CRISPR Genome Editing: Considerations for Therapeutic Applications

November 9, 2017
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Medicines that Aim to Repair Any Broken Gene

Potential to create the next major category of transformative medicines
CRISPR Provides Versatile Genome Editing Systems

- Complex of nuclease and guide RNA precisely locates and cuts genomic sites
- Ability to target several sites simultaneously using multiple guide RNAs
- Nuclease can be engineered to reach more sites and to modulate cutting
Non-homologous end joining typically **disrupts a gene or eliminates a disease-causing mutation**

Homology-directed repair and targeted insertion aim to **promote expression of correct DNA sequences**
**Broad Toolkit of CRISPR Nucleases**

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<thead>
<tr>
<th>MULTIPLE EDITING SYSTEMS</th>
<th>AsCpf1</th>
<th>LbCpf1</th>
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<tbody>
<tr>
<td>SpCas9</td>
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<td>SaCas9</td>
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<th>ADVANCED FORMS FOR FLEXIBLE TARGETING</th>
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<td>PAM</td>
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<td>eS</td>
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<tr>
<td>HiFi</td>
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AsCpf1: *Acidaminococcus* species Cpf1; LbCpf1: *Lachnospiraceae* bacterium Cpf1; PAM: Protospacer Adjacent Motif; HiFi: High Fidelity; eS: enhanced Specificity
SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

Platform Enables Broad Product Opportunities

- **Broad Range of Sites**
  - SpCas9
  - SaCas9
  - SpCas9 Variants
  - SaCas9 Variants
  - Cpf1
  - Cpf1 Variants
  - Editas Platform

- **Wide Delivery Options**
  - Viral Vector
  - Lipid Nanoparticle
  - Electroporation

- **Diverse Spectrum of Edits**
  - Disrupt
  - Remove
  - Replace
  - Insert

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Lead Finding for Nuclease/gRNA and Specificity

Identify, Measure, Minimize

Proprietary in silico Prediction of Cutting Sites

Testing of On-Target Cutting (Cas9, Cpf1 WT, nickase)

Targeted Panels for Detection of Sites from Biased & Unbiased Screens

Unbiased Detection of Off-Target Cuts and Genomic Alterations (e.g., GUIDE-Seq, UDiTaS™)

In silico Selection
Identification of Robust gRNAs

- S. pyogenes Cas9 RNPs in primary human T cells to knock-out PD-1
- Several gRNAs performed well as assessed by FACS
- Sequencing confirmed indels
- gRNAs analyzed by GUIDE-Seq to identify off targets
Screening of multiple Cpf-1 orthologs and variants

AsCpf1 emerging as the “go to” Cpf1 with Robust activity

% indels at four matched sites in U2OS

% indels by T7E1

0 10 20 30 40 50 60 70 80

MS1  MS5  MS11  MS18

AsCpf1  FnCpf1  LbCpf1  Lb2Cpf1  SpCas9
Control and Specificity to Drive Precision

- **GUIDE-Seq Read Count**

- GUIDE-Seq drives empirical demonstration of selectivity of product candidates
- Off-targets identified by GUIDE-Seq would not be accurately predicted by *in silico* methods alone
A simple question with a complex answer

- Sequence anchored detection approaches are limited to:
  - What is between the primers
  - Amplicon size

**Intra-chromosomal Events**
- Perfect repair
- Insertion^*
- Deletion^*
- Inversion
- Duplication

**Inter-chromosomal Events**
- Balanced Translocation
- Unbalanced Translocation

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UDiTaS™ (Uni-Directional Targeted Sequencing)

A simple, robust method for capturing complex editing events in a single reaction

- Measurement of small Indels correlates well with targeted sequencing and T7E1 assays

- Measurement of Inversions and Large Deletions
Platform Enables Broad Product Opportunities

- Broad Range of Sites
- Wide Delivery Options
- Diverse Spectrum of Edits

SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

SpCas9 | SaCas9 | SpCas9 Variants | SaCas9 Variants | Cpf1 | Cpf1 Variants | Editas Platform

- Viral Vector
- Lipid Nanoparticle
- Electroporation

~10x

Disrupt | Remove | Replace | Insert
Scalable, Consistent Engineered Cell Therapies

Optimized Delivery of RNP to Primary T cells Via Electroporation
Generating Synthetic Covalently-Coupled Dual gRNA

A completely non-enzymatic process for guide production

Why make a synthetic guide?

- Targeted chemistries anywhere in the molecule
- Unhindered ends and modifications
- Scale up and purity are more compatible with CMC requirements

covalently-coupled dual gRNA (dgRNA)
Cellular Editing Activity

_In vitro_ transcribed and synthetic covalently-coupled dgRNA are equivalent in cells.
Assessing gRNA purity and sequence fidelity

Development of an RNA-Seq based method for gRNA QC

- smRNA/Total RNA
  - 5' 3'OH
  - 3' Polyadenylation
  - First-strand synthesis and tailing by RT
  - Template switching and extension by RT
  - Forward PCR Primer
    - 5' 3'
  - Reverse PCR Primer
    - Addition of full length Illumina adapters by PCR

Graphs:
- synthetic 100mer “A”
- synthetic 100mer “B”
- covalently-coupled dgRNA
gRNA purity and sequence fidelity

Covalently-coupled dgRNA result in greater sequence fidelity in target region

A

B

Covalently-Coupled dgRNA
**Single gRNA**

Heterogeneous product (full-length, truncated, errors)

**Covalently-Coupled Dual gRNA**

Well-defined product (full-length)
Platform Enables Broad Product Opportunities

- **SpCas9**: *Streptococcus pyogenes* Cas9; **SaCas9**: *Staphylococcus aureus* Cas9

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**Wide Delivery Options**
- Viral Vector
- Lipid Nanoparticle
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**Diverse Spectrum of Edits**
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Cas9 Stimulates the Endogenous Repair Pathways

WT Cas9

DSB

5'

C-NHEJ

Locus Unaltered
Small Deletions
Small Insertions

Alt-NHEJ

Deletions
Blunt EJ
MMEJ
SD-MMEJ

3'

Resection

HDR

Large Deletions
SSA
HR

Correction
Cas9 is a Flexible Tool

- Could we engage different pathways by using these different variants?
- Could we selectively stimulate HDR?
DSBs Generated by D10A are Predominantly Repaired by HDR

Bothmer et al., Nat Comm 2017
Do Gene Conversion and Gene Correction have the same Genetic Requirement?

Gene Correction

Gene Conversion

Do they both dependent on the HR pathway?
Gene Conversion and Gene Correction have Different Genetic Requirements

HR is required for repair from double stranded donors (endogenous homology tracks or plasmids) but not single stranded donors.
Conclusions from the Dual Nick Analysis

- Different ends activate different DNA repair pathways

- Different donors stimulate different pathways
  Gene Correction mediated by ssODN is not HR dependent

Bothmer et al., Nat Comm 2017
Prioritization Principles

**Medical Need**
- Severe diseases where current treatments, if any, are poor
- Potential for durable therapies to provide unique benefit

**Biology & Clinical**
- Clear biological hypothesis for genomic intervention
- Favorable clinical and regulatory path

**Technical**
- Validated delivery approaches
- Mutation feasibly corrected

**Product Pipeline**

**Eye**
- LCA10 (EDIT-101)
- Ocular HSV
- Additional ocular indications

**Lung**
- Cystic Fibrosis

**Muscle**
- Duchenne Muscular Dystrophy

**Liver**
- Alpha-1 Antitrypsin Deficiency
- Infectious diseases of liver

**Bone Marrow & Blood**
- Hemoglobinopathies
- Engineered T cells for cancer
- Additional bone marrow and blood indications
Thank You

- Hayat Abdulkerim
- Luis Barrera
- Anne Bothmer
- Frank Buquicchio
- Dawn Ciulla
- Cecilia Cotta-Ramusino
- Georgia Giannoukos
- Kiran Gogi
- Jennifer Gori

- Fred Harbinski
- Hari Jayaram
- Eugenio Marco
- Carrie Margulies
- Tanushree Phadke
- Terence Ta
- Grant Welstead
- Chris Wilson

- Vic Myer

- I2 Pharmaceutical Team