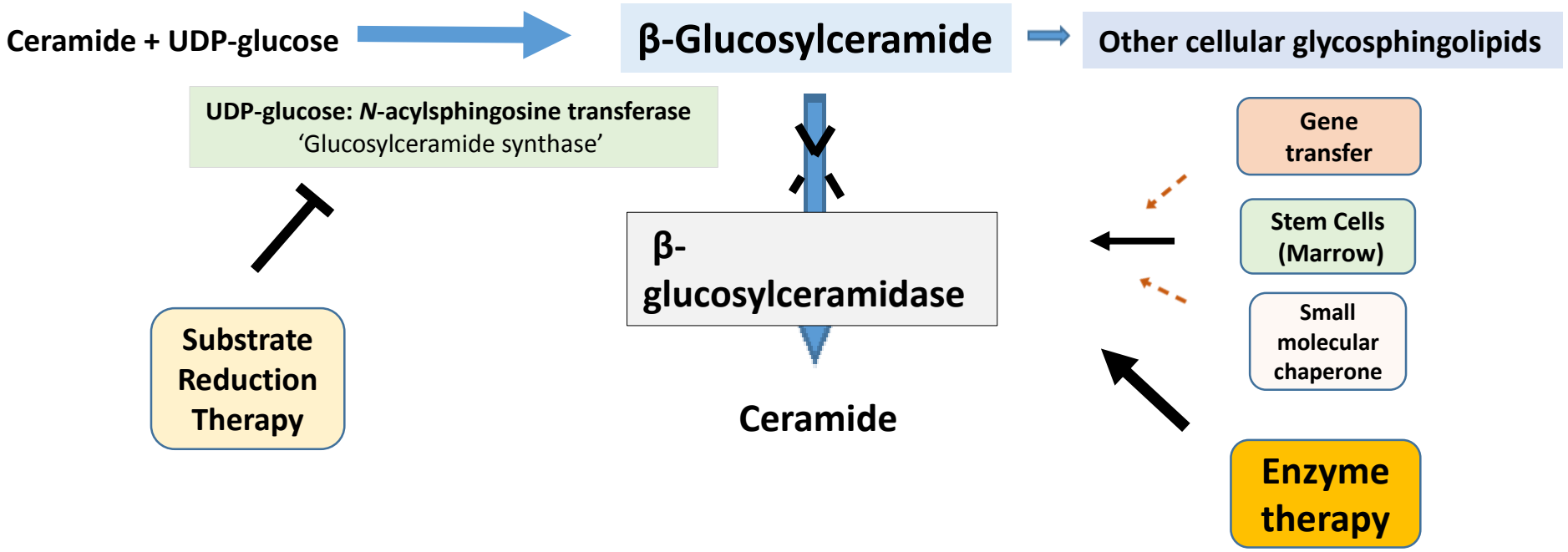
PDMP

1-phenyl-2-decanoylamino-3-morpholino-propanol

Vunnam R, Radin NS (1980) Analogs of ceramide that inhibit glucocerebroside synthetase in mouse brain. Chem Phys Lipids 26: 265-78.

This compound and analogues caused ceramide-mediated cell death

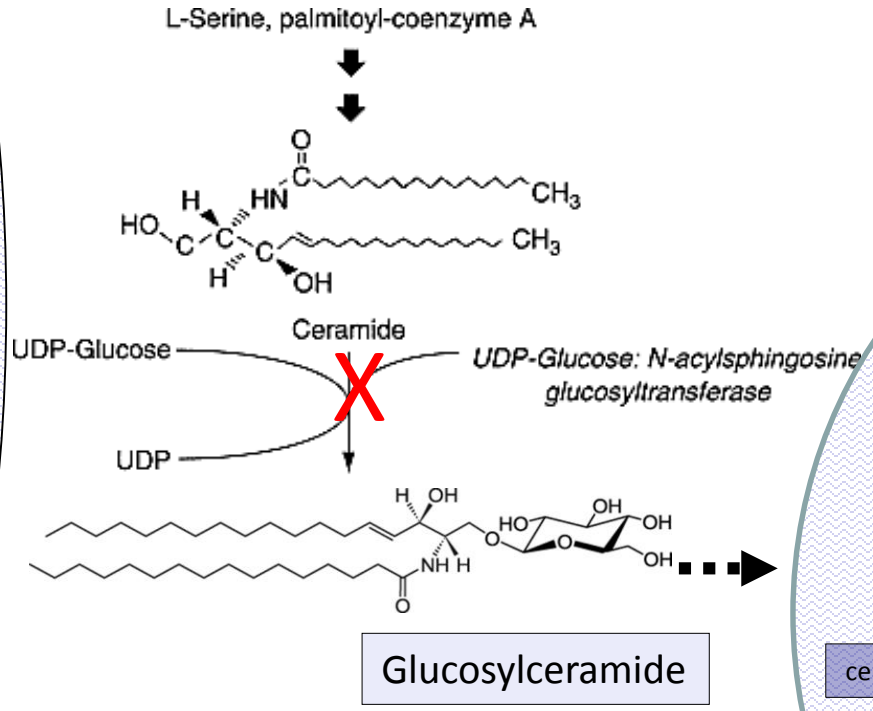
Modern therapeutic stratagems in Gaucher disease



TM Cox modified from James Shayman 2014

Attenuating glycosphingolipid biosynthesis

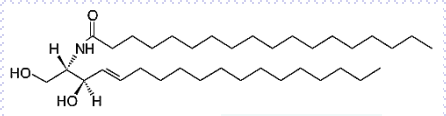
Golgi



De novo synthesis

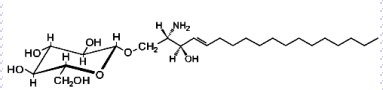
Recycling

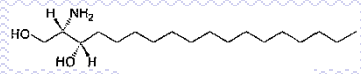
Lysosome



ceramidase

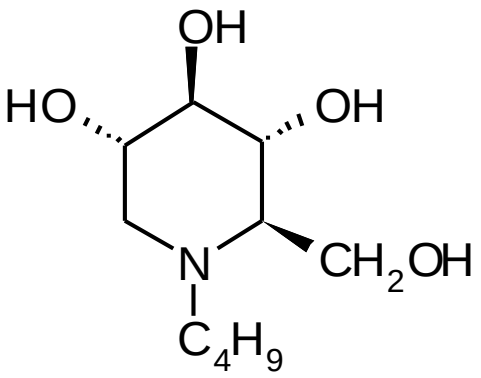
acid ß-glucosylceramidase





TM Cox ESGLD 2017 Presenter's Artwork

Miglustat: Zavesca™



N-butyldeoxynojirimycin

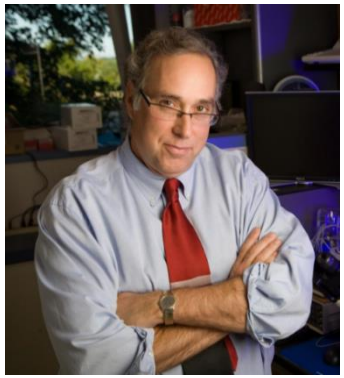
Miglustat

Oral agent Licensed 3/3/2003

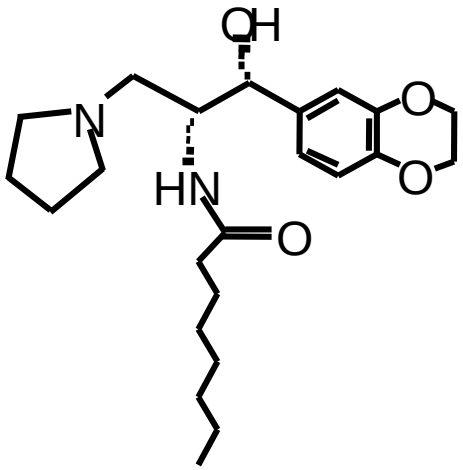
- Decreases production of storage material (glucosylceramide)
- Approved for those in whom ERT is unsuitable (unwilling/unable)
- Efficacy in mild-to-moderate disease
- Diarrhoea, tremor, weight loss
- Neuropathy (rare)

Eliglustat

D-threo-3'4'-ethylenedioxy-1-phenyl-2-octanoylamino-3-pyrrolidino-1-propanol (tartrate)



James Shayman MD PhD



Genz-112638

Orally active
IC_{50} ≈ 25nM

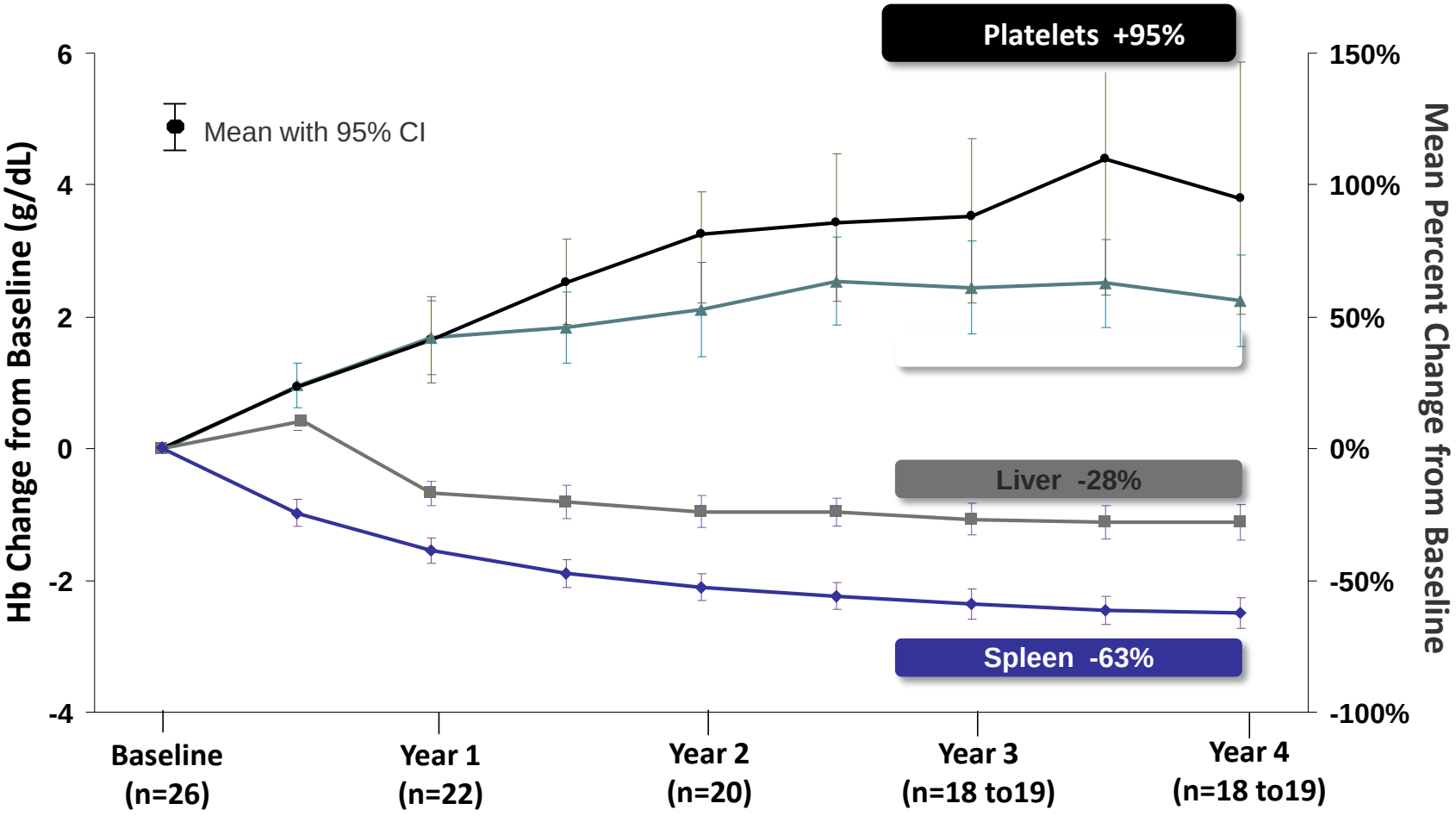
Selective inhibitor of uridine diphosphate-glucose: *N*-acylsphingosine transferase

Unlike PDMP series parent compound does not inhibit ceramide transacylase

(Lee L, Abe A, Shayman JA (1999). Improved inhibitors of glucosylceramide synthase. J Biol Chem; 274:14662-69)

Phase 2 Study: GENZ 112638 (Eliglustat) 4-Year Outcomes

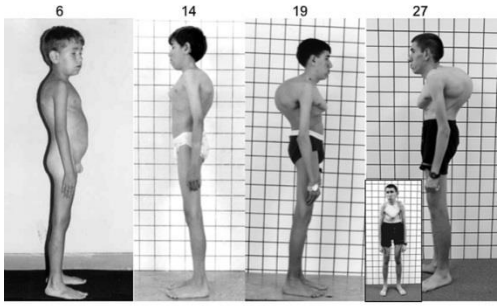
Improvements in Haematological variables and Organ volume



***P*<0.0001 for spleen, liver and haemoglobin and *P*=0.0003 for platelets at 4 years**

CI=Confidence Interval; Hb=haemoglobin

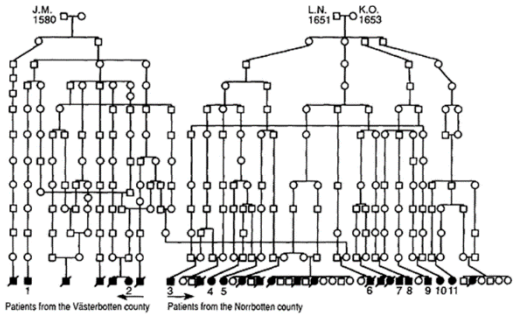
Norrbotten and Västerbotten Gaucher disease



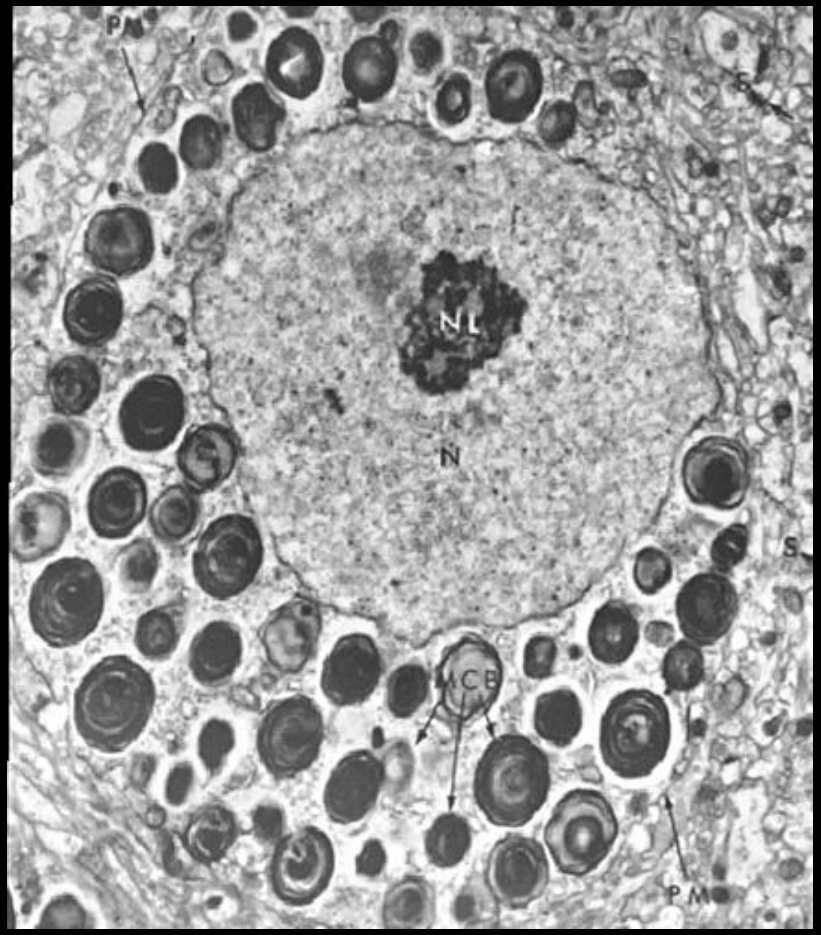
Patient 14

Subacute neuronopathic disease

- **Aggressive systemic childhod disease**
- **Slowly progressive neurological signs**
- **Horizontal supranuclear palsy**
- **Slow dementia**
- **Ataxia**
- **Spasticity in legs**
- **Myotonic or complex seizures**
- **L444P homozygotes**



About 60 patients identified in an extended pedigree established in 1651 and 1653
(Dahl N, Hillborg PO & Olofsson A (1993) Hum Genet 92: 513-5)



Transmission electron micrograph of brain of patient with Tay-Sachs disease (Dr. Robert Terry)

Terry RD & Weiss M (1963) Studies in Tay-Sachs disease. II. Ultrastructure of the cerebrum. J Neuropathol Exp Neurol 22:18-55.

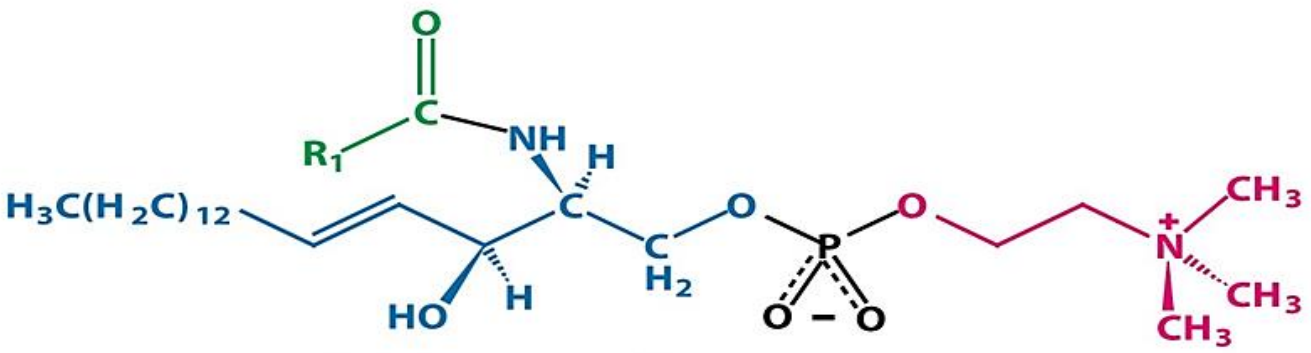
Luceroglustat

[GZ/SAR 402671]

(S)-Quinuclidin-3-yl (2-(2-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate

CNS penetrant inhibitor of UDP-glucosylceramide transferase

Sphingolipids



Sphingomyelin

Thudichum (1874)

The amine is linked to a fatty acid ; one hydroxyl group may be functionalized to a phosphate or sugar group with other substituents

Sphingolipids are an integral part of the membrane bilayer

Cell recognition, transmembrane signal transduction, antigen display, control of proliferation and cell death signalling, angiogenesis , senescence, autophagy. . .