Advancing Gene Editing Therapeutics: Pivotal Assessment of mRNA Analytics for Phase 1 to Commercialization

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Presentation Overview

Beam Introduction

- Our technology
- Our pipeline

Brief quality by design and critical quality attribute assessment overview, method lifecycle pre-IND to Pivotal

- Quality by Design (QBD)
- mRNA CQAA Examples

Focused discussion of two mRNA attributes as part of pivotal readiness assessment

- Poly A tail length and tail heterogeneity
- Covalent base modifications (Oxidation, deamination, depurination, etc.)
Beam Introduction (Our Technology)

- CRISPR-Deaminase Fusion Protein coded by our mRNA
- gRNA forms Ribonuclear Protein complex and binds near target
- Deaminase removes amine converting a single base

Many human genetic diseases are due to point mutations. In fact, amongst the over 50,000 human disease-causing variants described in a mutation database, about 30,000 are point mutations.
Beam Introduction
(Where we are 2024-Our Pipeline)

Mix of In-vivo and Ex-vivo programs

<table>
<thead>
<tr>
<th>PROGRAM / DISEASE</th>
<th>DELIVERY</th>
<th>EDITING 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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</thead>
<tbody>
<tr>
<td>BEAM-101 Sickle Cell Disease Beta Thalassemia</td>
<td>Ex vivo HSCs</td>
<td>Activation of fetal hemoglobin</td>
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<tr>
<td>ESCAPE Sickle Cell Disease Beta Thalassemia</td>
<td>Ex vivo HSCs</td>
<td>Multiplex CD117 edit-antibody pair</td>
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<td>BEAM-302 Alpha-1 Antitrypsin Deficiency</td>
<td>In vivo LNP</td>
<td>Correction of E342K mutation</td>
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<tr>
<td>BEAM-301 Glycogen Storage Disease Ia</td>
<td>In vivo LNP</td>
<td>Correction of R83C mutation</td>
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<tr>
<td>BEAM-201 T-ALL / T-LL CD7+ AML</td>
<td>Ex vivo T cells</td>
<td>Multiplex silenced CD7 CAR-T</td>
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<tr>
<td>Complement Pathway (Apellis)</td>
<td>In vivo LNP</td>
<td>Undisclosed</td>
<td></td>
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<tr>
<td>3 undisclosed targets (Pfizer)</td>
<td>In vivo LNP</td>
<td>Undisclosed</td>
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LNP = Lipid Nanoparticle; HSC = Hematopoietic Stem Cell; T-ALL / TLL = T-Cell Acute Lymphoblastic Leukemia / T-Cell Lymphoblastic Lymphoma; AML = Acute Myeloid Leukemia; ESCAPE: Engineered Stem Cell Antibody Paired Evasion.
Iterative Process of Quality by Design (QBD) and Critical Quality Attribute Assessment (CQAA)

QBD is an iterative process where product understanding and process understanding feed into each other to build and strengthen the product and process control strategy. QBD is iterative and responds to inputs from structure function studies, pre-clinical studies, clinical data, and process characterization studies (refer to ICH Q8R2).

Example of mRNA CQAA and Focus on Today’s Presentation

- Snippet of CQAA for mRNA detailing the attribute, safety/efficacy rationale summary, notes/references and data, and risk assessment for safety, efficacy, and uncertainty.
- All three risk assessment parameters (safety, efficacy, and uncertainty) are combined to determine criticality of the attribute.
- Document is living and subject to change/iteration as data becomes available. CQAs and justifications can be communicated to regulatory bodies for late-stage products.

<table>
<thead>
<tr>
<th>Attribute Class</th>
<th>Attribute</th>
<th>Rationale</th>
<th>Additional Notes and References</th>
<th>Safety</th>
<th>Activity/ Efficacy</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Purity</td>
<td>Tail length and heterogeneity</td>
<td>Safety: unknown impact&lt;br&gt; Longer tail can increase mRNA stability/half-life. Short poly A tail may result in exonuclease attack on 3' end and consequent product degradation. Generate internal data for different tail length impact on potency and safety</td>
<td>L</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covalent Base Modifications</td>
<td>Safety: Unknown&lt;br&gt; Oxidation products, depurination, crosslinking can reduce translation efficiency. Deamination will change the base ID and potentially affect the coding sequence. Oxidation, depurination, and deamination are strongly influenced by pH and temperature.</td>
<td>M</td>
<td>M</td>
<td>H</td>
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Additional Attributes that can affect safety and efficacy that are outside scope for today…
Tail Length and Tail Heterogeneity Background

Plasmid DNA to mRNA manufacturing

Plasmid design/orientation optimization may reduce heterogeneity
Instability of cell line during production or lack of clonality

IVT Enzyme infidelity on homopolymer tracts may impact tail heterogeneity

Liquid Chromatography Methods for Analysis of mRNA Poly(A) Tail Length and Heterogeneity

Anal. Chem. 2023, 95, 38, 14308–14316, September 11, 2023
https://doi.org/10.1021/acs.analchem.3c02552
Tail Length and Tail Heterogeneity CQAA

Is it a CQA?

- Literature consensus is that shorter tails on average lead to shorter mRNA half-life. Conclusions are mixed when it comes to translation efficiency (TE) except for tails <=30nt where TE drops
  - Provides endonuclease protection as well as poly A binding protein stable loop for translation initiation
  - Cell type play a major roll in mRNA half-life and TE
- ex-vivo vs. in-vivo (LNP) drug products differ dramatically making it difficult to leverage platform knowledge across modalities
- Structure-function studies using varied poly A Tail length constructs
  - For ex-vivo: perform mRNA potency/protein expression as well as electroporation of human cells at standard dosing concentration and sub-saturating doses to make more sensitive to potential changes in TE
  - For in-vivo (LNP): perform mRNA potency/protein expression testing as well as LNP DP potency testing to determine effect.

How to measure it?

- pDNA digestion-CGE, pDNA bidirectional sequencing of supercoiled
- mRNA digested IPRP, IEX, SEC, CGE, and ddPCR

How to control for it?

- In the pDNA production release and/or mRNA process release? Build process understanding and relationship with the attribute
- Average length? Distribution? Specifications (>eg.80nt)? Use process characterization and structure function studies to inform control strategy

Is it a QCQ? YES. Assessment is revisited as data comes in. Uncertainty should decrease as more information is gained
Covalent Base Modifications Background

• Bases A, G, C, and U can undergo many covalent modifications that impact the fidelity of the mRNA

• “Covalent base modifications” is a general term to encompass several types of chemical changes generated by different mechanisms
  o Oxidization
  o Deamination (in unbuffered water)
  o Depurination
  o Cross-linking or adduct formation

• Rate of formation are affected by pH, dissolved oxygen, metal impurities, and UV.

• Translation efficiency is reduced by interruption of the coding sequence with base modifications (G→8oxG, A→I, G→X, etc.) caused by oxidation, deamination (water), oxidative crosslinking, UV degradation products (can include radical crosslinking), etc.

<table>
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<th>Degradation products</th>
<th>Source</th>
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<tr>
<td>Oxidation of bases</td>
<td>Auto-oxidation, Metal residues, Light</td>
</tr>
<tr>
<td>Depurination (abasic site)</td>
<td>Hydrolysis (acid), Oxidation</td>
</tr>
<tr>
<td>Deamination-hydrolyzed bases</td>
<td>Hydrolysis (acid)</td>
</tr>
<tr>
<td>RNA Fragments</td>
<td>Hydrolysis (base), Heat, Peroxides, H2O2, RNase enzymes</td>
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Covalent Base Modifications CQAA

Is it a CQA?

- There are many literature sources detailing the negative effects of base oxidation and side reactions on TE. This potentially can influence efficacy and requires the following considerations:
  - Manufacturing process may impact this attribute class, exposure to metal ions and other catalysts for oxidation. Process ranges and hold times should be evaluated
  - Perform forced degradation (FD) studies, generally keep conditions close to process extremes and monitor to inform process characterization studies
  - Utilize the mRNA formulation or in-process matrix as this affects degradation pathways
  - Long term storage conditions and stability studies should also be evaluated
- Structure function studies
  - Determine influence on potency and/or protein expression (Rabbit Reticulocyte or target cell based protein expression) using FD material
  - Correlate % base modification to protein expression

How to measure it?

- RP/IPRP Single nucleotide or nucleoside analysis, Nuclease digested mRNA-LC/MS

How to control for it?

- Initially use structure function, process ranging, and any pre-clinical data to drive control strategy. Influence on potency may lead to testing on release and stability

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Summary

- Quality by design concepts can be used to build safer products through iterative cycles of studies focused on process and product understanding.
- In general structure function study parameters should be based on the production process as well as the patient dose administration.
- CQAA are built over time and are integral to building the product control strategy.
- Tail length is a CQA affecting TE, however, the degree of which needs to be evaluated for each specific molecule and target system.
- Covalent modifications as a class of attributes are also CQAs and can lead to a reduction in TE. Specific forced degradation studies in formulation and in-process conditions should be assessed to determine the risk level.