GLYCOMINE

CONGENITAL DISORDERS OF GLYCOSYLATION WORLD CONFERENCE

The power of advancing patient-oriented research united

FAMILIES AND PROFESSIONALS

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

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Agenda

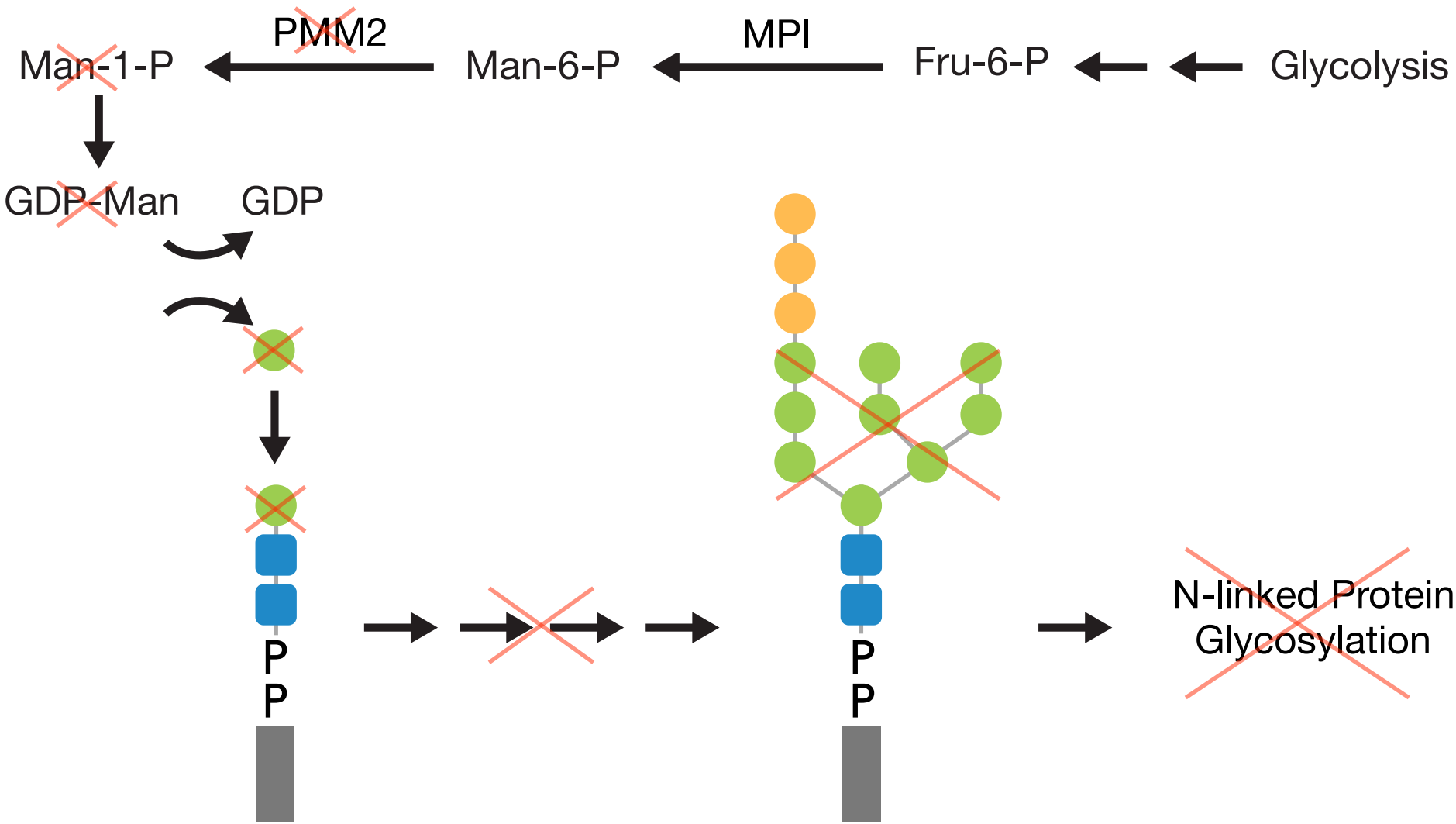
- Glycomine's mission
- CDG-Ia disease and rationale for substrate replacement therapy (SRT)
- Our approach to SRT for CDG-Ia
- Preliminary data and findings
- Potentials and next steps

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  Grace Wilsey Foundation

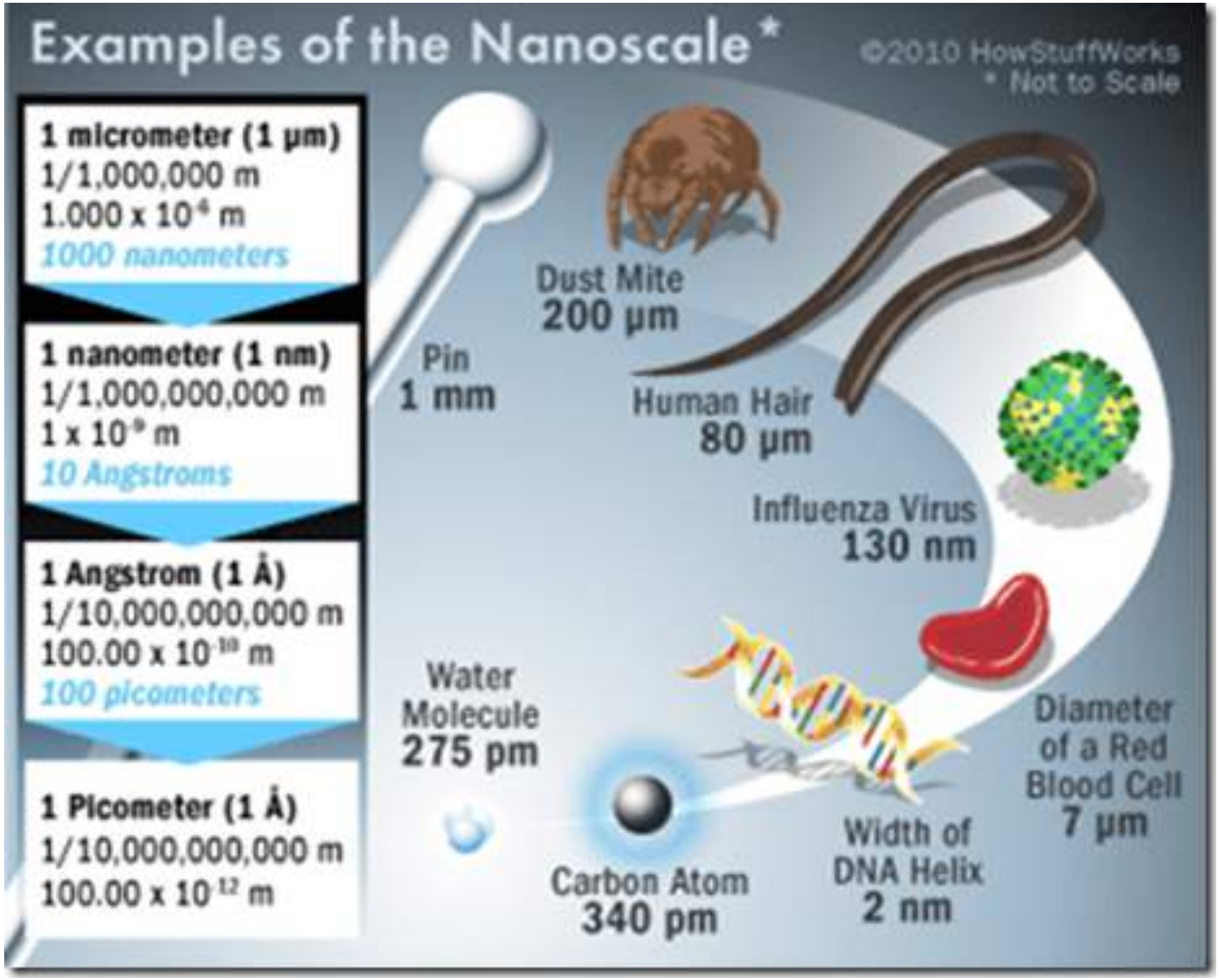
Molecular Basis of CDG-Ia Disease

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



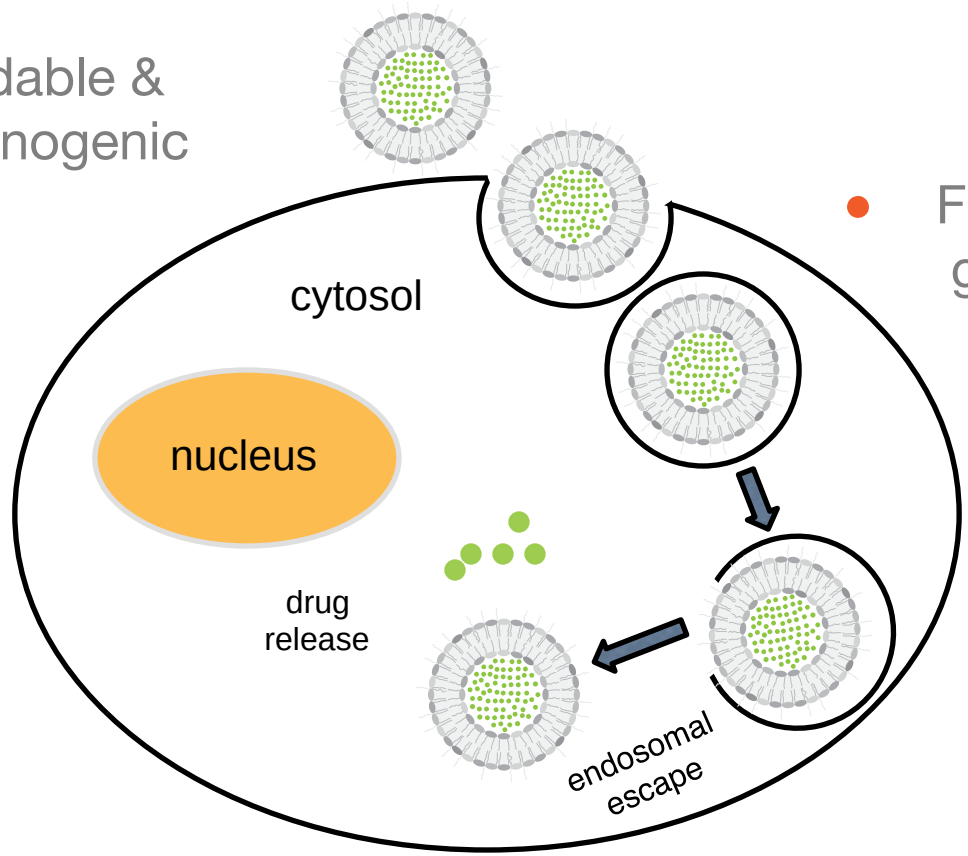
Previous Efforts Toward a Therapy for CDG-Ia

- Mannose-1-phosphate (Man-1-P), the missing substrate, can not 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)


Examples of the Nanoscale*
©2010 HowStuffWorks
* 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 m
100.00 x 10⁻¹² m
Pin
1 mm
Dust Mite
200 µm
Human Hair
80 µm
Influenza Virus
130 nm
Water
Molecule
275 pm
Carbon Atom
340 pm
Width of
DNA Helix
2 nm
Diameter
of a Red
Blood Cell
7 µm

Cellular Uptake of Lipo-Man-1-P by Endocytosis

- Biodegradable & non-immunogenic



- FDA-approved drug & gene delivery carriers
- Efficient carriers of small molecule drugs, stable, and non-toxic
- Can be functionalized to target the blood-brain barrier

Evaluation of Liposomal Formulation of Man-1-P

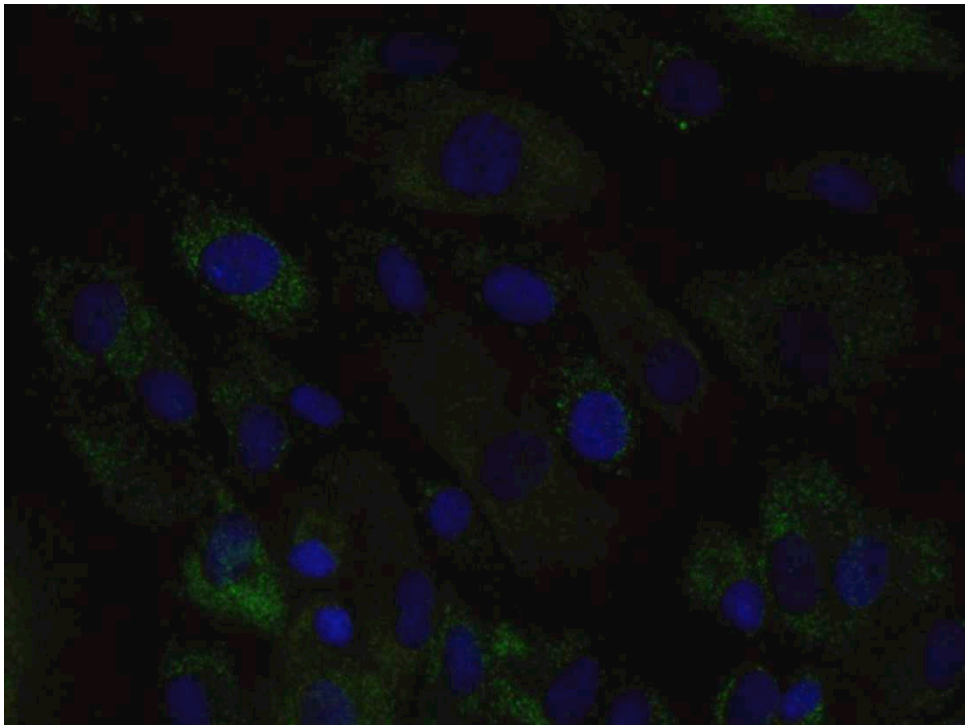
Critical Parameters:

- **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

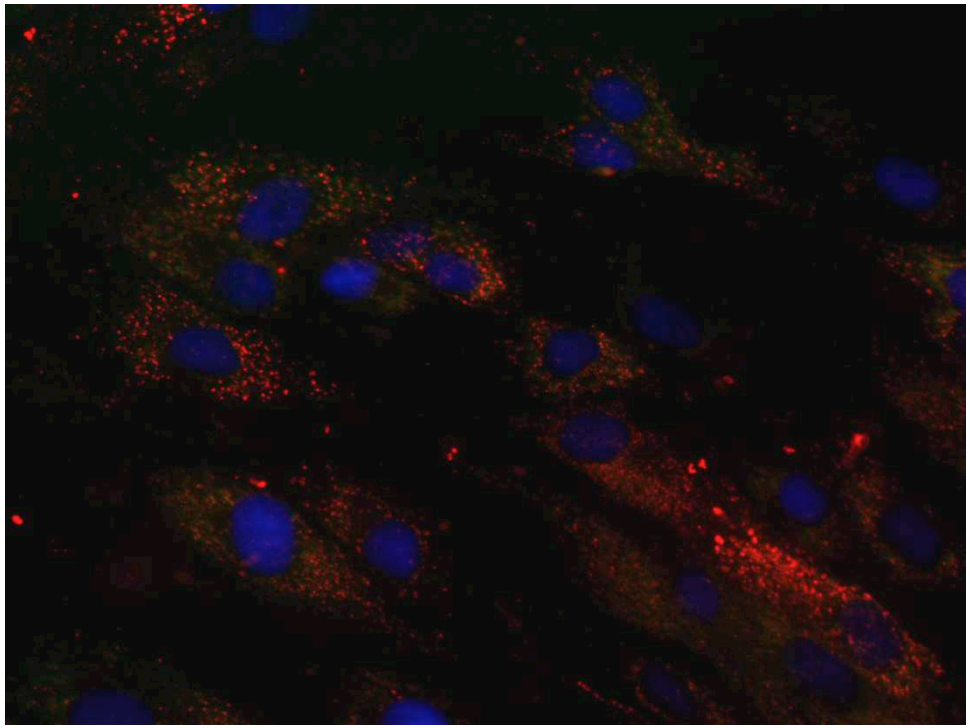
- In order to be effective, Man-1-P needs to be delivered to cell interior (cytosol)

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

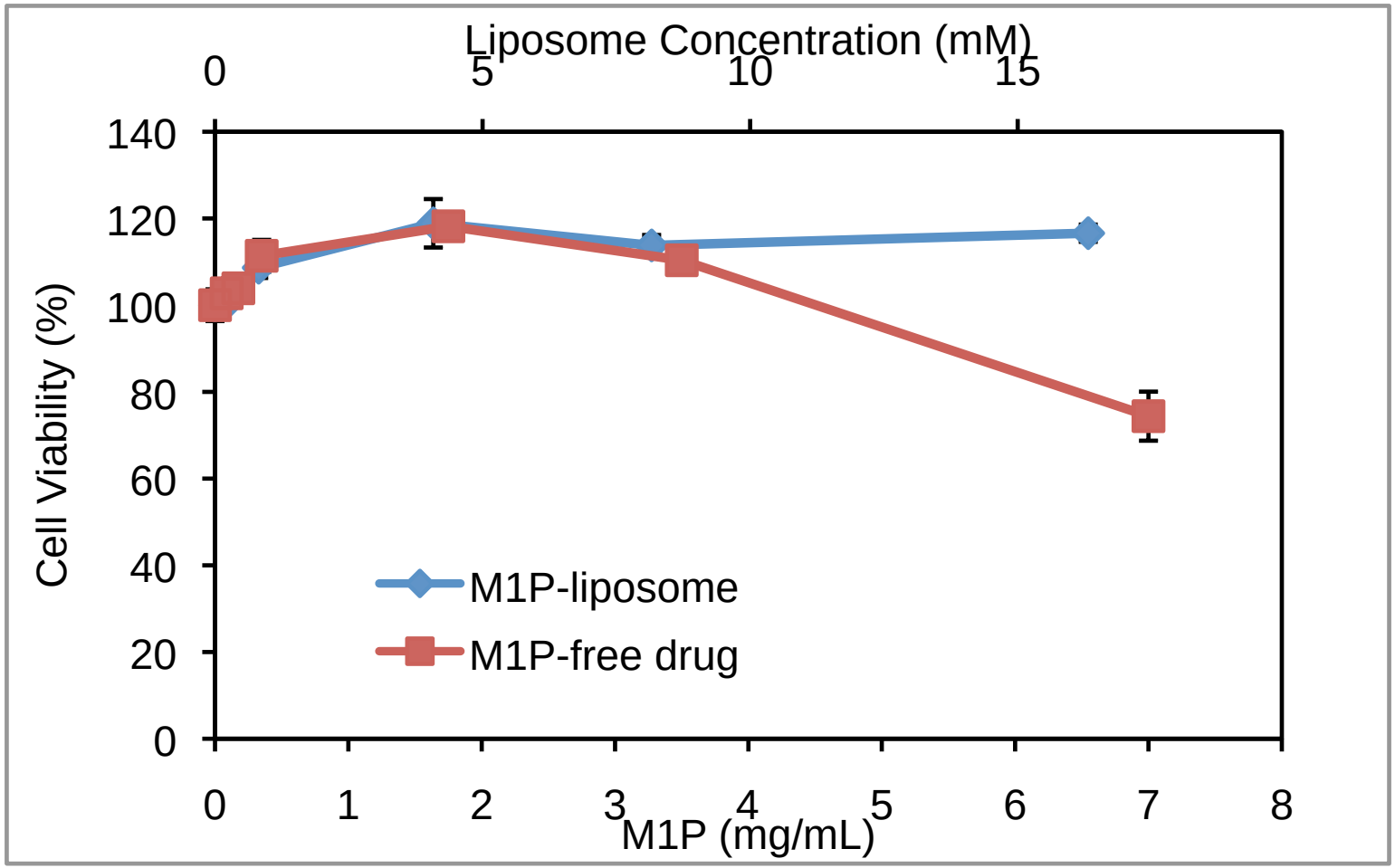


lysosomes (green)
nuclei (blue)

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

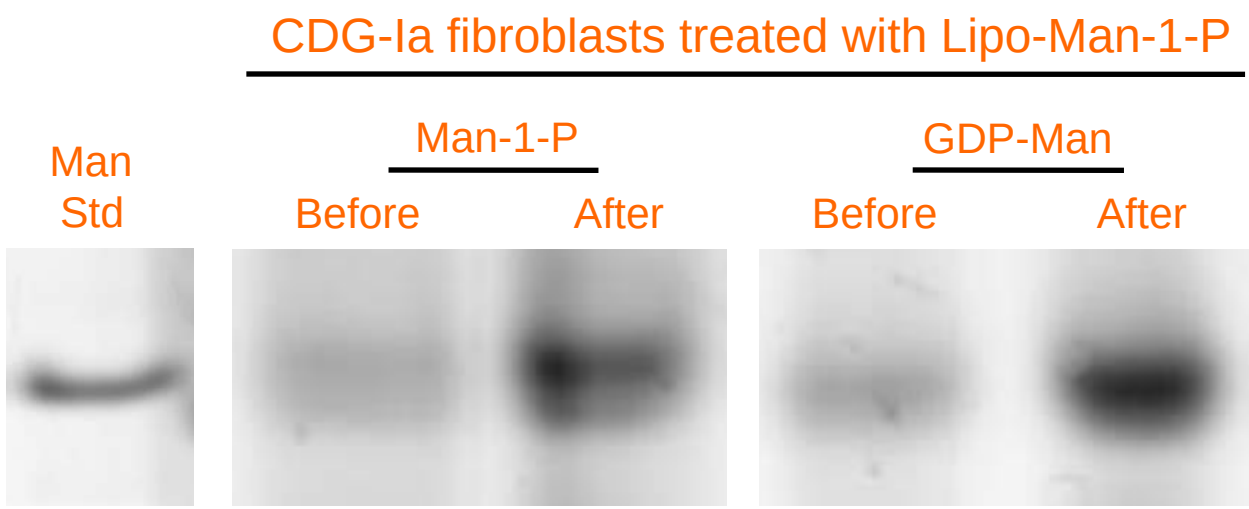


Lipo-Man-1-P Toxicity

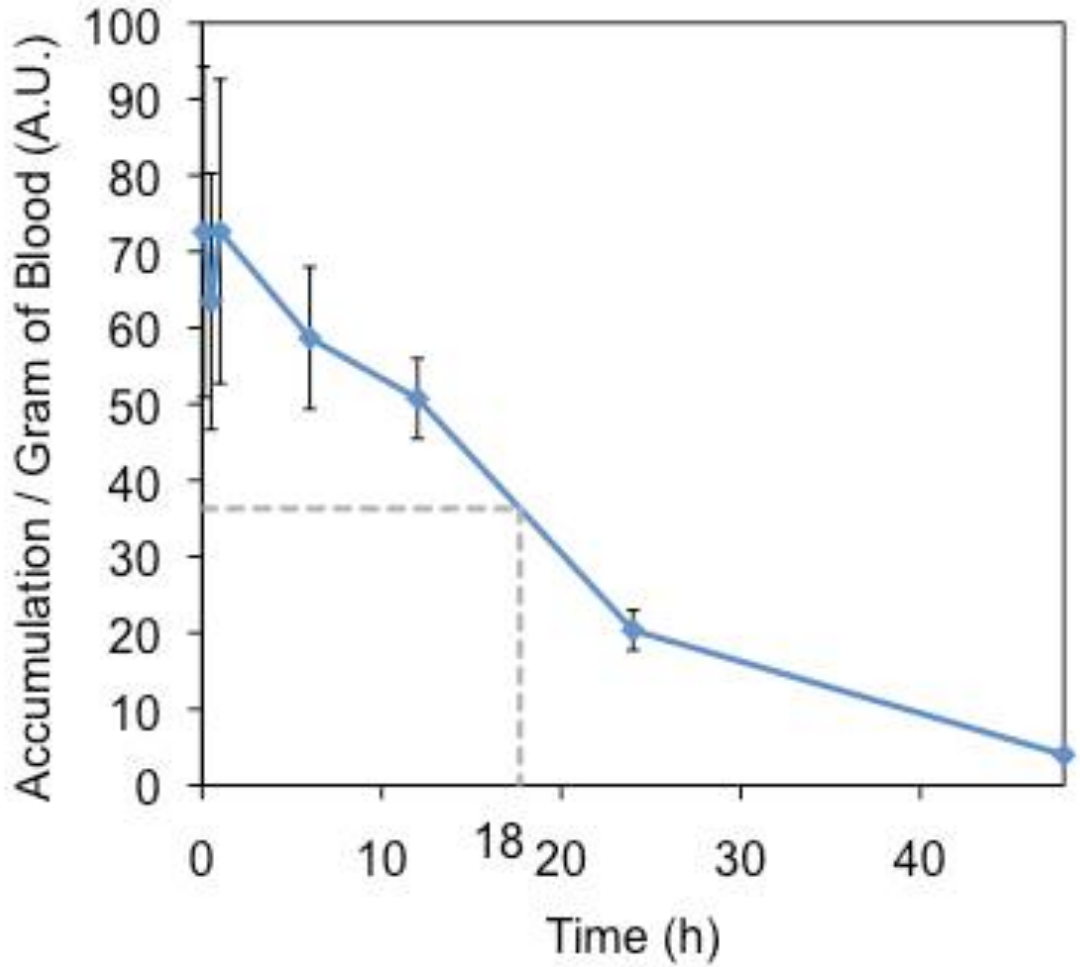


Lipo-Man-1-P Ability to Increase Man-1-P and GDP-Man Levels (*in vitro*), "Efficacy"

- 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

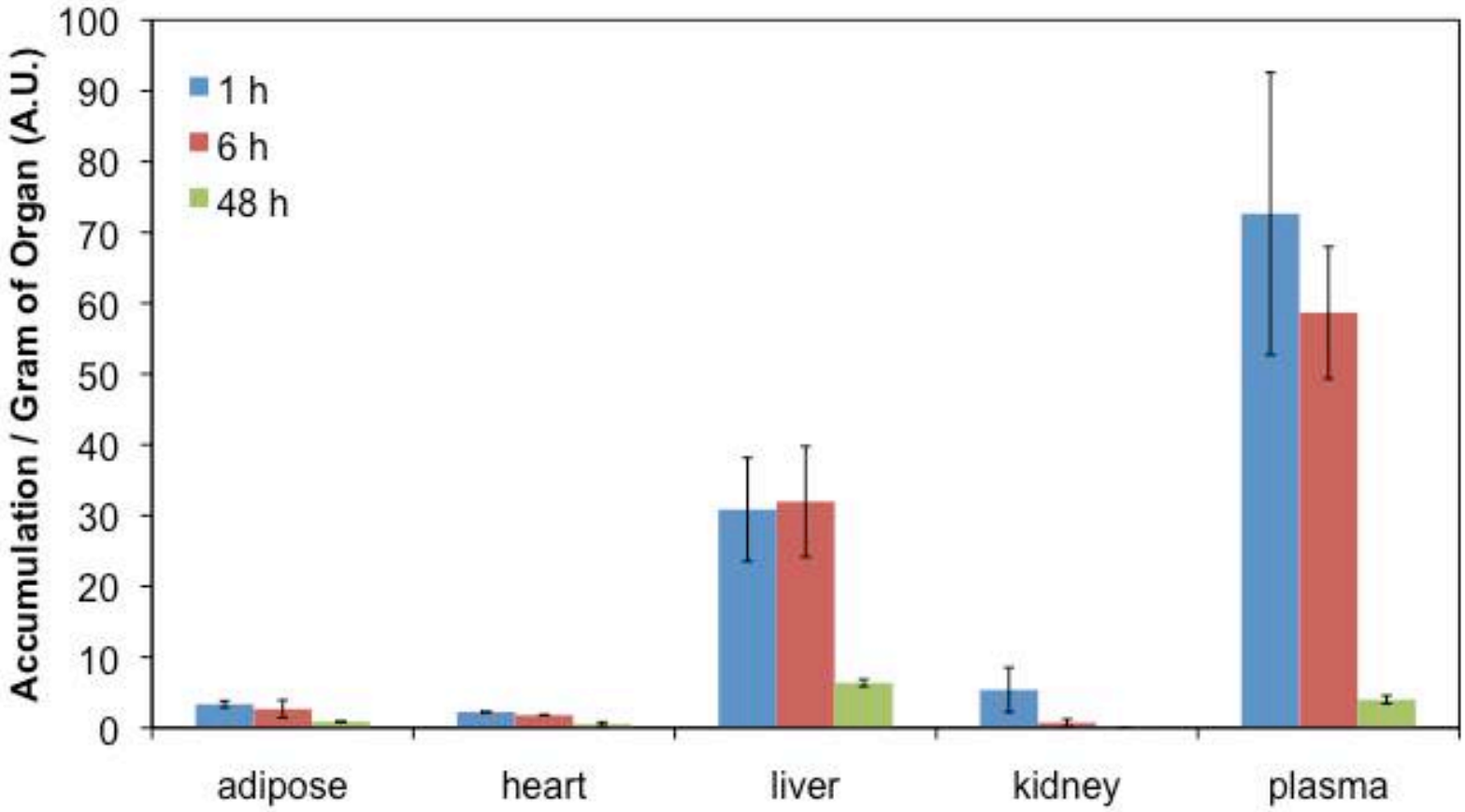


Lipo-Man-1-P Pharmacokinetics – Retention Time in Blood of Healthy Mice



- 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

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