GENETICALLY MODULATED SUBSTRATE REDUCTION THERAPY

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THE **ENZYME** AS A **DRUG**?
THE **ENZYME AS A DRUG?**

- **1996**
- **Norman Radin**

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**Treatment of Gaucher disease with an enzyme inhibitor**

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The hypothesis is offered predicting that Gaucher patients could be treated with a drug that slows the synthesis of glucosylceramide, the lipid that accumulates in this disorder. The present therapeutic approach involves augmenting the defective enzyme, glucocerebrosidase β-glucosidase, with exogenous β-glucosidase isolated from human tissue. This spectacularly expensive mode of treatment should be replaceable with a suitable enzyme inhibitor that simply slows formation of the lipid and lessens the rate of synthesis with the rate of the defective, slowly working β-glucosidase. Several drugs that possess this ability are available, the best known of which is 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a designer inhibitor that resembles the synthase's substrate and product. PDMP has been found to be effective in mice, rats, fish, and a wide variety of cultured cells. Its use, at suitable dosages, seems to be harmless, although long-term tests have not been made. The lack of suitable animal models of Gaucher disease has made it difficult to test the hypothesis adequately, but PDMP does rapidly lower the levels of glucosylceramide in normal animal tissues and the animals evidently do well with the lowered levels of glucosylceramide and its more complex glycolipid metabolites.
substrate reduction  enzyme replacement
N-Butyldeoxyojojirimycin Is a Novel Inhibitor of Glycolipid Biosynthesis

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Safety, Tolerability, and Pharmacokinetics of Eliglustat Tartrate (Genz-112638) After Single Doses, Multiple Doses, and Food in Healthy Volunteers

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Three phase 1 studies of eliglustat tartrate (Genz-112638), an oral inhibitor of glucosylceramide synthase under development for treating Gaucher disease type 1 (GD1), evaluated the safety, tolerability, and pharmacokinetics in healthy volunteers. After escalating single doses (n = 99), escalating multiple doses (n = 36), and food (n = 24). Eliglustat tartrate was well tolerated at single doses ≤20 mg/kg and multiple doses ≤200 mg bid, with 50 mg bid producing plasma concentrations in the predicted therapeutic range—2 hours, followed by a monophasic decline with a ~6-hour terminal half-life. Unchanged drug in 8-hour urine collections was <1.5% of administered doses. Food did not significantly affect the rate or extent of absorption. Multiple-dose pharmacokinetics was nonlinear, showing higher than expected plasma drug concentrations. Steady state was reached ~60 hours after bid dosing. Higher drug exposure occurred in slower CYP2D6 metabolizers. Based on favorable results in healthy participants, a phase 2 trial is planned.
## SUBSTRATE REDUCTION THERAPY (SRT)

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Approved SRT</th>
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| Gaucher              | Zavesca®<sup>®</sup>  
(Miglustat; Actelion)                                                        |
|                      | Cerdelga®<sup>®</sup>  
(Eliglustat tartrate; Genzyme)                                                 |
| Niemann-Pick C       | Zavesca®<sup>®</sup>  
(Miglustat; Actelion)                                                        |
SUBSTRATE REDUCTION THERAPY (SRT)

1. NAME OF THE MEDICINAL PRODUCT

Zavesca 100 mg hard capsules

4.4 Special warnings and precautions for use

**Tremor**
Approximately 37% of patients in clinical trials have developed tremor. In a clinical trial in Niemann-Pick type C disease patients treated with Zavesca, these tremors were described as an exaggerated Parkinson's disease. The tremors began within the first month, and in many cases continued over the following 3 months. Dose reduction may ameliorate the tremor, but in some cases, treatment may sometimes be required.

**Gastrointestinal disturbances**
Gastrointestinal events, mainly diarrhoea, have been observed at the onset of treatment or intermittently during treatment. This is likely inhibition of intestinal disaccharidases such as lactase, leading to reduced absorption of dietary disaccharides. Gastrointestinal events have been observed to resolve with a reduction of sucrose, lactose and other carbohydrates. In addition, anti-diarrhoeal medicinal products such as loperamide may be necessary. Patients with chronic diarrhoea not responding to these interventions should be monitored. The efficacy has not been evaluated in patients with a history of inflammatory bowel disease.

Effects on **spermatogenesis**
Male patients should maintain reliable contraception during treatment. Studies have shown that miglustat adversely affects spermatogenesis. Until 3 months.

**What are the risks associated with Cerdelga?**

The most common side effect seen with Cerdelga (which may affect more than 1 in 10 people) is diarrhoea, in approximately 6 out of 100 patients. The majority of side effects are mild and transient. For the full list of all side effects reported for Cerdelga, see the package leaflet.

Cerdelga must not be taken together with certain medicines that can interfere with the ability of the body to break down the medicine, which may affect the levels of Cerdelga in blood. For the full list of restrictions, see the package leaflet.
gSRT PROOF OF PRINCIPLE - GD

- Spanish group
- Target: GCS

Glucosylceramide synthase
(=UDP-glucose ceramide synthase)

Diaz-Font et al., (2006)
gSRT PROOF OF PRINCIPLE - GD

- Spanish group

- Target: *GCS*

Diaz-Font *et al.*, (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: \textit{GCS}
- Test in: HeLa cells

Human \textit{GCS} gene

http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core\&g=ENSG00000133937\&r=14:94768216-94770230\&t=ENST00000238558 (adapted)
gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs
gSRT PROOF OF PRINCIPLE - GD

- **Target:** \(GCS\)
- **Test in:** HeLa cells
- **Compound(s):** si/shRNAs

Diaz-Font *et al.*, (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs

*dose-dependent effect*

Diaz-Font et al., (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs

*dose-dependent effect*

Diaz-Font et al., (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs

**Effect on GCS activity**

(72h)

![Graph showing GCS enzyme activity](chart)

Diaz-Font *et al.*, (2006)
**gSRT PROOF OF PRINCIPLE - GD**

- **Target:** *GCS*
- **Test in:** HeLa cells
- **Compound(s):** si/shRNAs

**Effect on glucosylceramide formation**

(TLC)

Diaz-Font *et al.,* (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

**Effect on glucosylceramide formation**

(TLC)

Diaz-Font *et al.*, (2006)
Target: GCS
Test in: HeLa cells
Compound(s): si/shRNAs
coded in a DNA expression vector
stable transfected cells
permanent reduction of expression

Diaz-Font et al., (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

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**Graph: GCS mRNA (%)**

- untransfected
- psiRNA
- shRNA11
- shRNA68

Diaz-Font *et al.*, (2006)
gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs

**Effect on GCS activity**

![Graph showing GCS enzyme activity](image)
Target: GCS
Test in: HeLa cells
Compound(s): si/shRNAs

effect on GCS activity

GCS enzyme activity (%)

untransfected  psiRNA  shRNA11  shRNA68

Diaz-Font * et al., (2006) **

GCS PROOF OF PRINCIPLE - GD
gSRT PROOF OF PRINCIPLE - GD

SUMMARY

si/shRNAs

- GCS mRNA levels
- GCS enzyme activity
- glucosylceramide synthesis
Mucopolysaccharidoses (MPS)

- Chronic
- Progressive
- Large spectrum of severity & symptoms

MPS III
(= Sanfilippo Syndrome)
A, B, C and D
Available Therapies

- **Enzyme Replacement Therapy**
  - Only available for 4 MPSs!
  - No effect on neurological symptoms!

... antibodies!
AVAILABLE THERAPIES

- Enzyme Replacement Therapy

Perfect Target for Substrate Reduction Approaches!
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Polish group

- Targets: XYLTO, XYLTT, GALTt, GALTII
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Polish group

- Targets: XYLTL1, XYLTL2, GALTI, GALTII
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Polish group

- Targets: \textit{XYLT1}, \textit{XYLT2}, \textit{GALTI}, \textit{GALTII}
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSSES

- Polish group

- Targets: *XYLT1, XYLT2, GALTI, GALTII*

- Test in: MPS IIIA fibroblasts

gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSSES

- Polish group
- Targets: XYLT1, XYLT2, GALTI, GALTII
- Test in: MPS IIIA fibroblasts
- Compounds: siRNAs

Dziedzic et al., (2010)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Polish group

- Targets: XYLT1, XYLT2, GALTI, GALTII
- Test in: MPS IIIA fibroblasts
- Compounds: siRNAs

proteins levels

Dziedzic et al., (2010)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Polish group

- Targets: XYLT1, XYLT2, GALTI, GALTII

- Test in: MPS IIIA fibroblasts

- Compounds: siRNAs

**GAG synthesis**

![Graph showing relative GAG synthesis](image)
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

SUMMARY

“early stages” GAGs biosynthesis genes

↓ synthesis

Dziedzic et al., (2010)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Targets: **XYLT1, XYLT2, GALTI, GALTII**
- Test in: MPS IIIA fibroblasts

Dziedzic et al., (2012)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Targets: *XYLT1, XYLT2, GALTI, GALTII*
- Test in: MPS IIIA fibroblasts

Dziedzic et al., (2012)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Targets: XYLT1, XYLT2, GALT1, GALTII
- Test in: MPS IIIA fibroblasts

≠ not statistically significant

Dziedzic et al., (2012)
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

Would the simultaneous use of 2 species of siRNAs be more effective than the use of single siRNAs?

Potential benefit: doubtful

Cost-benefit?
Side-effects?

Dziedzic et al., (2012)
gSRT FOR OTHER LSDS - MUCO POLY SACCHARIDOSES

- What about...

- cytotoxic effects?...
- efficacy gSRT vs ERT?...
- effect of combiatory treatment?...

MPS I MPS IIIA and MPS IIIB

Chmielarz et al., (2012)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Cytotoxic effects?...

(cell survival %)

Chmielarz et al., (2012)
Efficacy gSRT vs ERT and effect of combiatory treatment?

![Graphs showing GAG level as percentage of untreated control for different cell lines and treatments.](image)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Efficacy gSRT vs ERT and effect of combiatory treatment?

\[\text{GAG level as percentage of untreated control [\%]}\]

**MPS I – cell line 1**

- **MPS I – cell line 2**

- **MPS I – cell line 3**

Chmielarz et al., (2012)
Efficacy gSRT vs ERT and effect of combinatory treatment?...

Chmielarz et al., (2012)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Cytotoxic effects?…

  No!

  However,
  
  *some cytotoxic effects could occur in particular cell lines*

- Efficacy gSRT vs ERT and effect of combinatorial treatment?…

  Depends on specific features of each cell line

  *Thus,*
  
  *the combined therapy might potentially result in various effects in different patients*
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Australian group

- Targets: \textit{EXTL2} and \textit{EXTL3}

Kaidonis \textit{et al.}, (2012)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Australian group
  - Targets: *EXTL2* and *EXTL3*
  - Test in: 293T cells; MPS I and MPS IIIA fibroblasts

[Image: https://upload.wikimedia.org/wikipedia/commons/8/85/HEK_293_cells_grown_in_tissue_culture_medium.jpg]


Kaidonis et al., (2012)
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Australian group

- Targets: $EXTL2$ and $EXTL3$
- Test in: 293T cells; MPS I and MPS IIA fibroblasts
- Compounds: shRNAs

![Graph showing percent of psiCHECK-2 target gene only luminescence for different shEXTL samples.](image_url)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Australian group

- Targets: *EXTL2* and *EXTL3*
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts
- Compounds: shRNAs

![Graph showing percent of target gene expression](chart.png)

*(endogenous)*
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Australian group

- Targets: EXTL2 and EXTL3

- Test in: 293T cells; MPS I and MPS IIIA fibroblasts

- Compounds: shRNAs

GAG synthesis

Kaidonis et al., (2012)
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Australian group

- Targets: EXTLE2 and EXTLE3
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts
- Compounds: shRNAs

GAG synthesis

Kaidonis et al., (2012)
gSRT FOR OTHER LSDS - MUCOPOlySACCHARIDOSes

- Spanish group
  - Targets: *EXTL2* and *EXTL3*

Canals et al., (2015)
gSRT FOR OTHER LSDS - MUCOPOlysACCHARIDIOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS III\textcolor{red}{C} fibroblasts 2 ≠


Canals et al., (2015)
gSRT FOR OTHER LDS - MUCOPOLYSACCHARIDOSIS

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts
- Compounds: siRNAs

Canals et al., (2015)
**gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES**

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIc fibroblasts
- Compounds: siRNAs

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![Graph showing GAG synthesis](image)

Canals *et al.*, (2015)
gSRT FOR OTHER LSDS - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIc fibroblasts
- Compounds: siRNAs

Canals et al., (2015)
gSRT FOR OTHER LSDs - MUCOPOlysACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIc fibroblasts
- Compounds: siRNAs

Canals et al., (2015)
**gSRT FOR OTHER LSDS - MUCO PolySACCHARIDOSES**

**SUMMARY**

si/shRNAs

- mRNA levels
- enzyme activity
- GAGs synthesis

**Reverse the cellular phenotype!!!**
gSRT FOR OTHER LSDS - POMPE

- American group

- Target: *muscle specific glycogen synthase (Gys1)*

- Test in: Pompe mice

GAA−/− (Charles River Laboratories, Wilmington, MA)
gSRT FOR OTHER LSDS - POMPE

- American group
- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

phosphorodiamidate morpholino oligonucleotide conjugated to a cell penetrating peptide

≈ therapy for Duchene muscular dystrophy
gSRT FOR OTHER LSDS - POMPE

- American group

- Target: *muscle specific glycogen synthase (Gys1)*

- Test in: Pompe mice

- Compound(s): GS-PPMO

  phosphorodiamidate morpholino oligonucleotide conjugated to a cell penetrating peptide

Exon skipping

PTC
gSRT FOR OTHER LSDS - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

Tail vein injection every 2 weeks

$\Delta t = 12$ weeks
gSRT FOR OTHER LSDS - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO
gSRT FOR OTHER LSDS - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

---

**Skeletal muscles**

Clayton et al. (2014)
gSRT FOR OTHER LSDS - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

*cardiac muscles*

Clayton et al. (2014)
gSRT FOR OTHER LSDS - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

* tissue-specific effect

Clayton et al. (2014)
gSRT FOR OTHER LSDS - POMPE

- **Target:** muscle specific glycogen synthase (Gys1)
- **Test in:** Pompe mice
- **Compound(s):** GS-PPMO

*lysosomal glycogen accumulation*

Clayton *et al.* (2014)
gSRT FOR OTHER LSDS - POMPE

SUMMARY

↓ Target protein levels
↓ Iglycogen levels

Tissue-specific effect
A LOOK FORWARD…

- Vector design & si/shRNA encapsulation
  - ↑ bioavailability of siRNAs;
  - protection from degradation
  - control of
    - circulation time
    - release rate

Lipid nanoparticles?
siRNA-peptide conjugates?
  Cell-penetrating peptides (CPP)
A LOOK FORWARD…

- Vector design & si/shRNA encapsulation
  - ↑ bioavailability of siRNAs;
  - protection from degradation
  - control of
    - circulation time
    - release rate

- Genotoxic evaluation of the si/shRNA+nanocarrier formulation(s)
  - internalization
  - cell viability
  - genotoxic potential
A LOOK FORWARD…

- *In vivo* studies
**The Open Questions:**

- **Delivery**
  - Appropriate vector

- **Animal models**
  - Recapitulate human disease
  - *In vivo* testing

- **Mode of administration**
  - Local?
  - Systemic?
  - Intravenous?
  - Intrathecal?
THE FUTURE…

genetic substrate reduction  +  enzyme replacement
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