

A microscopic image of cells, likely from a bone marrow or tissue sample, stained with a combination of pink and blue dyes. The cells are irregular in shape, with some showing prominent nuclei. The background is a deep blue.

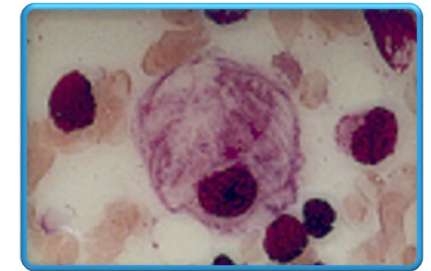
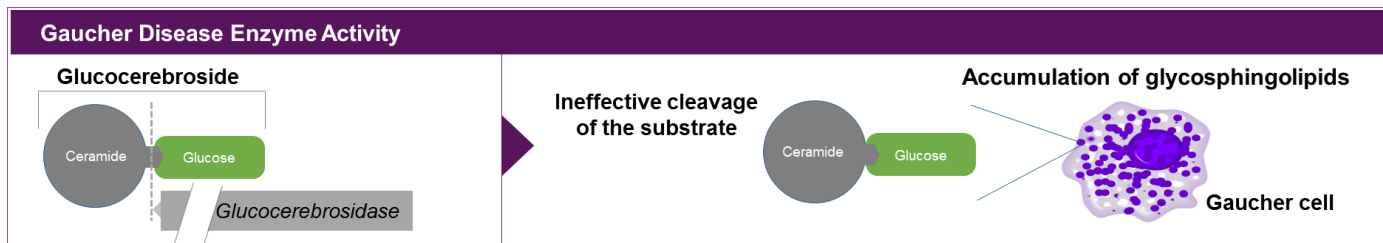
Clinical Challenges and Unmet Medical Needs in Neuropathic Gaucher Disease

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Gaucher disease: Pathophysiology

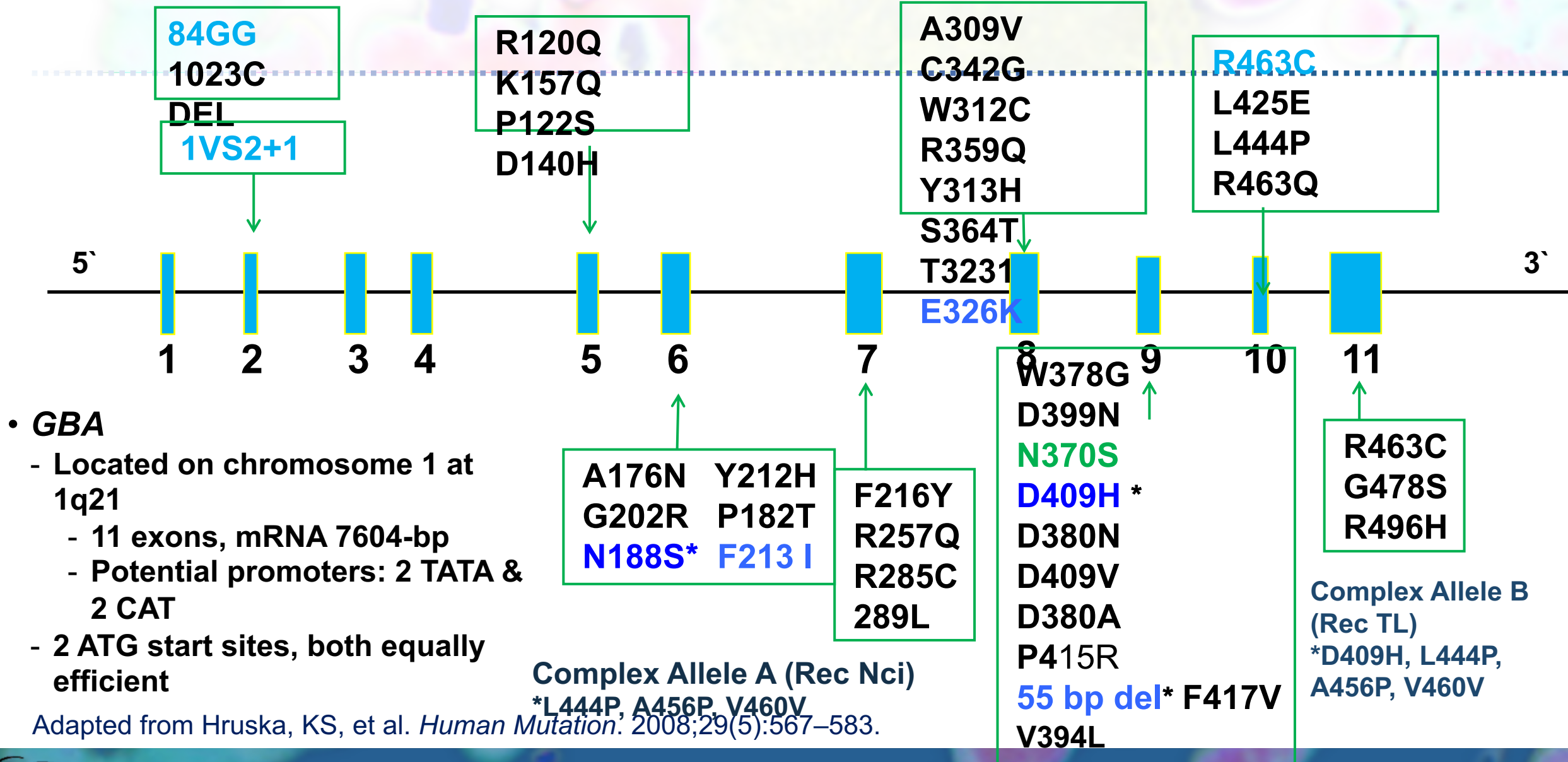
- Pathogenic variants in the *GBA1* gene reduce the activity of the glucocerebrosidase resulting in inefficient breakdown of glucocerebroside and progressive
- In the lysosome, certain levels glucocerebrosidase (GCase) catalyzes the normal breakdown of glucocerebroside
- Deficiency of GCase results in Intra-lysosomal accumulation of glucosylceramide in different tissues^{2,3}
- Macrophages stimulated with the effects of the stored lipid substrate are called “Gaucher cells”^{2,3,4}



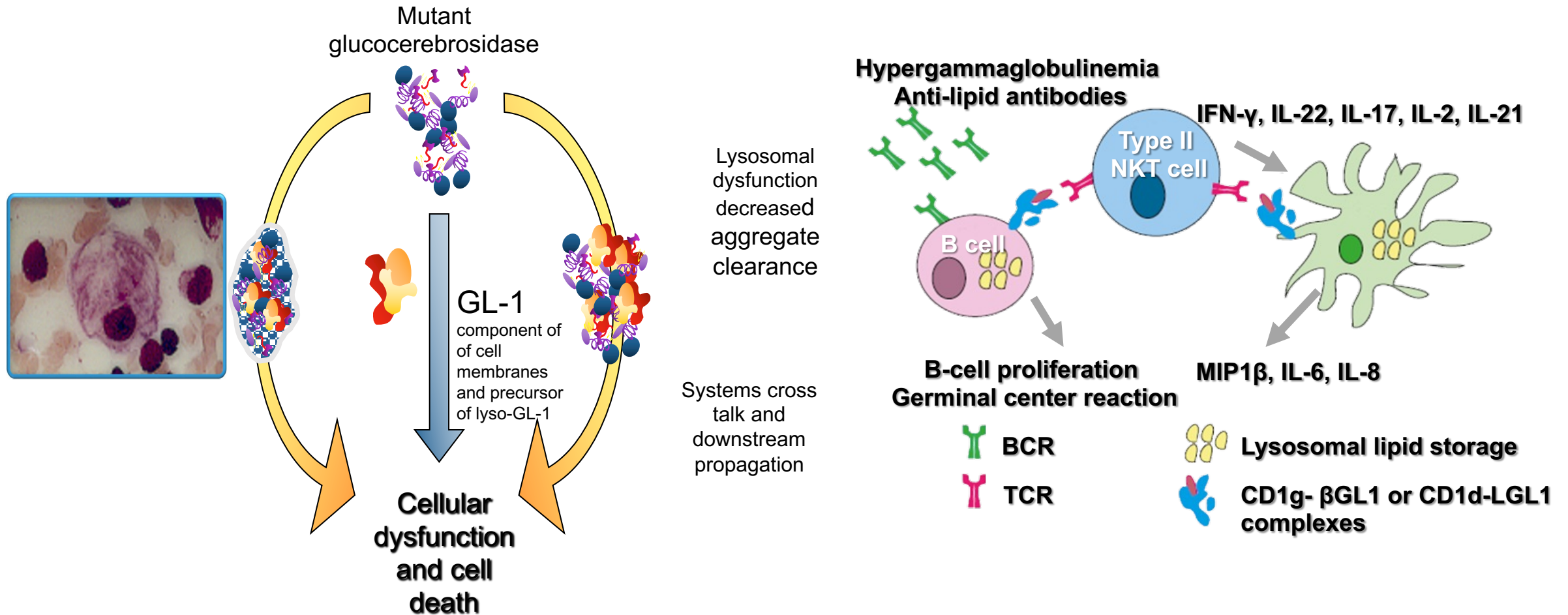
Gaucher cell

1. Cooper GM. *The cell: A molecular approach*. 2nd edition. Sunderland MA: Sinauer Associates; 2000. <http://www.ncbi.nlm.nih.gov/books/NBK9953/>. Accessed August 29, 2016. 2. Huang WJ et al. *Eur Rev Med Pharmacol Sci*. 2015;19:1219-1226 3. Jmoudiak M, Futerman AH. *Br J Haematol*. 2005;129(2):178-188. 4. Kotler T et al. *Biochim Biophys Acta*. 2006; 1758:2057-2079.

GBA1 mutations result in Gaucher disease



Downstream events in Gaucher disease

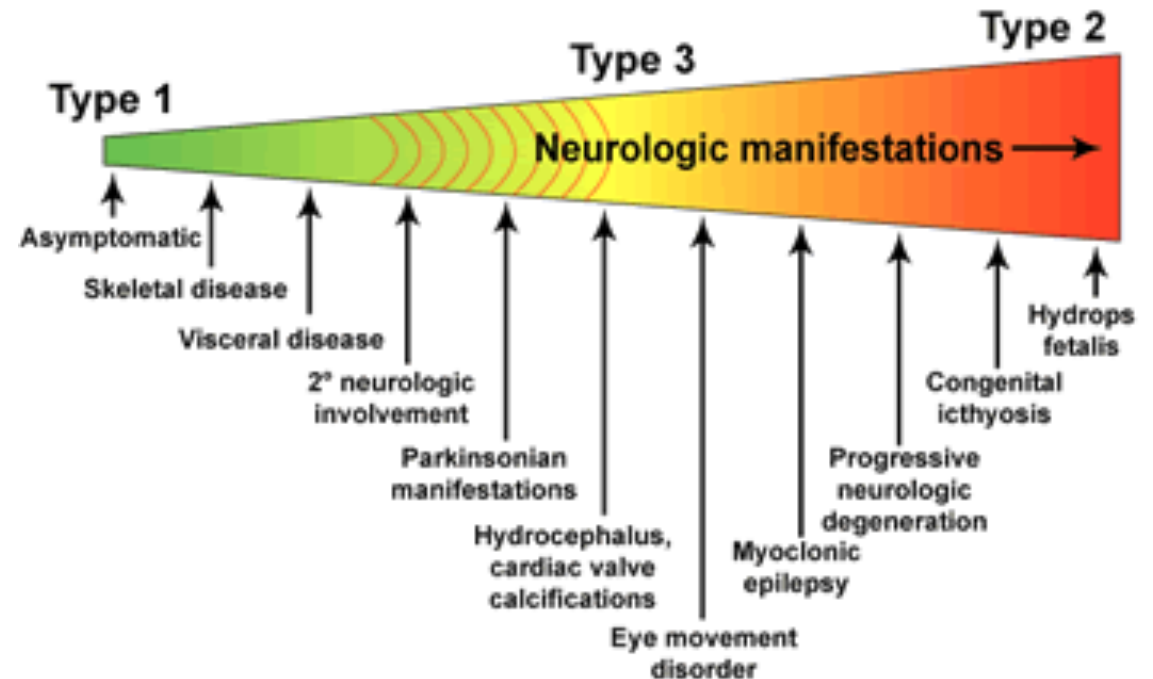


Nair et al (2015) Blood, 125: 1200-1202

Gaucher disease is subdivided into 3 clinical types

Clinical subtypes are delineated by the involvement of the Central Nervous System and its progression¹:

Prevalence	Type
>95%	<ul style="list-style-type: none">■ Type 1 non-neuronopathic^{2,3}<ul style="list-style-type: none">— Symptoms range from asymptomatic to patients with childhood-onset disease
~1%	<ul style="list-style-type: none">■ Type 2 acute neuronopathic²<ul style="list-style-type: none">— Acute neuronopathic form with severe prognosis with limited survival (up to 4 yrs)
~5%	<ul style="list-style-type: none">■ Type 3 subacute neuronopathic^{2,4}<ul style="list-style-type: none">— Neurological involvement, which generally appears later in life than in type 2 disease— Severe systemic involvement

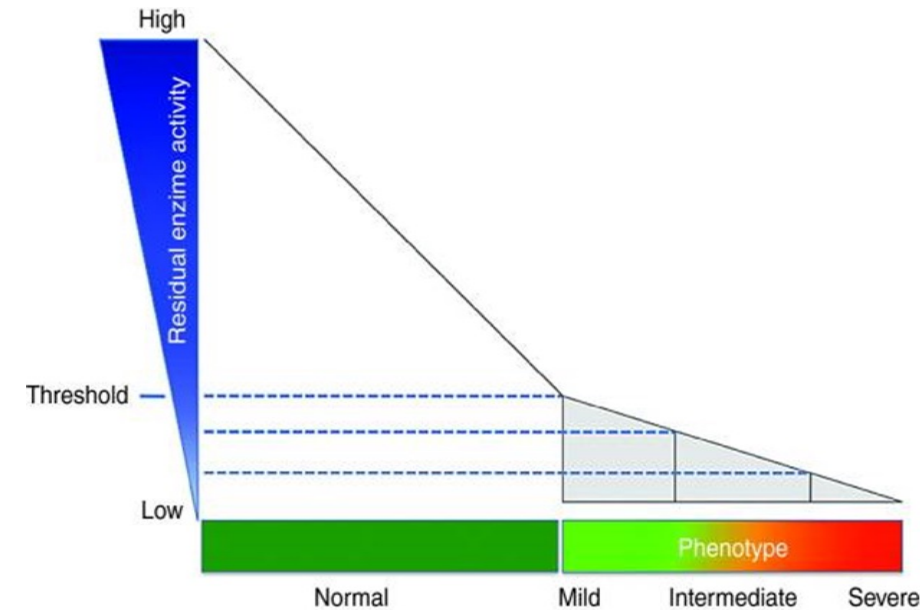


Reprinted with permission from ©2004, Academic Press, Elsevier: Sidransky E. Gaucher disease: complexity in a "simple" disorder. *Molecular Genetics and Metabolism*. 1969;83(1-2):6-15.

1. Linari S et al. *Clin Cases Miner Bone Metab*. 2015;12:157-164. 2. Huang WJ et al. *Eur Rev Med Pharmacol Sci*. 2015;19:1219-1226. 3. Mehta A. *Eur J Internal Med*. 2006.;17:S2-S5. 4. Baris HN et al. *Pediatr Endocrinol Rev*. 2014;12(10):72-81.

Why is there a clinical spectrum of in neuronopathic Gaucher disease?

- For most LSDs, correlations have been observed between residual enzyme activity and disease severity.
- Null and severe *GBA1* pathogenic variants result in a very low GCase enzymatic activity.
- Patients with two severe mutations or a combination of a severe- variant with very low detectable residual GCase activity and null mutations are predicted to have severe disease.
- This is not an absolute predictor of outcome, especially for the missense variants.
- The homozygosity for L444P (L483P) is commonly linked to the neurological forms of GD, however, the phenotypes associated with this genotype range from non-neuronopathic to severe progressive neurological disease.
- Many factors including how GCase processed in the cell could be responsible for the clinical variability .



Parenti G, et al. *Mol Ther.* 2015;23(7):1138-1148.

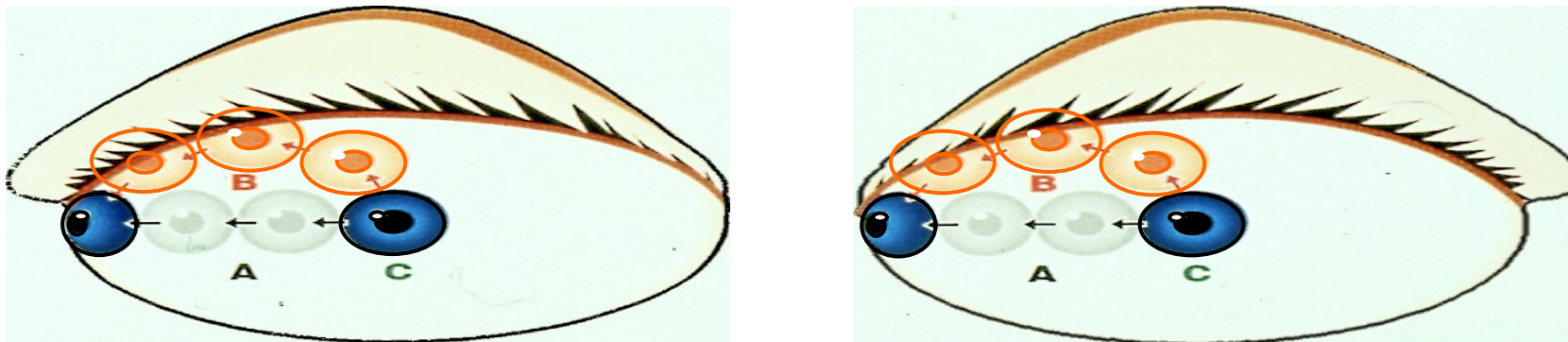
Bley AE, et al. *Pediatrics.* 2011;128:e1233-e1241.

Ron I. and Horwitz M. ,2005. *Hum Mol Genet*

Definition of Neuronopathic GD

- Acid β -glucosidase deficiency and bi-allelic *GBA1* gene pathogenic variants.
- Gaze palsy, predominantly horizontal, with slow or absent saccades.

Movement of the Horizontal Saccades with Right Gaze



A. In normal individuals, eyes move straight horizontally.

B. In patients with neuronopathic Gaucher disease, eyes loop up when looking horizontally.

C. With severe involvement, there is no horizontal movement at all.

Other suggestive features at presentation

- Background slowing on EEG. While epileptogenic activity, is often observed in nGD. EEG may be noncontributory as seizures can have subcortical origin.
- Homozygosity of the p.Leu483Pro *GBA1* variant (L444P) is associated with GD3.
- Progressive increased curvature of the spine (kyphosis, kyphoscoliosis)
- Auditory dysfunction: Abnormal BAER (brain stem dysfunction) with normal peripheral hearing (central auditory processing disorder)
- Hearing loss
- Severe systemic disease with associated comorbidities (Gaucheromas, mesenteric lymphadenopathy; infiltrative lung disease and vitreous opacities).
- Cardiac valvular and aortic calcifications, hydrocephalus, corneal opacity (only in p.Asp448His/p.Asp448His- previously known as D409H/D409H)



Rare presentations of neuronopathic Gaucher disease

-
- Hydrops fetalis
 - Congenital ichthyosis (Collodian baby phenotype)
 - Myoclonic epilepsy (N188S variant)
 - Cardiac valve calcification (D409H variant)
 - Psychiatric disease
 - Protein losing enteropathy



Acute neuronopathic Gaucher disease (GD2) presentations



- Onset prenatal to 3 - 6 months of age
- Hepatosplenomegaly
- Ichthyosis
- Brain stem involvement
- Autosomal recessive - rare
- No ethnic predilection



Definition of GD2

- Supranuclear gaze palsy (HSGP), convergent squint*.
- Rapid neurological deterioration over months in the first 2 years of life*.
- Severe stridor and apnea at some point, frequently necessitating tracheostomy by age 2 years*.
- Feeding difficulties due to abnormal swallowing often requiring tube feedings.
- Development of spasticity, opisthotonus and/or progressive myoclonic epilepsy by age 2 years.
- Failure to achieve independent gait (GD3 always achieve independent gait).



Unmet medical challenges in GD2

- Acute progressive disease (\approx 8 months)
- Correct and timely diagnosis
- Seizures: EEG may be normal (subcortical seizures)
- Feeding, recurrent aspirations
- Management of inter-current illness: infections
- Invasive interventions (tracheostomy, G/J tube placement, ventilatory support)
- Autonomic dysfunction (temperature regulation, diarrhea)
- A progressive fatal disease without an existing treatment or cure



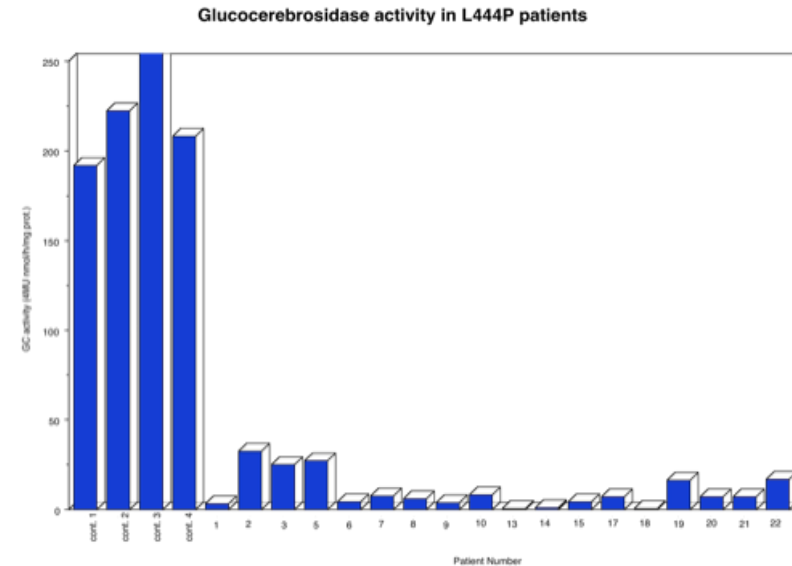
Clinical Manifestations of Chronic Neuronopathic Gaucher Disease (GD3)

- Heterogeneous (Types 3 a, b, c)
- Usually presents in late infancy/early childhood
- Common signs and symptoms severe hepatosplenomegaly, skeletal involvement with progressive spine deformity (kyphosis/gibbus)
- Neurological presentations may later arise
- Panethnic
- Type 3 c is a distinct type with progressive involvement of heart valves associated with a certain genotype (D409H/D409H)



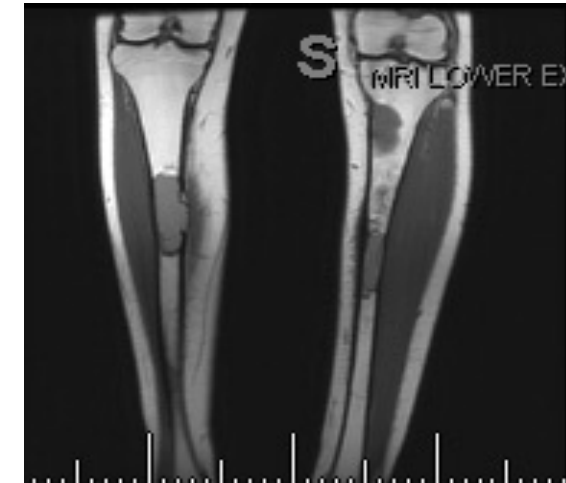
The clinical spectrum of GD3

- 32 children homozygous for the point mutation L444P (L483P)
- The residual enzyme activity was variable and did not correlate with the clinical course well.
- There was a wide variety in clinical presentations .
- Average age at diagnosis was 15 months,
- Abnormalities of the saccadic eye movements were the uniform (Goker-Alpan, 2006)



Unmet medical challenges in GD3

- Progressive disease despite intervention
- Seizure disorder
- Myoclonic encephalopathy
- Hearing loss
- Cognitive impairment
- Behavioral and psychiatric problems
- Pain
- Skeletal disease (AVN and other structural changes)
- Infiltrative pulmonary disease
- Cardiac involvement associated with rare genotypes (D409H/D409H)
- Progressive kyphoscoliosis
- Eye involvement (vitreal opacities, cataracts. corneal involvement)
- Gaucheroma (Gaucher cell tumors)
- Giant lymphadenopathy
- Protein losing enteropathy
- Social/psychosocial burden of therapy



Newborn Screening (NBS) for Gaucher disease

State health department initiative

- Individual states decide which disorders to include

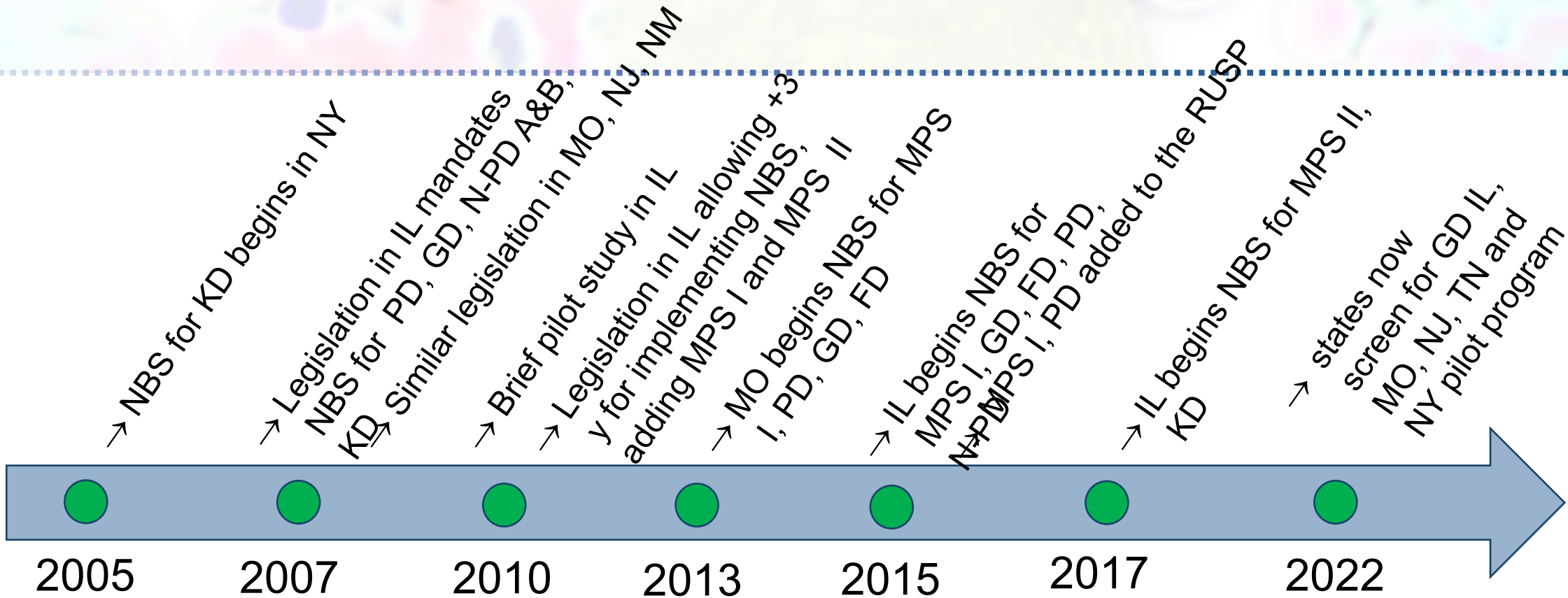
Recommended Uniform Screening Panel (RUSP): list of disorders recommended by the Department of Health and Human Services (Advisory Committee on Heritable Disorders in Newborns and Children) for states to screen as part of NBS programs

Most states follow federal recommendations and eventually implement screening for disorders on the RUSP

As of 2018, lists 35 core conditions including two LSDs (Pompe disease and MPS I), and 26 secondary disorders are in RUSP

- Core conditions: primary screening targets
- Secondary conditions: those that could be identified when screening, or as part of confirmatory testing, for a core condition
- A RUSP review request was placed for Gaucher disease

History of LSD NBS in the US



KD = Krabbe disease; FD = Fabry disease; GD = Gaucher disease; PD = Pompe disease; N-PD= Niemann-Pick disease; MPS = mucopolysaccharidosis.

1. Burton BK, et al. *J Pediatr*. 2017;190:130-135; Millington D, et al. *Int. J. Neonatal Screen*. 2018;4:24; Missouri Newborn Screening 2013 Annual Report.

2. HRSA 2013. <https://health.mo.gov/living/families/genetics/newbornscreening/pdf/newbornscreeningreport2013.pdf>; HRSA. 2013.

3. HRSA 2013. <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/pompe-27-june-2018.pdf>; HRSA. 4. HRSA 2015. <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/mps-i-27-june-2018.pdf>;

5. NewSTEPS. <https://www.newsteps.org/resources/newborn-screening-status-all-disorders>.

Current and Emerging Treatments for Gaucher disease

	FDA-Approved	Human Trials	Pre-Clinical	“Off label”
Gaucher disease	ERT – IV SRT	<ul style="list-style-type: none">• in vivo gene therapy*• SRT (brain penetrant)• in vivo gene therapy• ex vivo gene therapy	<ul style="list-style-type: none">• in vivo gene therapy• ex vivo gene therapy• ERT brain penetrant	Chaperone+
+ In Europe and other countries				

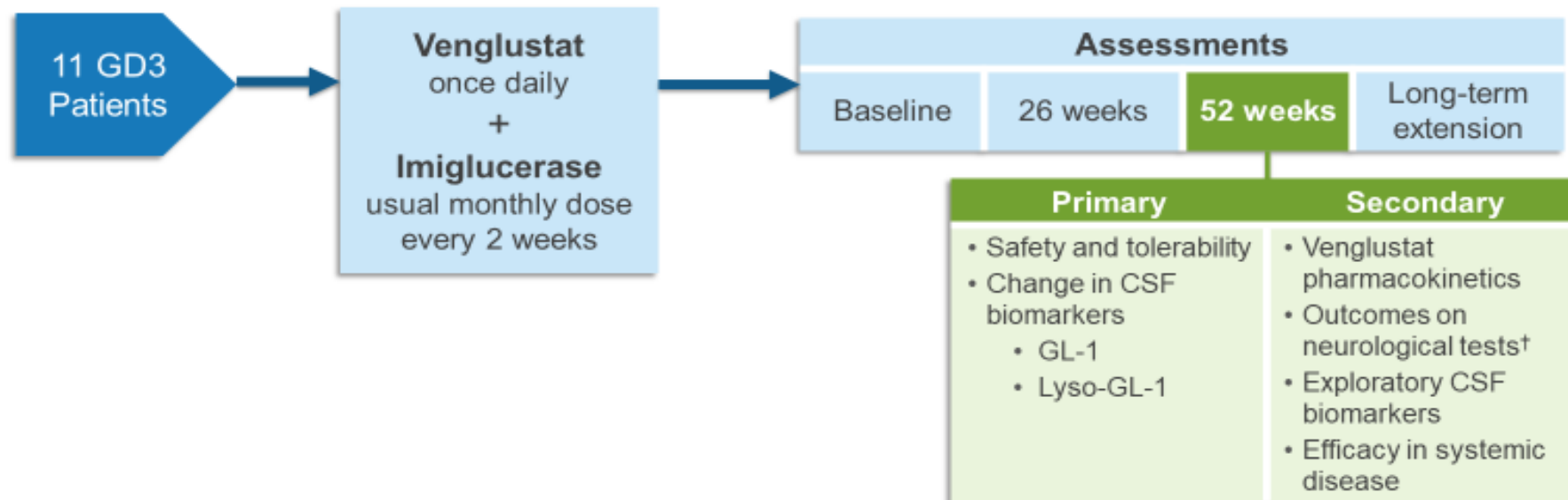


Venglustat in combination with imiglucerase ERT in adult patients with GD3

LEAP trial design (NCT02843035)

Current analysis at 52 weeks

Ongoing phase 2 trial of venglustat, an oral glucosylceramide synthase inhibitor, in combination with intravenous imiglucerase (Cerezyme) in adult GD3 patients*



*Trial also includes biomarker evaluation for differences between GD3 and GD1 patients (GD1 patients do not receive study medication).

†Scale for Assessment and Rating of Ataxia (SARA), brain functional MRI, and trail-making test

CSF: cerebrospinal fluid; GD3: Gaucher disease type 3; GL-1: glucosylceramide; Lyso-GL-1: glucosylsphingosine



Venglustat SRT in combination with imiglucerase ERT in adult patients with GD3

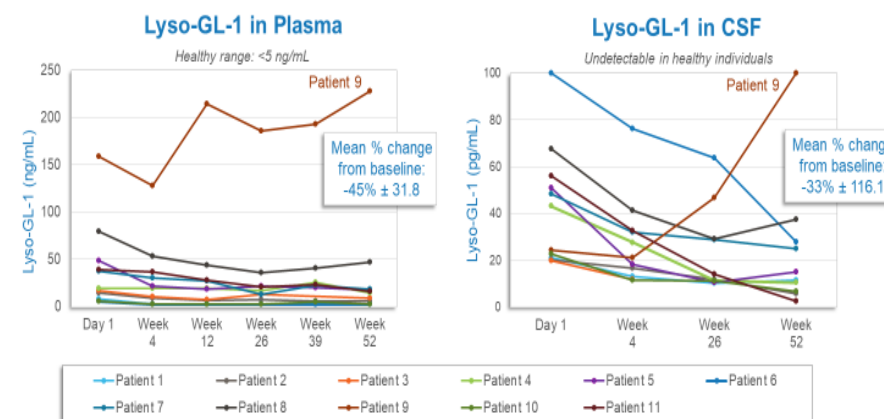
Conclusions from the LEAP Study

At 1 year, venglustat added to imiglucerase in adult GD3 patients showed:

- Favorable safety and tolerability
- Evidence that venglustat crosses the blood–brain barrier
- Robust reduction in CSF sphingolipids
- Resting state fMRI (baseline to Week 52) showed enhanced connectivity across brain regions including cerebellum, sensorimotor and cognitive regions
- Potential to ameliorate neurological manifestations of GD3
- Stable systemic manifestations

CSF: cerebrospinal fluid; GD3: Gaucher disease type 3

Lyso-GL-1 in CSF (primary endpoint) and plasma
Levels decreased for all patients, except Patient 9

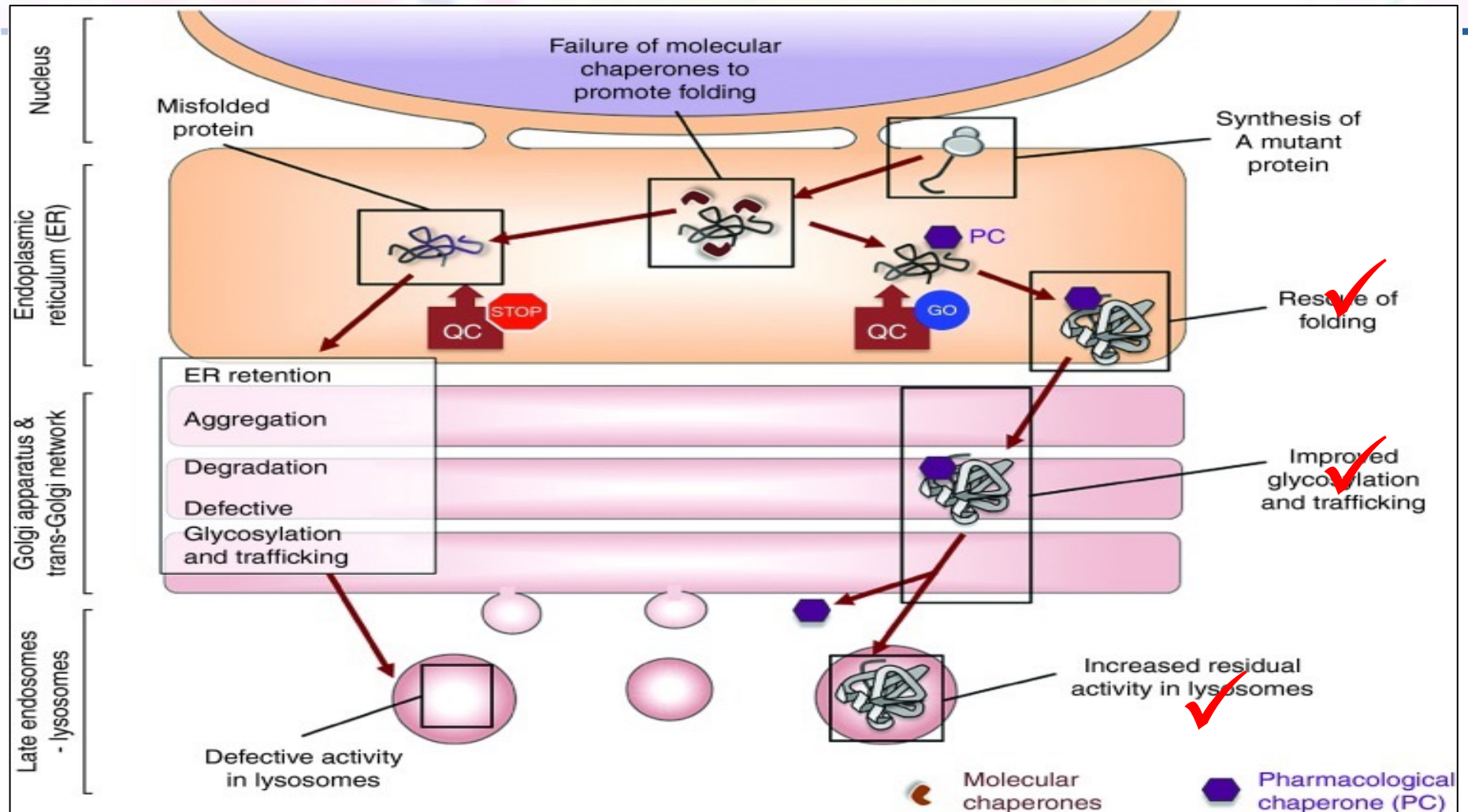


LLOQ: plasma lyso-GL-1: 5.0 ng/mL, CSF lyso-GL-1: 5 pg/mL
GL-1: glucosylceramide; Lyso-GL-1: glucosylsphingosine; CSF: cerebrospinal fluid; LLOQ: lower limit of quantification

Schiffmann R et al. *Mol Gen Metab.* 2020;129:S144.



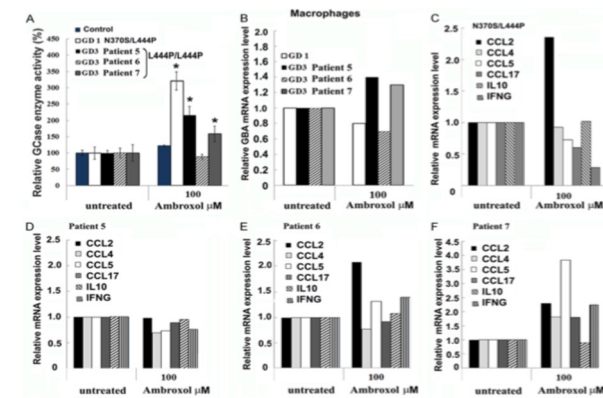
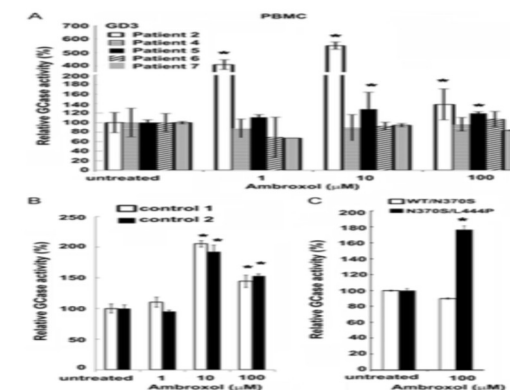
Chaperone Therapy in LSDs



Source: Parenti G, et al. *Mol Ther.* 2015;23(7):1138-1148.

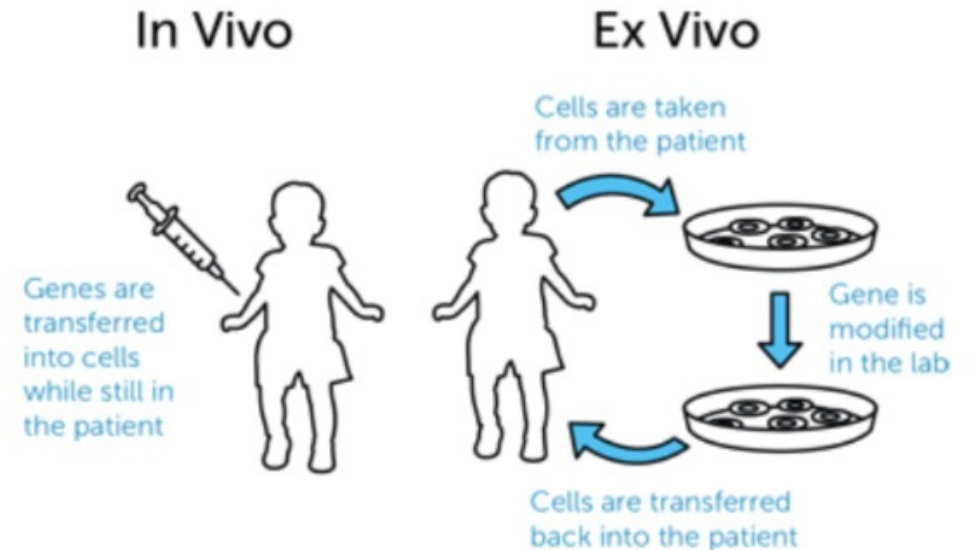
CHAPERONE ACTIVITY IS "PERSONALIZED"

- Ambroxol interacts the enzyme GCase in a mixed type of activation and inhibition, and pH dependent manner (Maegawa et al, JBC, 2009)
- High-dose oral ambroxol increased lymphocyte glucocerebrosidase activity, and decreased glucosylsphingosine levels (Narita et al, 2016, Charkhand et al, 2019, Kim et al, JMedGenet2019)
- Ambroxol levels could be influenced by multiple medications, especially antiseizure drugs which may impact the efficacy (Kim et al, J Med Genet 2019)
- Ambroxol was ineffective in cell lines with complex GBA alleles.
- In cells from patients with neuropathic GD and L444P/L444P genotype, the response to ambroxol was varied .
- Different cell types to screen efficacy, toxicity, and inflammatory response in GD: Macrophages respond to ambroxol by changing expression profile of immune system genes.
- A favorable *in vitro* response to a small molecule chaperone such as ABX , that the *GBA1* variants are functionally rescued as shuttled to the lysosome, also could predict more favorable outcomes.



Gene Transfer Therapy

- **Phase 1/2 Clinical Trial of PR001 in Infants With Type 2 Gaucher Disease (PROVIDE)**
- **A clinical trial of PR001 (LYS3884961) in (adult) patients with peripheral manifestations of Gaucher disease (PROCEED)**



Brain penetrant ERT for neuronopathic LSDs

- Animal models provided preclinical evidence of the delivery of enzymes into the brain via receptor mediated transcytosis (insulin and transferrin receptors)
- Pabinafusp alfa (IZCARGO[®]), a recombinant fusion protein of an antibody against the human transferrin receptor crosses the BBB through transferrin receptor-mediated transcytosis, and its uptake into cells is mediated through the mannose-6-phosphate receptor.
- Preclinical development of brain penetrant ERT for neuronopathic GD

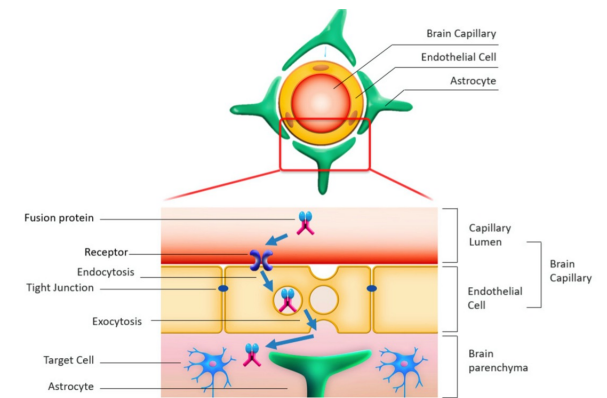


Figure 2. Schematic representation of the transcytosis mechanism.

Sato and Okuyama, Int J Mol Sci, 2020

