Substrate Replacement Therapy (SRT) for Congenital Disorder of Glycosylation Type Ia (CDG-Ia)

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Agenda

• Glycomine’s mission
• CDG-Ia disease and rationale for substrate replacement therapy (SRT)
• Our approach to SRT for CDG-Ia
• Preliminary data and findings
• Potentials and next steps
Glycomine’s Mission

- We are committed to helping patients that have no treatment options
- Glycomine develops therapeutics for rare genetic disorders of protein glycosylation
- There are 100 known glycosylation disorders, most of which cause severe debilitation in patients
- We are currently addressing two different CDGs:
  - CDG-Ia
  - NGLY1 deficiency
Molecular Basis of CDG-Ia Disease

- Deficiency of phosphomannomutase 2 (PMM2), enzyme responsible for the conversion of mannose-6-phosphate into mannose-1-phosphate

\[ \text{Man-1-P} \xrightarrow{\text{PMM2}} \text{Man-6-P} \xrightarrow{\text{MPI}} \text{Fru-6-P} \]

\[ \text{GDP-Man} \xrightarrow{\text{GDP}} \text{GDP} \]

\[ \text{N-linked Protein Glycosylation} \]
Previous Efforts Toward a Therapy for CDG-Ia

- Mannose-1-phosphate (Man-1-P), the missing substrate, cannot cross the cell membrane on its own due to its hydrophilic “water-loving” properties
- Several attempts toward a therapy focused on chemical modification of Man-1-P to make it cell membrane permeable
- Such approach works (tested in vitro only), but toxic and unsafe
- Additionally, chemical derivatives made thus far are unstable and exhibit short half-lives of about 2.5 minutes (at best)
Glycomine’s Approach to Therapy for CDG-Ia

- We deliver unmodified Man-1-P via liposomes – nano-vehicles composed of lipids that resemble the cell membrane, ~100 nm in size.

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**Examples of the Nanoscale***

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*Not to Scale*

- 1 micrometer (1 µm): 1/1,000,000 m, 1.000 x 10⁻⁶ m, 1000 nanometers
- 1 nanometer (1 nm): 1/1,000,000,000 m, 1 x 10⁻⁹ m, 10 Angstroms
- 1 Angstrom (1 Å): 1/10,000,000,000 m, 100.00 x 10⁻¹⁰ m, 100 picometers
- 1 Picometer (1 Å): 1/10,000,000,000,000 m, 100.00 x 10⁻¹² m
Cellular Uptake of Lipo-Man-1-P by Endocytosis

- Biodegradable & non-immunogenic
- Can be functionalized to target the blood-brain barrier
- FDA-approved drug & gene delivery carriers
- Efficient carriers of small molecule drugs, stable, and non-toxic
Evaluation of Liposomal Formulation of Man-1-P

Critical Parameters:

- **Subcellular localization** – delivery into a specific compartment within the affected cell, i.e., cytosol

- **Toxicity** – all drugs have adverse effects at normal doses, but some can produce positively dangerous effects which are referred to as toxic

- **Efficacy** – drug’s capacity to produce an effect, i.e., increase levels of Man-1-P and GDP-Man and correct abnormal protein glycosylation

- **Pharmacokinetics** – the movement of drug into, through, and out of the body – the time course of its absorption, bioavailability, distribution, metabolism, and excretion
Lipo-Man-1-P Cellular Localization

- In order to be effective, Man-1-P needs to be delivered to cell interior (cytosol)

CDG-1a fibroblast cells without Lipo-Man-1-P

CDG-1a fibroblast cells with Lipo-Man-1-P (red)

lysosomes (green)
nuclei (blue)
Lipo-Man-1-P Toxicity

![Graph showing cell viability (%)](image)

- **Y-axis:** Cell Viability (%)
- **X-axis:** M1P (mg/mL)
- **Legend:**
  - M1P-liposome
  - M1P-free drug
Lipo-Man-1-P Ability to Increase Man-1-P and GDP-Man Levels (in vitro), “Efficacy”

- The delivered Man-1-P is not radiolabeled, which poses difficulties with tracing it within the glycan biosynthetic and processing pathways.
- Development of alternative quantitative methods, labels, and test conditions is underway.

CDG-Ia fibroblasts treated with Lipo-Man-1-P
Lipo-Man-1-P Pharmacokinetics – Retention Time in Blood of Healthy Mice

- Half-life: period of time required for the amount of drug in the body to be reduced by one-half
- Lipo-Man-1-P half-life = 18 hrs
Lipo-Man-1-P Pharmacokinetics – Distribution in Tissue of Healthy Mice

- >30% of drug was taken up by the liver, which is the powerhouse for synthesis of blood glycoproteins
Lipo-Man-1-P Concentration in Blood and Liver of Healthy Mice

- We are currently working on a method to distinguish between exogenous Man-1-P and endogenous sugar-1-phosphates found in blood and liver tissue.
- Our initial data suggests that Lipo-Man-1-P treatment increases levels of Man-1-P in liver tissue while blood levels remain low.
- We hope to report on this study in the coming weeks.
Lipo-Man-1-P Findings and Potential

• Liposomal formulation of Man-1-P
  • Delivers missing Man-1-P where needed, i.e., into the cell interior (cytosol)
  • Non-toxic at high concentrations of the nano-vehicle (lipid) and Man-1-P
  • Replaces missing Man-1-P, increases levels of GDP-Man, and has the potential to correct abnormal glycosylation
  • Is retained in blood for extended periods of time
  • Targets liver tissue where majority of plasma proteins are synthesized
Lipo-Man-1-P Areas of Improvement and Next Steps

- Work on extending formulation half-life and retention in blood
- Develop methods to demonstrate drug efficacy without the use of radiolabeled Man-1-P (both in vitro and in vivo)
- Work on delivery to brain by either
  - vehicle modification with ligands capable of penetrating the blood-brain barrier
  - AND/OR
  - explore routes of drug administration other than intravenous
Timeline for Next Steps with CDG-Ia Lipo-Man-1-P Therapy

- **2015**
  - Sep: Formulation optimization
  - Nov: Efficacy in healthy mice

- **2016**
  - Early: Therapeutic Candidate
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