Optimization of LNP for in vivo base editing

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TIDES USA
Disclosure

- I am a Beam employee and shareholder
Overview

- Introduction to Base Editing and Beam’s program portfolio
- Optimization of LNP platform for potent in vivo base editing in the liver of NHPs
- Develop LNPs for in vivo delivery outside the liver
Base editing is a new approach to gene editing

**Nuclease editing**
Creation of double-stranded breaks in DNA at a target location to disrupt, delete, insert, or modify genes

CRISPR, Zinc Finger Nucleases, TALEN, ARCUS

**Base editing**
Direct conversion of one base pair to another at a target location, without double-stranded breaks
Base editors chemically modify target bases, permanently and predictably

**CRISPR** – established guide RNA-driven DNA targeting:
- Opens a short stretch of single strand DNA window
- Modified to not cause double stranded breaks

**Deaminase** – operates on single stranded DNA to completes chemical modification at predictable target DNA base

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**A-to-G base editor (“ABE”)**

- Adenine (A) to Inosine (I) (read as G by DNA polymerase)
- Amine and Deamination

**C-to-T base editor (“CBE”)**

- Cytosine (C) to Uracil (U) (read as T by DNA polymerase)
# Diversified portfolio of wholly-owned base editing programs

<table>
<thead>
<tr>
<th>DELIVERY</th>
<th>THERAPEUTIC AREA</th>
<th>PROGRAM / DISEASE</th>
<th>APPROACH</th>
<th>RESEARCH</th>
<th>LEAD OPTIMIZATION</th>
<th>IND ENABLING</th>
<th>PHASE I/II</th>
<th>PIVOTAL</th>
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LNP = Lipid Nanoparticle; AAV = Adeno Associated Virus; CNS = Central Nervous System
We deliver base editor mRNA and gRNA using LNP to enable in vivo base editing

Optimization of LNP components led to potent A→G editing in NHP liver
- mRNA production process
- gRNA chemical modification
- LNP formulation

Surrogate payload

target: CAGG\textcolor{red}{A}TCCGCACAGACTCCA GGG
- Rodent-NHP conserved region on liver-expressed ALAS1*
- 5A→G edit causes a I491T mutation of unknown functional consequences

*ALAS1: 5’-aminolevulinate Synthase 1
### Goal of mRNA process optimization

The goal of mRNA process optimization is to improve activity and reduce immune stimulation.

<table>
<thead>
<tr>
<th>Goal</th>
<th>Purpose</th>
<th>Tunable parameters</th>
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<tbody>
<tr>
<td>↑ Translation: higher base editor expression, higher editing</td>
<td>↑ full length product</td>
<td>5’cap</td>
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<tr>
<td>↓ Immune stimulation: reduce toxicity</td>
<td>↑ capping</td>
<td>5'/3’-UTR</td>
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<td></td>
<td>↓ dsRNA</td>
<td>codon optimization</td>
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<td></td>
<td>↓ short transcripts</td>
<td>poly(A)</td>
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<tr>
<td></td>
<td>↓ dsRNA</td>
<td>modified nucleosides</td>
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<tr>
<td></td>
<td>↓ process residuals</td>
<td>modified nucleosides</td>
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#### Process steps

- **design mRNA construct**
- **Plasmid production**
- **In vitro transcription**
- **Purification**

- • oligo dT
- • IPRP
- • HIC
- • other…

- • cap
- • modified NTP
- • reaction condition
- • other…

- • 5’cap
- • 5'/3’-UTR
- • codon optimization
- • poly(A)
- • modified nucleosides

#### Parameters

- • cap
- • modified NTP
- • reaction condition
- • other…

- • oligo dT
- • IPRP
- • HIC
- • other…
Optimization of IVT and purification increased full-length product and eliminated immune stimulation in vitro

% Full length mRNA

IVT1

IVT2

mRNA purification

In vitro immune stimulation

IFN-β (fg/mL)

mRNA purification

IVT1

IVT2

P1 P2 P2 P3 buffer
Optimized mRNA is active and does not induce inflammatory response in vivo

ALAS1 edit in mouse liver
(0.1mg/kg total RNA)

Mouse serum [IP-10]
6hr-post injection

<table>
<thead>
<tr>
<th>%A-G edit</th>
<th>P1</th>
<th>P2</th>
<th>P2</th>
<th>P3</th>
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<tbody>
<tr>
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<td>15</td>
<td>20</td>
<td>25</td>
<td>30</td>
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<td>IVT2</td>
<td>18</td>
<td>22</td>
<td>28</td>
<td>32</td>
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<table>
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<th>Serum [IP-10], pg/mL</th>
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<tbody>
<tr>
<td>IVT1</td>
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<td>IVT2</td>
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mRNA purification
gRNA can be chemically modified to increase its stability

Stabilizing modification types

- **End mods**: Stabilize gRNA against exonucleases
  - Modifications at the first three nts of the 5’ end and first three nts of the last four at the 3’ end

- **Heavy (internal) mods**: Stabilize gRNA against endonucleases
  - Can inhibit Cas activity and thus must be placed at specific locations
  - Particularly important for in vivo studies

Beam proprietary sgRNA modifications increase base-editing potency in vivo

Liver editing in mice

![Graph showing liver editing in mice with various gRNA chemical modifications and dosages.]

- End mods
- Lit M1
- Lit M2
- Beam M1
- Beam M2

Dosages:
- 0.01mg/kg
- 0.03mg/kg
Produce potent, stable, and consistently manufactured LNP

- **Scope of process optimization**
  - Lipid composition
    - Helper lipid components
    - Molar % of lipids
    - N:P ratio
  - Formulation process
    - Mixing of components
    - Purification and concentrating
  - Buffer and excipients

- In this work, mRNA and sgRNA are co-encapsulated in the same LNP at 1:1 mass ratio
Consistency of LNP formulations was improved through process optimization.

Size

Polydispersity index

Encapsulation efficiency

![Graphs showing size, polydispersity index, and encapsulation efficiency comparisons between LNP1 and LNP2.]
LNPs remain stable after 3-month storage at -80°C and -20°C.
High degrees of hepatocyte editing is detected via BaseScope in liver of LNP-treated NHP

Untreated NHP liver  
LNP-treated NHP liver  
(47% whole liver editing)
LNPs appear well tolerated in NHPs based on clinical pathology

- Minimal to mild transient increases in AST and/or ALT at 24hr (Day 2) post-dose resolving by Day 15
- No other significant changes in clinical pathology parameters were observed

**ALT**

**AST**

- • payload 1
- ▲ payload 2
- × payload 3
- Δ payload 4

1.5mg/kg
Improvements to LNP processes increase LNP potency up to 60% editing at clinically relevant dose

previously shared at 2021 ASGCT

1.5mg/kg

% A→G edit

Bars represent mean +/- standard deviation

n=2 or 3
Improvements to LNP processes increase LNP potency up to 60% editing at clinically relevant dose

Previously shared at 2021 ASGCT

n=2 or 3
Bars represent mean +/- standard deviation
We optimized the LNP platform for in vivo base editing in the liver

The optimized platform consists of
• Potent, immunosilent mRNA
• Chemically modified sgRNA
• Consistent, stable LNP

Optimized LNP produced up to 60% A→G editing in NHP liver at 1.0mg/kg

Optimization is a continuous journey
Developing LNPs for extrahepatic tissues

An ideal LNP discovery process would be (i) very high throughput, (ii) *in vivo* (mice → NHPs), and (iii) analyze delivery to any desired combination of on- / off-target cell types.
High-throughput *in vivo* screening of LNPs using DNA barcodes

**mRNA Screening System**

- **Material N**
- **Barcode N**
- **Cre mRNA**

**NP library; each carries Cre mRNA+barcode**

**Cre-LoxP reporter mouse**

**FACS**

- **tdTom− cells = not targeted**
- **tdTom+ cells = targeted**

**Sequence barcodes in tdTom+ cells**

NP1 < NP2 < NP3

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**Nanoparticles That Deliver RNA to Bone Marrow Identified by in Vivo Directed Evolution**

Cory D. Sago, Melissa P. Lokugamage, Fatima Z. Islam, Brandon R. Krupczak, Manaka Sato, and James E. Dahlman

**High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing**

Developing LNPs for the delivery of mRNA to Hematopoietic Stem & Progenitor Cells (HSPCs)

- The development of LNPs for the targeting of HSPCs could meaningfully impact the treatment of hemoglobinopathies
- Using our DNA barcoding approaches, we identified a family of LNPs that delivers to HSPCs in mice.
- In Cre-reporter mice, hit ‘LNP A’ transfected in a dose-dependent manner with >40% HSPCs transfected at 1.0mg/kg
Thank you

mRNA
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- Jason St. Laurent
- Krishna Sapkota
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- Shefal Parikh

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- Ho Yau

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- Emma Wang
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- Cory Sago

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- Dominique Leboeuf

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- Colin Lazzara
- Bob Gantzer

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- Jeff Marshall
- Carlo Zambonelli

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- Robert Dorkin

Cell Biology
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Toxicology
- Brian Johnson

Leadership
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- Mano Singh
- Francine Gregoire
- Rodrigo Laureano
- Steve Prescott