IND-enabling Small-Scale Guide RNA Production Under GMP for CRISPR Based Cell Therapies

Sept 23rd 2021

Keith Jarvis
Senior Director, Process Chemistry
I am an employee and shareholder of Editas Medicine
IND-enabling Small-Scale Guide RNA Production Under GMP for CRISPR Based Cell Therapies

This presentation will focus on the appropriate scale, final purity release specifications and GMP compliance for internal small-scale guide RNA production necessary to support our pre-clinical programs. It will also highlight the quality management system we have created and the guide RNA production clean rooms we have implemented at the Editas Boulder location.
Editas Medicine Research Pipeline

- Cas12a advantage and how it relates to guide RNA production
- Large scale manufacturing advantage of ~40/60mers vs 100mers
- Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs
- Quality Systems to support GMP manufacturing
- Internal GMP manufacturing will accelerate our pre-clinical programs
Editas Research and Pipeline

- **In Vivo Gene Edited Medicines**
- **Ex Vivo Gene Edited Cell Medicines**
- **Cellular Therapy Medicines**
# In Vivo Research Pipeline

## In Vivo Gene Edited Medicines

### Ocular

<table>
<thead>
<tr>
<th>Program</th>
<th>Discovery</th>
<th>Lead Optimization</th>
<th>IND Enabling</th>
<th>Early-Stage Clinical</th>
<th>Late-Stage Clinical</th>
<th>Commercial Partner</th>
<th>Enabling Tech</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EDIT-101</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leber Congenital Amaurosis 10 (LCA10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EDIT-102</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usher Syndrome 2a (USH2A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autosomal Dominant Retinitis Pigmentosa 4 (RP4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Other Organs

- Neurological Diseases
### Ex Vivo Research Pipeline

#### Ex Vivo Gene Edited Cell Medicines

**Hemoglobinopathies**

- **EDIT-301:** Sickle Cell Disease (SCD)
- **EDIT-301:** β-Thalassemia

#### Cellular Therapy Medicines

**Oncology**

- αβ T Cells
- iPSC NK (iNK) Cells

---

© 2021 Editas Medicine
§ Remove an individual’s cells, edit them, then reintroduce them to the patient.

§ Create a universal cell population that can be edited and then given to any patient who needs it, without needing the patient to donate cells first.
Overview

Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs
• gRNA has differences in protospacer adjacent motifs
• Staggered DNA cuts increase efficiency and accuracy for gene repair

• Programmable protein that specifically locates, binds to and edits the DNA of targeted genes
• Pairs with a guide RNA molecule that recognizes and initiates a double stranded break at target DNA sequence
The AsCas12a Nuclease has Higher Intrinsic Specificity and Higher Sequence Fidelity in the Shorter Chemically Synthesized Guide RNA

**Specificity:**

Matched Target Site (20-Ns):

\[
\text{TTTV} \text{NNNNNNNNNNNNNNNNNNNNGG}
\]

**Takeaway:**

AsCas12a is more specific across matched sites in the genome in contrast to SpCas9

**Guide RNA synthesis:**

Chemical synthesis of gRNAs occurs in the 3’ → 5’ direction and purity and yield of the entire gRNA sequence drops with increasing length

Cas12a gRNAs (~40mer) are much shorter than SpCas9 gRNAs (~100mer)

Cas9 guide is most sensitive to mismatches at 5’ end which is the location of lowest sequence fidelity as this is where synthesis ends

Cas12a guide is most sensitive to mismatches at 3’ end which is the location of highest sequence fidelity as this is where synthesis starts

Lack of sequence fidelity will lead to unanticipated off-target editing due to errors in RNA sequence targeting the protospacer region

**Takeaway:**

AsCas12a synthetic gRNAs have reduced risk of off-target editing that results from synthesis errors

References on Cas12a specificity:
Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs
Ex Vivo Gene Edited Cell Medicine

gRNA (~40-60mer) + Nuclease (Cas12a) → RNP

RNP → Active Pharmaceutical Ingredient
Sterile Fill

→ Patient Cell → Engineered Patient Cell
Chemistry of guide RNA

RNA targeting region

DNA

2’Omethyl modifications

PS/PO

~40 to 60mers
Effect of coupling efficiency on yield

yield \sim (\% \text{ coupling efficiency})^n
where n = \# of bases

<table>
<thead>
<tr>
<th>length</th>
<th>90%</th>
<th>95%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>2.8%</td>
<td>17%</td>
<td>71%</td>
</tr>
<tr>
<td>66</td>
<td>0.1%</td>
<td>3.4%</td>
<td>51%</td>
</tr>
<tr>
<td>100</td>
<td>&lt;0.01%</td>
<td>0.6%</td>
<td>36%</td>
</tr>
</tbody>
</table>

- shorter sequences:
  - superior fidelity
  - higher maximum yield
- 631 µmol scale synthesis
- Crude gross yield: 81700 ODU
- Crude full-length purity by UPLC: 51%
- Adjusted crude yield: 41667 ODU / 1287.02 mg
- Full length mass confirmed
Typical Guide RNA Final

<table>
<thead>
<tr>
<th>OLI #</th>
<th>Batch#</th>
<th>Length</th>
<th>(A260−blank)</th>
<th>Pthlnth</th>
<th>Dil'n Fctr</th>
<th>Extinction Coefficient</th>
<th>moles/liter</th>
<th>uM</th>
<th>uL</th>
<th>pmol</th>
<th>nmol</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>40735</td>
<td>D</td>
<td>41</td>
<td>0.649</td>
<td>1</td>
<td>200</td>
<td>420700</td>
<td>0.00030853</td>
<td>308.53</td>
<td>98000</td>
<td>30236272.86</td>
<td>30236.27</td>
<td>392.91</td>
</tr>
</tbody>
</table>

- 631 µmol scale synthesis
- Final yield: ~393 mg
- Final purity by UPLC: ~85%
- Full length mass confirmed
Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs
New Boulder Facility

7,669 sf

Phase 1
Office Space

Phase 2
PD/AD Lab

Phase 3
GMP Manufacturing
Production Overview

Supply Planning → Reagent Prep → Synthesis → Cleavage & Deprotection → Crude Workup*

Purification* → Ultrafiltration* → Lyophilization → Suspension and Batch Pooling* → Sterile Filtration*

Aseptic Fill and Finish → Packaging → Final Release Testing → Shipment

* denotes an in-process testing point
Synthesis
• Akta™ Oligopilot™ 100 synthesizer
• Cleavage and Deprotection within enclosed hood space
• Located in controlled PD lab space

Purification
• Gilson Reverse Phase Prep System
• Ultrafiltration
• Located in Clean Room

Aseptic Fill and Finish
• Formulation
• Sterile Filtration
• Lyophilization
• Located in Clean Room
PD Lab Process Steps:
• Synthesis
• Cleavage / Deprotection
• Crude Ultrafiltration

Cleanroom Process Steps:
• Purification
• Final Ultrafiltration
• Lyophilization
• Final Fill & Finishing

In-Process QC analysis will be completed at Editas.

Final release testing will be completed at an external contract lab.

First GMP batch initiation expected in Q1 2022
Dual Duty: Lead Guide PD/AD and Synthesis Upstream Processing

PROCESS DEVELOPMENT/ANALYTICAL DEVELOPMENT
- Guide process optimization once a lead is declared by research
- Phosphoramidite and Reagent Prep
- Synthesis
- Cleavage and Deprotection
- Crude Ultrafiltration
- Purification
- Fraction Collection
- Final Ultrafiltration
- Lyophilization

SYNTHESIS UPSTREAM PROCESSING
- Phosphoramidite and Reagent Prep
- Synthesis
- Cleavage and Deprotection
- Crude Ultrafiltration
• CNC area (ISO 9)
• First airlock (ISO 8)
• Purification room (ISO 7)
• Fill / Finish Room (ISO 7)
• BSC (ISO 5)
<table>
<thead>
<tr>
<th>Category</th>
<th>Attribute</th>
<th>Method</th>
<th>Current Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Molecular Weight</td>
<td>LCMS (TM-0001)</td>
<td>Consistent w/ Theor. Mass ±0.2%</td>
</tr>
<tr>
<td>Strength</td>
<td>Assay % w/w (anhydrous) by UV Assay</td>
<td>$A_{260}$ UV/Vis (TM-0002)</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td>Total Impurities (Product Related)</td>
<td>IP-RP-UHPLC (TM-0001)</td>
<td>Report to 1 decimal: a) Early Eluting Cluster b) Late Eluting Cluster c) other peaks ≥ 0.1 %</td>
</tr>
<tr>
<td></td>
<td>Impurity Quant/ID</td>
<td>IP-RP-UHPLC (TM-0001) (custom slow gradient methods)</td>
<td>Report Results</td>
</tr>
<tr>
<td>Category</td>
<td>Attribute</td>
<td>Method</td>
<td>Current Acceptance Criteria</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------------------</td>
<td>---------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Safety</td>
<td>Bioburden</td>
<td>USP &lt;61&gt;</td>
<td>≤100 CFU/100 mg TAMC/ TYMC</td>
</tr>
<tr>
<td></td>
<td>Endotoxin</td>
<td>USP &lt;85&gt;</td>
<td>≤ 0.25 EU/mg (to two decimal places)</td>
</tr>
<tr>
<td>General</td>
<td>Appearance</td>
<td>Agilent QC-GNM-6005</td>
<td>White to off-white solid, free of visible particulates</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>USP &lt;791&gt;</td>
<td>Report Results</td>
</tr>
<tr>
<td>Identity</td>
<td>Molecular Weight</td>
<td>LCMS</td>
<td>Consistent with Theoretical Mass ±5 Da</td>
</tr>
<tr>
<td></td>
<td>Sequence</td>
<td>Sanger</td>
<td>&gt;99% accuracy for each base call</td>
</tr>
<tr>
<td>Strength</td>
<td>Assay % w/w (anhydrous) by UV Assay</td>
<td>A&lt;sub&gt;260&lt;/sub&gt; UV/Vis</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td>Moisture Content</td>
<td>Karl Fisher</td>
<td>≤10% w/w</td>
</tr>
<tr>
<td>Purity</td>
<td>Purity</td>
<td>IP-RP-UHPLC</td>
<td>≥ 80.0% Main Peak</td>
</tr>
<tr>
<td></td>
<td>Impurities (Product Related)</td>
<td>IP-RP-UHPLC</td>
<td>Report to 1 decimal each of the following:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a) Early Eluting Cluster</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b) Late Eluting Cluster</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c) peaks ≥ 0.1 % area not included in other peaks</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>Flame AA Spectroscopy</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td>Residual Solvents</td>
<td>GC-FID</td>
<td>2.6-lutidine: Report Acetonitrile: ≤ 410 ppm Ethanol: ≤ 5000 ppm Isopropanol: ≤ 5000 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pyridine: ≤ 200 ppm Toluene: ≤ 890 ppm</td>
</tr>
<tr>
<td></td>
<td>Elemental Impurities (Metals)</td>
<td>ICP-MS</td>
<td>As: ≤ 19                                      Cd: ≤ 03                                      Cr: ≤ 1383  Cu: ≤ 377  Fe: Report</td>
</tr>
<tr>
<td></td>
<td>Triethylamine</td>
<td>GC-MS</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(note: first two GMP lots of OLI21036: 4, 6 ppm)</td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethyl Formamide</td>
<td>GC-FID</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(note: first two GMP lots of OLI21036: &lt;170 ppm)</td>
</tr>
<tr>
<td></td>
<td>Dimethyl Sulfoxide</td>
<td>GC-FID</td>
<td>Report Results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(note: first two GMP lots of OLI21036: &lt;0.004, &lt;50 ppm&lt;)</td>
</tr>
</tbody>
</table>
Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs
GMP Systems Implementation for gRNA Production

Materials Management System
- Procure appropriate grade of materials for gRNA production
- Create Internal Material Specifications
- Procedures for receipt and disposition of incoming raw materials
- Vendor Qualification for suppliers/manufacturers

Production Records
- Master Batch Records

Cleaning Program
- Evaluation of cleaning agents
- Development of cleaning procedures
- Utilization of contract cleaning company

Gowning Program and Gowning Qualification
GMP Systems Implementation for gRNA Production

Environmental Monitoring Program and Qualification
  • HVAC operation
  • Monitoring of particulates and air sampling
  • Aseptic conditions

Equipment
  • Operation, Calibration and Maintenance procedures
  • IQ/OQs

Data Integrity
  • Data backup

Validation
  • CSV for GMP equipment
  • Server and network

QC
  • In Process Testing performed in-house
  • Method Development and Qualification
  • Release testing and stability testing to occur at a contract test facility
Overview

Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers in terms of cost, purity and time

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs
Internal GMP Manufacturing Accelerates our Pre-Clinical Programs

- Appropriate scale customized to actual need
- 6 to 12 month lead time required to hold a manufacturing slot
- Ability to prioritize internal and collaborative programs based on evolving research timelines
- All IP retained internally (every gRNA is unique so process development efforts can turn into important IP)
Process Chemistry Team Acknowledgement

Medicinal Chemistry
- Vy Le (Scientist)
- Austin McFarlin (Research Associate)

Research Scale Guide RNA Production
- McKenzie Weiss (Research Associate)
- Mark Jones (Research Associate)
- Shelby Beer (Research Associate)
- Research Associate

Process Development
- Stephen Pietrasiewicz (Scientific Technical Leader)
- Jill Fletcher (Research Associate)
- Research Associate

GMP Manufacturing
- Tim Corre (Manufacturing Lead)
- Manufacturing Associate
- Manufacturing Associate