in vivo Base-Editing Corrects Metabolic Defects in Glycogen Storage Disease Type-1a

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I am a Beam employee and shareholder
GSD-la overview

- **Molecular**
  - Protein
  - RNA
  - DNA
  - Gene: WT G6PC gene

- **Liver**
  - G6P ↔ Glucose

- **Circulation**

- **Outcome**
  - Glucose homeostasis is primarily maintained by the liver
  - When blood sugar drops below normal, G6P, produced in the terminal step of glycogenolysis and gluconeogenesis is converted to glucose by glucose-6-phosphatase-α (G6Pase) in the liver
Glucose homeostasis is primarily maintained by the liver.

When blood sugar drops below normal, glucose-6-phosphatase-α (G6Pase) in the liver converts glucose-6-phosphate (G6P) to glucose.

G6PC patients lack G6Pase and cannot produce endogenous glucose, leading to fasting hypoglycemia which may result in seizures or death.

Current treatment is dietary therapy.

Hepatomegaly is a clinical manifestation of GSD-Ia caused by glycogen accumulation.

**GSD-Ia overview**

<table>
<thead>
<tr>
<th>Normal G6PC Function</th>
<th>Molecular</th>
<th>Liver</th>
<th>Circulation</th>
<th>Outcome</th>
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**Physiologic euglycemia**

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**Coma, seizure**

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GSD-la overview

Normal G6PC Function

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G6PC Loss-of-function

| Protein   |       |             |         |
| RNA       |       |             |         |
| DNA       |       |             |         |
| Gene      | Mutated G6PC gene (R83C) |             |         |

Today’s agenda

1. **Base editing**
   Optimization of base editors for precise correction of G6PC-R83C

2. **Disease model**
   Characterization of an R83C transgenic mouse model of GSD-la

3. **In vivo correction**
   In vivo base-editing and correction of metabolic defects associated with GSD-la

4. **Next steps**

   - Glucose homeostasis is primarily maintained by the liver
   - When blood sugar drops below normal, G6P, produced in the terminal step of glycogenolysis and gluconeogenesis is converted to glucose by glucose-6-phosphatase-α (G6Pase) in the liver
   - GSD-la patients lacking G6pase cannot produce endogenous glucose and suffer from fasting hypoglycemia which may result in seizures or death
   - Current treatment is dietary therapy
   - Hepatomegaly is a clinical manifestation of GSD-la caused by glycogen accumulation
Base Editors Generate Permanent and Predictable Single Nucleotide Substitutions

Base editor binds the target DNA and exposes a narrow editing window

A-to-G base editor ("ABE")

Deaminase chemically modifies target base, A>G edit made permanent by DNA repair/replication

Gene Correction – Direct repair of point mutations to restore gene function

Abnormal Protein Expression

Functional Protein Expression
Base Editing: Lead optimization in immortalized HEK293 cells yields significant rate of precise correction of R83C

- Lead optimization yields ~60% targeted base-editing efficiency, reduced bystander editing
- What is the functional benefit of R83C correction via base-editing in a GSD-Ia mouse model?
Disease Model: 3-week-old homozygous huR83C mice exhibit expected growth impairment and metabolic defects

The homozygous huR83C mouse is a novel GSD-Ia model in which a human G6PC-R83C transgene replaces mouse G6pc.

Relative to littermate controls, GSD-Ia mice homozygous for huG6PC-R83C exhibit:
- Postnatal lethality
- Lower body weight
- Enlarged livers
- Significant G6Pase inhibition
- Abnormal serum metabolites

* p<0.05
** p<0.005
**In vivo correction:** Efficient LNP-mediated base editing in livers of adult and newborn heterozygous huR83C mice

- Given neonatal lethality of the GSD-Ia mouse model, we explored LNP-dosing shortly after birth via the temporal vein.
- LNP administered via tail vein (adult) or temporal vein (newborn) in heterozygous huR83C mice.
- Next-gen. sequencing analysis in total liver extracts yield:
  - ~40% base-editing efficiency in adults
  - A range, up to ~60% in newborns
- Next step: Correction in newborn homozygotes.
**In vivo correction:** LNP-mediated R83C correction is associated with survival of homozygous huR83C mice

- LNP-dosed homozygous huR83C mice survived to 3 weeks of age without glucose therapy
- Up to ~60% R83C correction
In vivo correction: Base editing reverses GSD-Ia pathology

R83C correction is associated with restoration of near-normal serum metabolites, G6PC activity, hepatic morphology and lipid deposition.
Summary

- Base editor and guide RNA optimized for correction of R83C in vitro
- Transgenic huR83C mice exhibit expected GSD-la phenotypes
- LNP-mediated base editing yields up to ~60% R83C correction and restoration of function in treated homozygous huR83C mice

Next steps

- *in vivo* fasting challenge studies
- Correlation of base-editing efficiency and metabolic function
Thank You

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