Overview of Genome Editing Products and CMC Considerations

Phillip Ramsey

VP Technical Development

2022 CASSS CGTP Meeting

June 06, 2022

Presentation Outline

1. Introduction
2. Introduction to Genome Editing Platforms
3. Genome Editing Use & Safety Evaluation
4. On-target Activity
5. Off-target Identification
6. CMC Considerations & Case Studies

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Sangamo THERAPEUTICS

Sangamo's Robust Pipeline with Thoughtful Balance of Partnered and Wholly Owned Programs

Wholly Owned Programs

INDICATION	TECHNOLOGY	PRECLINICAL	PHASE I/2	PIVOTAL
Fabry Disease *(Isaralgagene civaparvovec)*	*Gene Therapy*	*Updated clinical data expected in 2H 2022*		
Renal Transplant *(TX200; Auto)*	*T_{REG} Cell Therapy*	*First patient dosed. Second patient planned mid-year 2022*		
Renal Transplant *(Allogeneic)*	*T_{REG} Cell Therapy*			
Inflammatory Bowel Disease	*T_{REG} Cell Therapy*			
Multiple Sclerosis	*T_{REG} Cell Therapy*			
Prion	*ZF Genome Engineering*			
Neurology	*ZF Genome Engineering*			

Partnered Programs

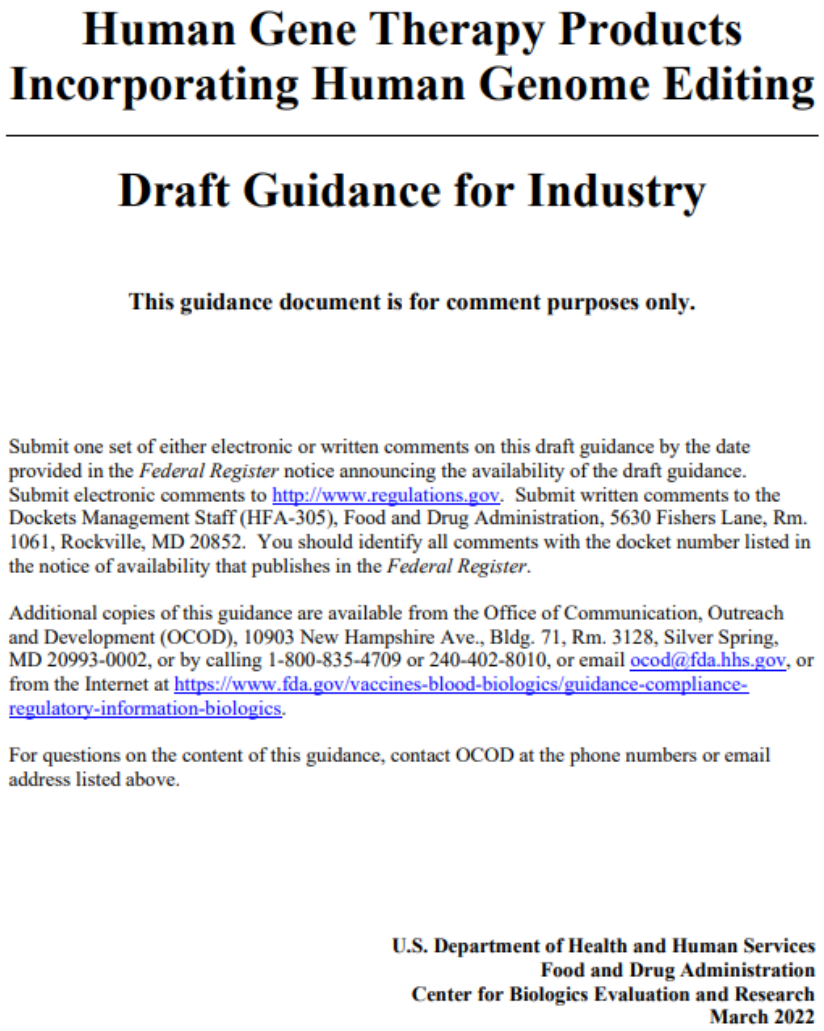
INDICATION	TECHNOLOGY	PRECLINICAL	PHASE I/2	PIVOTAL
Hemophilia A *(Giroctogene fitelparvovec)*	*Gene Therapy*	Pfizer		
Sickle Cell Disease* *(SAR445136)*	*Cell Therapy*			
Oncology *(Kite-037)*	*Cell Therapy*	Kite		
Oncology *(Undisclosed)*	*Cell Therapy*	Kite		
Neurodevelopmental Disorders	*ZF Genome Engineering*	NOVARTIS		
ALS/FTD	*ZF Genome Engineering*	Pfizer		
Huntington's Disease	*ZF Genome Engineering*	Takeda		
a-Synuclein *(ST-502)*	*ZF Genome Engineering*	Biogen		
Tauopathies *(ST-501)*	*ZF Genome Engineering*	Biogen		
Neurology *(DMI)*	*ZF Genome Engineering*	Biogen		
Neurology *(Undisclosed)*	*ZF Genome Engineering*	Biogen		

FIRST-GENERATION SECOND-GENERATION

*transitioning to wholly owned program in June 2022

New FDA Guidance for Development of Human Gene Therapy Products Incorporating Genome Editing (March 2022)

- Key considerations:
  - While the potential benefit of genome editing (GE) products are clear, the potential risks are not as well understood
  - Some of the risks specific to GE products include unintended consequences of on- and off-target editing, and unknown long-term effects of on- and off-target editing
  - Recommendations are made for how to assess these potential risks as well as safety and quality



Human Gene Therapy Products Incorporating Human Genome Editing

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics.

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
March 2022

New FDA Guidance for Development of Human Gene Therapy Products Incorporating Genome Editing (March 2022) (con't)

- Use a science-based approach weighing the benefits and risk of each product based on
    - Proposed indication,
    - Patient population
    - Extent and duration of therapeutic benefit achieved
    - Availability of alternative therapeutic options
- Evaluate the investigational product in definitive POC and safety studies, when feasible
- Differences in genomic sequences between animals and humans, may warrant use of surrogate GE products
- Ex vivo GE – use the clinical cell source for definitive preclinical studies; justify if an alternative cell source used
- Characterize each GE lot evaluated in preclinical studies according to specifications, consistent with stage of product development

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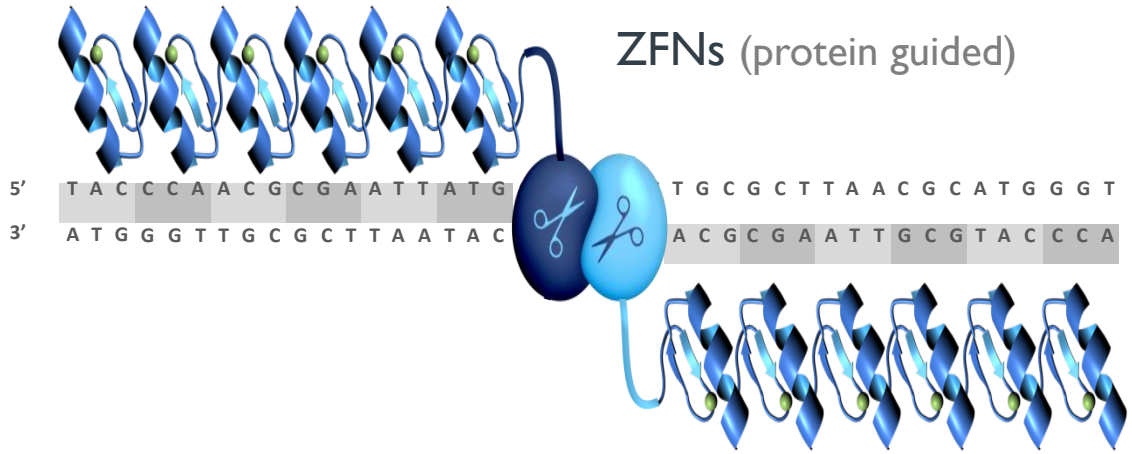
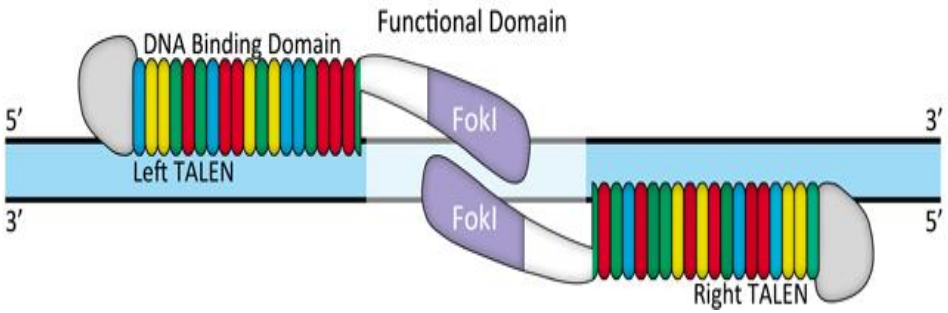
Clinical Stage Nuclease Genome Engineering Platforms

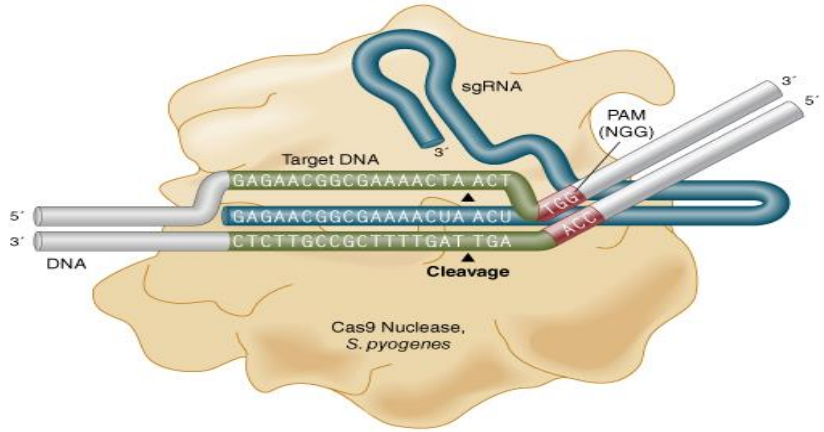
ZFNs (protein guided)



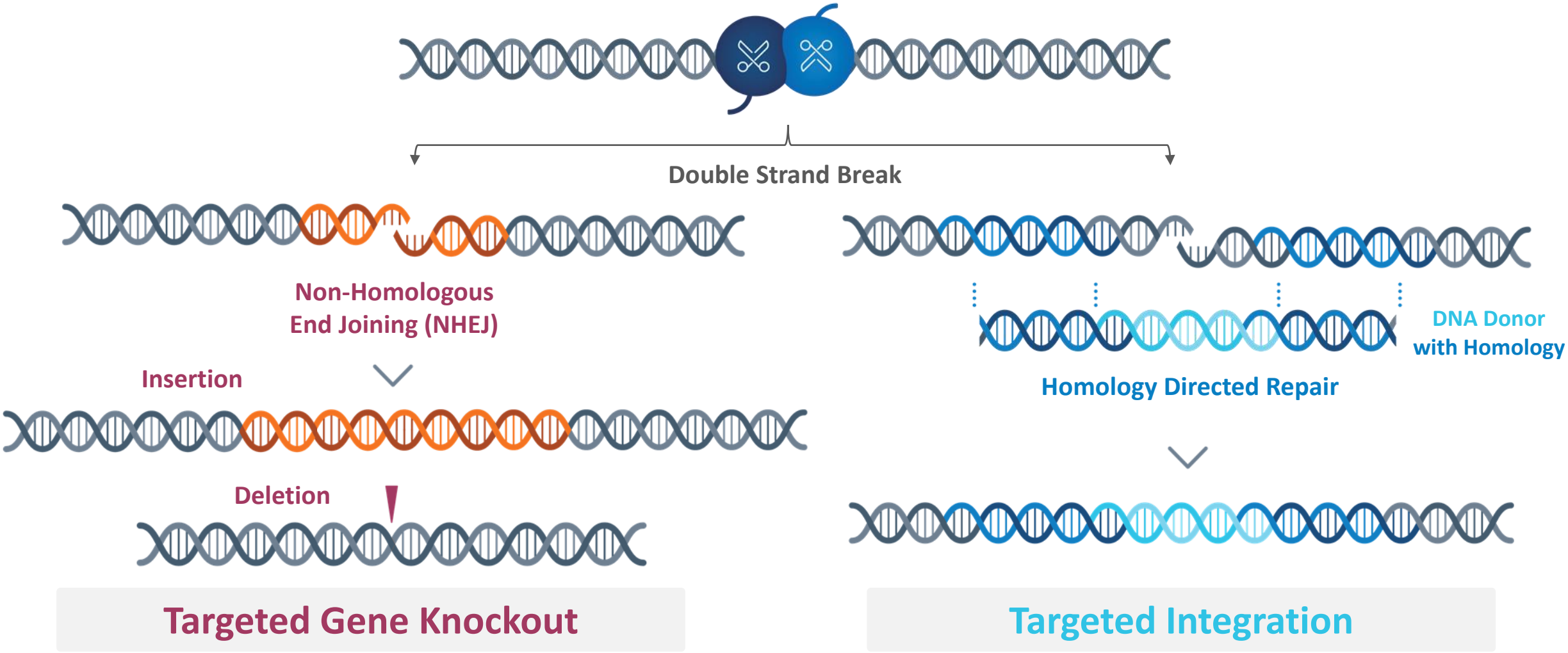
TALENs (protein guided)



CRISPR-Cas9 (RNA guided)



Overview of gene editing technologies – editing mechanisms



Nuclease GE - DNA Repair Using Non-homogenous End-joining (NHEJ)

Nuclease methods that induce DNA breaks are repaired by the same repair-mechanisms; no benefit of one type of nuclease modality over another with respect to downstream consequences of inducing DSBs (FDA Genome Editing Guidance 2022)

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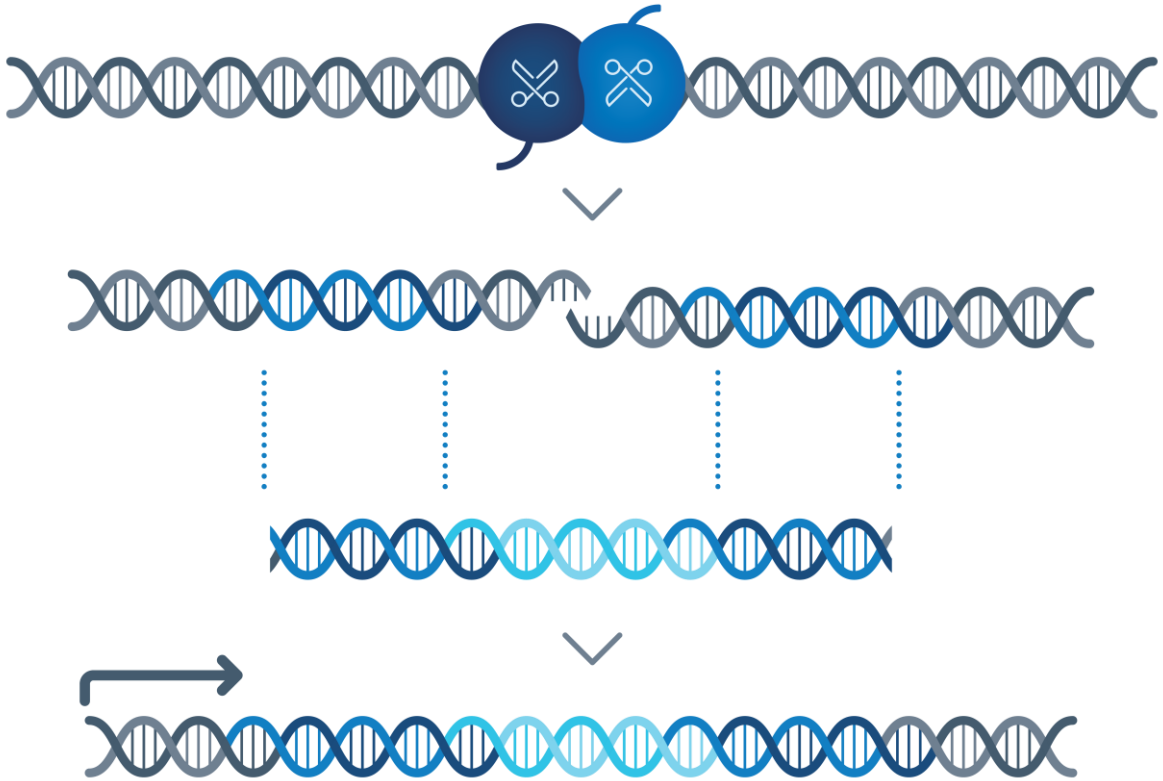
Nuclease GE - DNA Repair Using Homology Directed Repair (HDR)



Double Strand Break (DSB)

Homology Directed Repair (HDR)

Human Therapeutic Transgene with Homology Arms

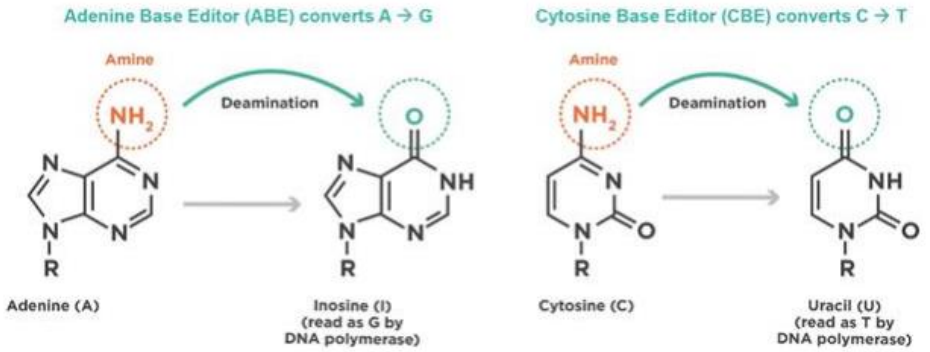
Insertion of Transgene into Specific Locus

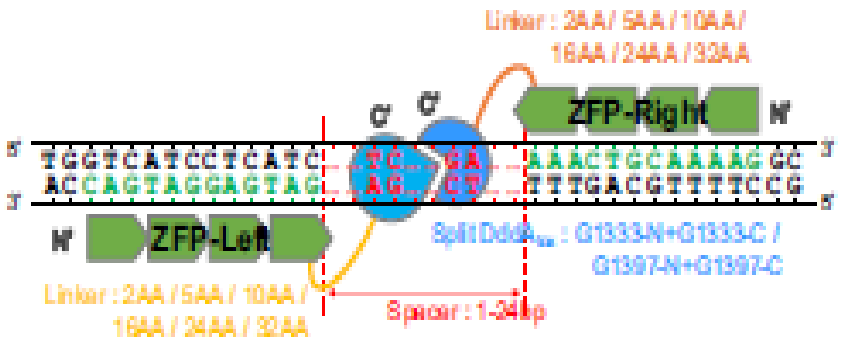
Research Stage Non-nuclease Genome Editing Platforms

No Induction of DSB

Nucleobase Editors

(deaminase enzymes)





Peptide Nucleic Acid (PNA) Editors

(synthetic DNA analogs)

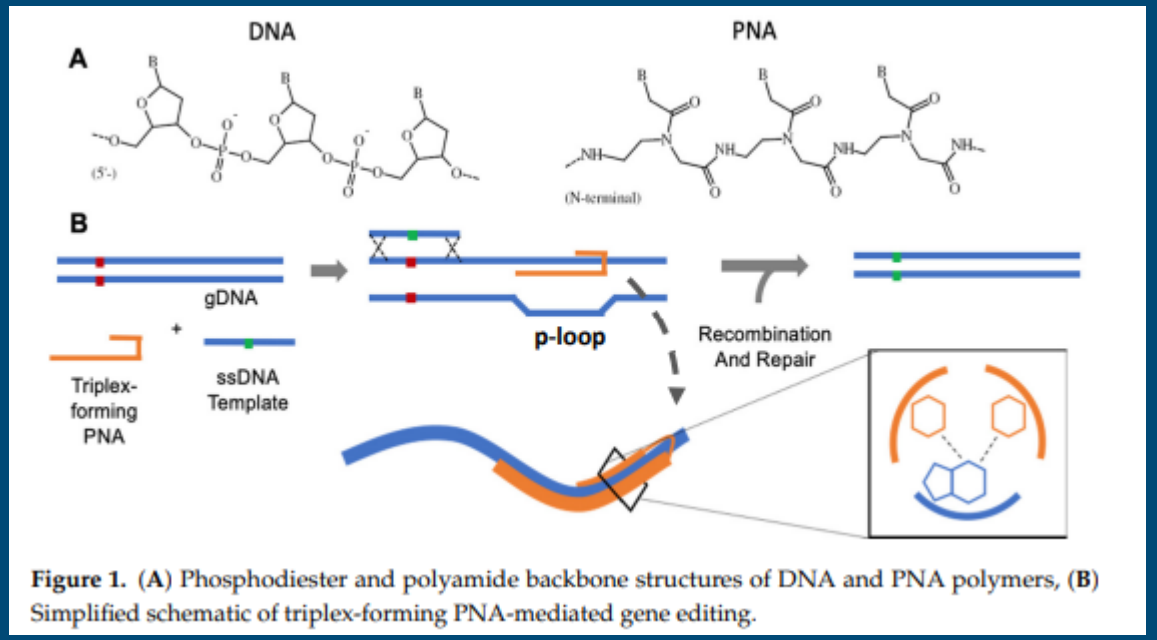


Figure 1. (**A**) Phosphodiester and polyamide backbone structures of DNA and PNA polymers, (**B**) Simplified schematic of triplex-forming PNA-mediated gene editing.

Nicholas Economos Molecules 2020, 25, 735

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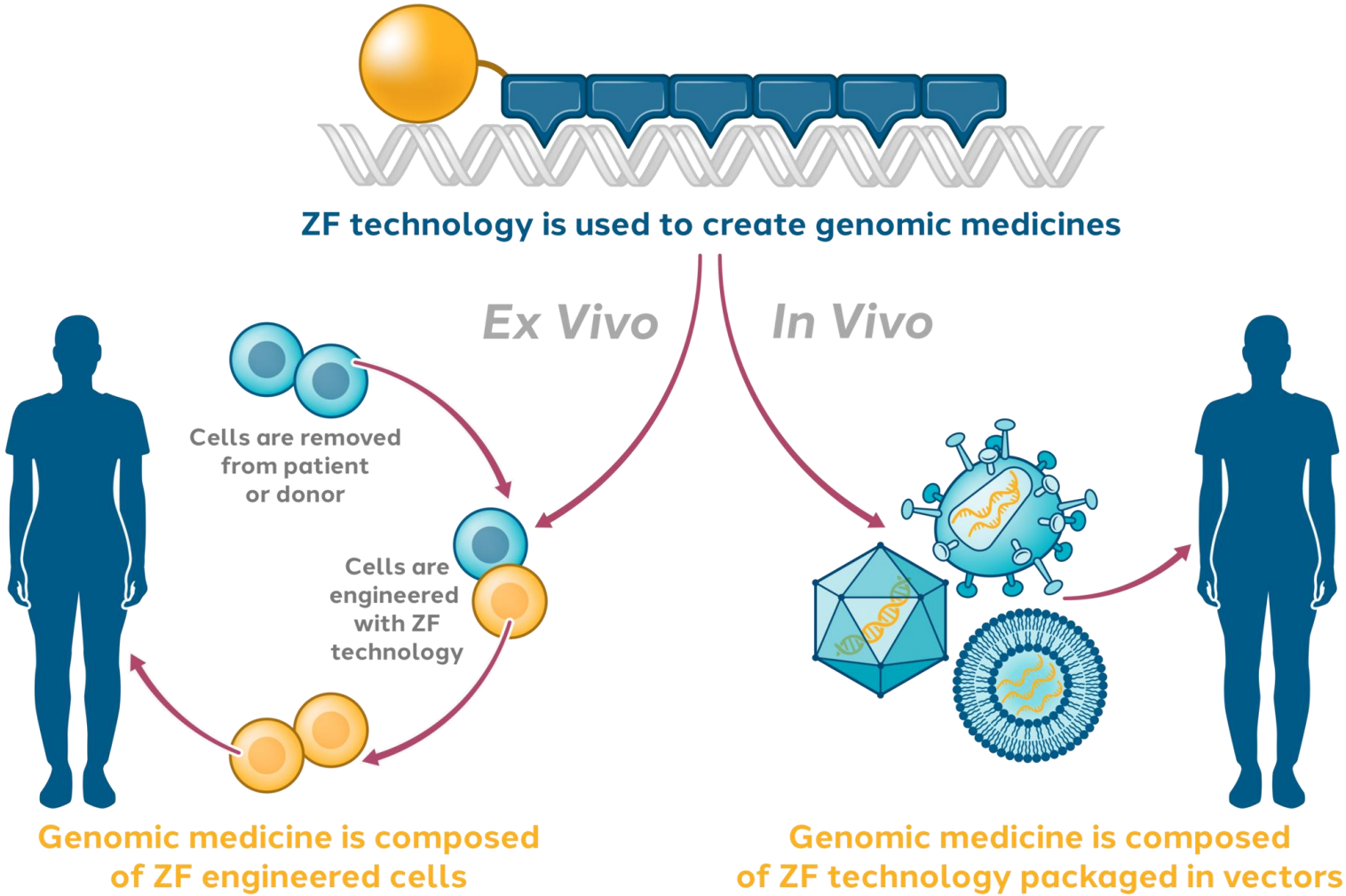
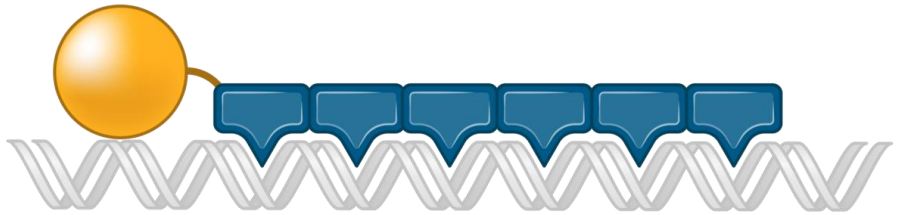
GE Products For Ex Vivo or In Vivo Clinical Applications



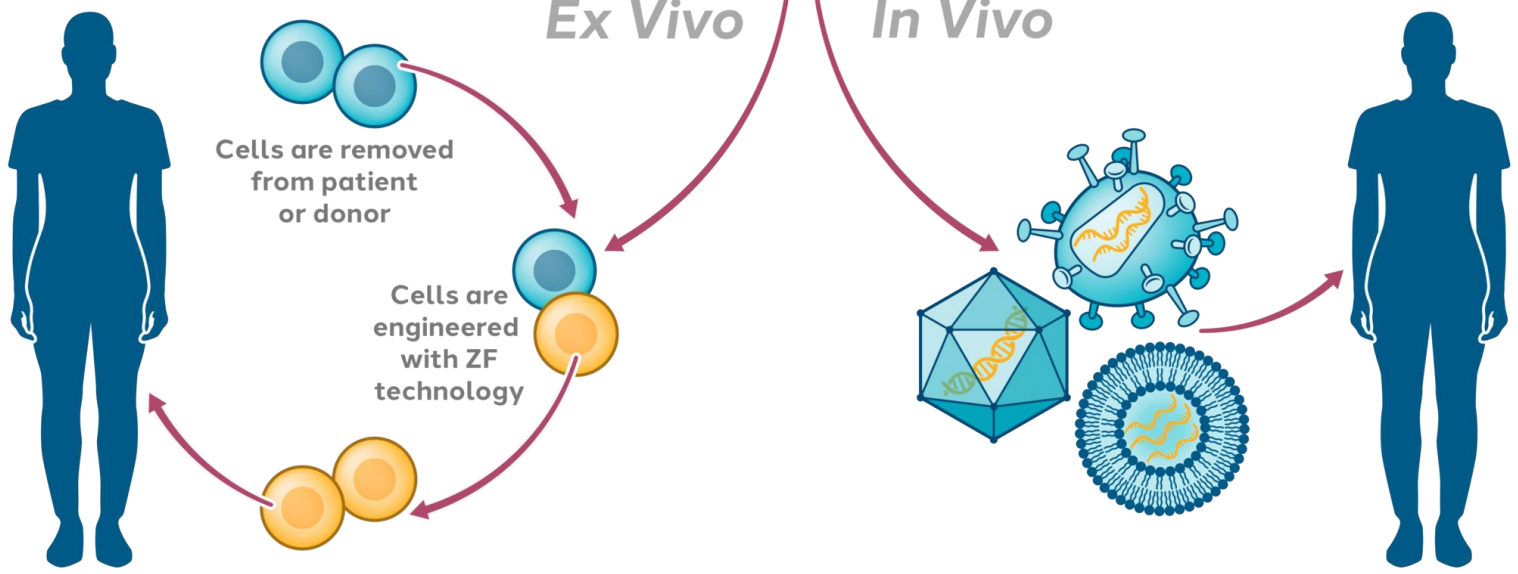
Genome Editing Products For Ex Vivo or In Vivo Clinical Applications

GE form: mRNA, DNA, protein or ribonucleoprotein
Route: Electroporation
Cell types: HSPC, CAR-T cells, CAR-T reg cells, iPSC cells and more



ZF technology is used to create genomic medicines

GE form: DNA, mRNA
Route: IV, CNS, ocular, IM and more
Vectors: AAV, lentivirus, lipid nanoparticles
Target cell: driven by tissue-specific or ubiquitous promoter



Genomic medicine is composed of ZF engineered cells

Genomic medicine is composed of ZF technology packaged in vectors

Nonclinical Safety Considerations

Ex Vivo

- Target cells for therapeutic benefit
- Intended therapeutic use of edited cell
- Transient editors
- Target editing level and impact
- Pharmacology and cell phenotype
- Persistence of editing
- Proof-of-concept in animal disease model
- Biodistribution and cell persistence
- **Genotoxicity assessment (more details to follow)**
- Immunogenicity (graft vs host or host vs graft)
- Toxicology/pathology

In Vivo

- Target cells for therapeutic benefit
- Intended therapeutic use of edited cell
- Transient or permanent editors
- Target editing levels and impact
- Pharmacology
- Persistence of editing
- Proof-of-concept in animal disease model
- Biodistribution
- **Genotoxicity assessment (more details to follow)**
- Immunogenicity (viral vector, nuclease components, and/or transgene)
- Toxicology/pathology

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Genotoxicity Risk Assessment

Ex Vivo

- Clinical candidate cell available to assess
- Bioinformatics
- In vitro off-target (e.g., oligonucleotide capture)
- Molecular translocation analysis
- Integration risk assessment
- Karyotype
- Tumorigenicity
  - In vitro methods (examples)
    - Soft agar assay for nucleases*
    - IL-2 independent growth for T cell products
  - In vivo (based on robustness/lifespan of edited cell in immunodeficient mice)
- Clonality (selective advantage of GE cells)
- Post-dose cell samples

*Lemmens, 2021

In Vivo

- Bioinformatics
- In vitro off-target (e.g., oligonucleotide capture)
- Molecular translocation analysis
- Integration risk assessment
- Kinetics of GE component expression and activity
- Post-dose tissue biopsy samples

Can genotoxicity assay results support GE platform? Most likely if same GE components/nucleases used among programs

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What's New for GE Products Compared to Gene Therapy Products? (1)

- Assessment of toxicity related to expression of GE components and modification of genomic structure
- Identification and characterization of on- and off-target editing and consequences (both observed and theoretical consequences)
  - Including type, frequency and location of all off-target editing events
  - Using orthogonal including unbiased genome-wide analysis, verification of bona fide off-target sites in human target cells
  - Biodistribution including evaluation of editing in target and non-target tissues
  - Assessment of genomic integrity including chromosomal rearrangements, insertions/deletions, integration of exogenous DNA, potential oncogenicity or insertional mutagenesis

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What's New for GE Products Compared to Gene Therapy Products? (2)

- Preservation of cell functionality following GE
- Immunogenicity of GE components
- Characterization of kinetics of GE component expression and editing activity
- Assessment of viability and any selective survival advantage of edited cells
- Evaluation for potential for inadvertent germline modification

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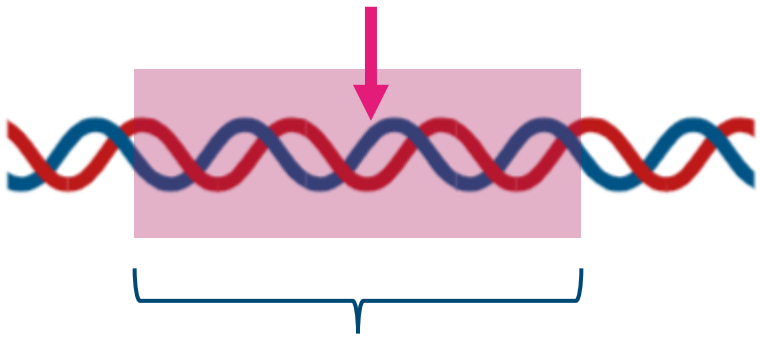
On-Target Activity

- Evaluate the consequence of ZFN activity at specific target site within genome
- Deep sequencing of specific target site
- Determine the % of genes modified (insertions and deletion mutations [indels])
- Evaluate the pharmacological impact of editing

Nuclease Target Cut Site



Deep Sequencing Region
(~200 basepairs)

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Example of Indel Analysis at ZFN Target Cut Site

```
ttttgtgggcaacatgctggtcatcctcatcctgataaactgcaaaaggctgaagagcatgactgaca wt

Deletions (43):
gttttgtgggcaacatgctggtcatcctcat-ctgataaactgcaaaaggctgaagagcatgactgaca -1
gttttgtgggcaacatgctggtcatcctcatcctgat--actgcaaaaggctgaagagcatgactgaca -2
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Insertions (16):
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gttttgtgggcaacatgctggtcatcctcatcctgataTAaactgcaaaaggctgaagagcatgactga +2
gttttgtgggcaacatgctggtcatcctcatcctgatCTGATaaactgcaaaaggctgaagagcatgac +5  13X
```

Holt *Nature Biotechnology* 2010

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On-Target Editing: How Much is Enough?

- What type of genomic modification(s) is needed for desired therapeutic effect?
- How much genomic modification is needed for desired therapeutic effect?
- Use in vitro studies and animal models of disease to establish proof-of-concept
- Justify strategy to regulatory authorities

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Presentation Outline

Off-Target Identification

Can't we just sequence the entire genome to find off-targets?

No. Whole genome sequencing is not sensitive enough for the large cell populations encountered in therapeutic contexts

- Solution = only sequence where cuts happen

Off-Target Identification

Bioinformatics – find similar sites by computer-based genomic sequence comparison

Biochemical – edit purified genomic DNA, trap and sequence where cuts happen

Cellular – edit cells, conduct karyotype analysis, sequence where cuts happen

Develop list of candidate off-target sites for quantitation

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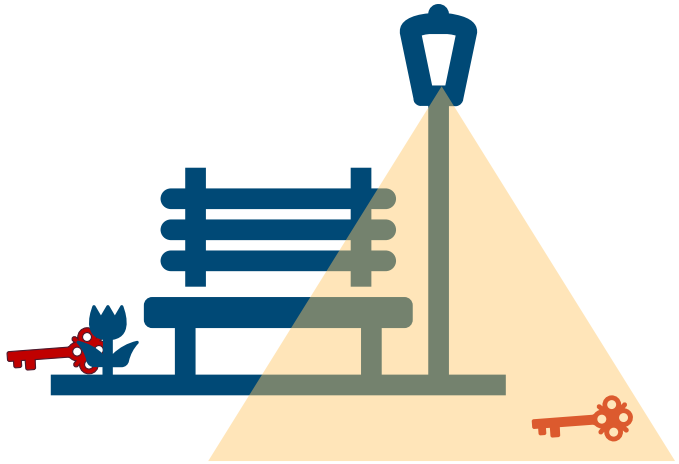
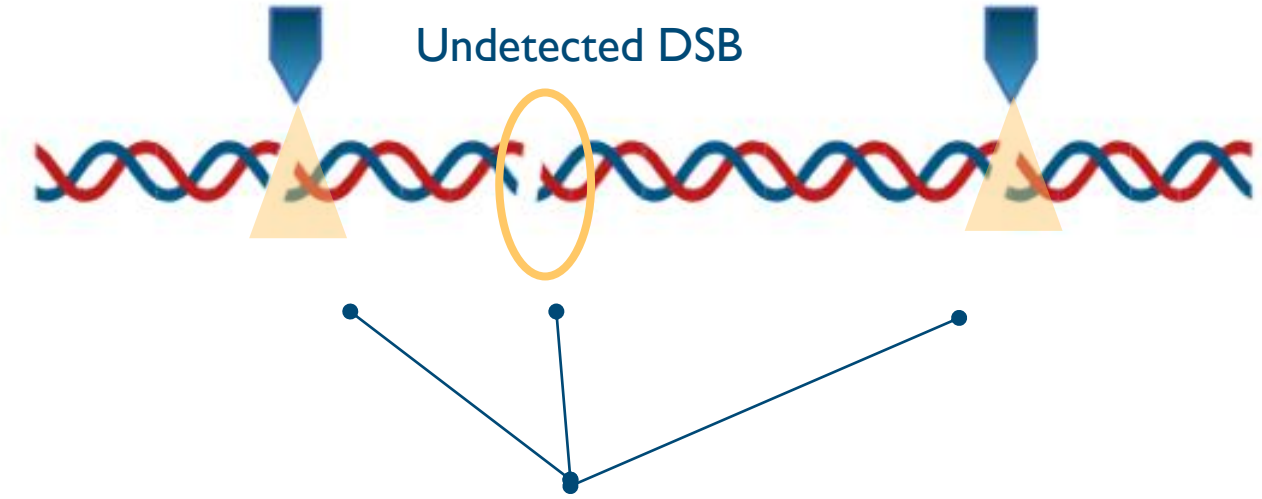
Off-Target Activity: Biased Approach

- Select potential off-target regions to assess using bioinformatics approach
- Evaluate these potential sites by deep sequencing
- Sequence the selected regions and determine presence/absence of mutations

Biased approach is akin to looking for a lost item under a lamp post

Not all cut sites are detected





Sites of double-strand breaks (DSB)

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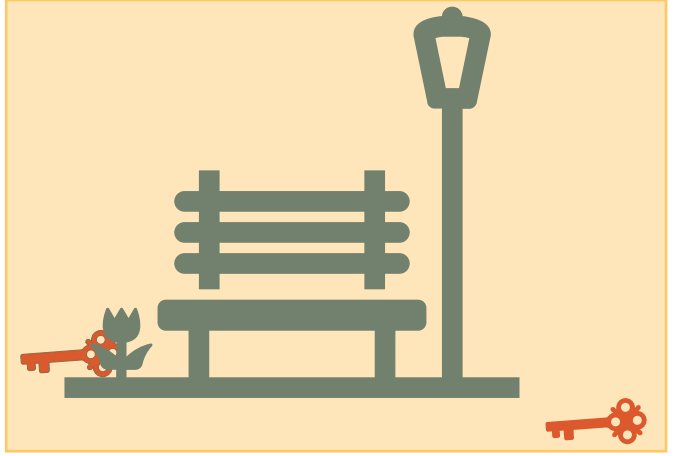
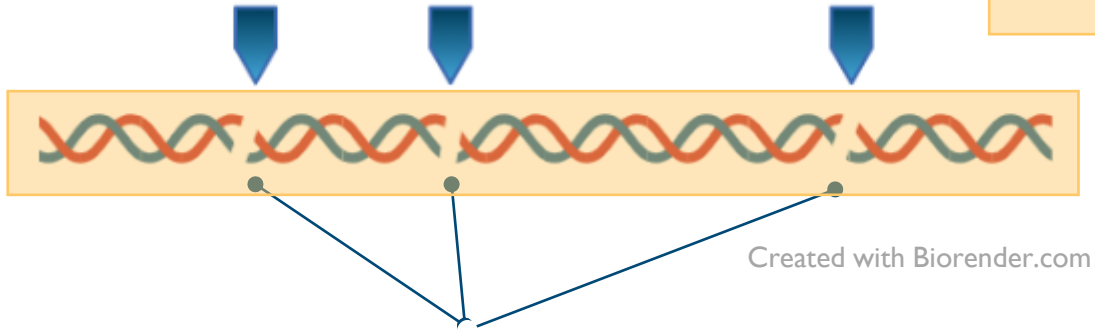
Off-Target Activity: Unbiased Approach

- Evaluate the consequence of off-target nuclease activity at each double-strand break site



Unbiased approach detects all cut sites



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Sites of double-strand breaks

Examples of technology for unbiased assessment:

- Oligonucleotide capture (e.g., GUIDESeq)
- LAM-PCR - Linear amplified mediated PCR
- Immunostaining for double-strand breaks
- IDLV/AAV capture – vector captured at break site

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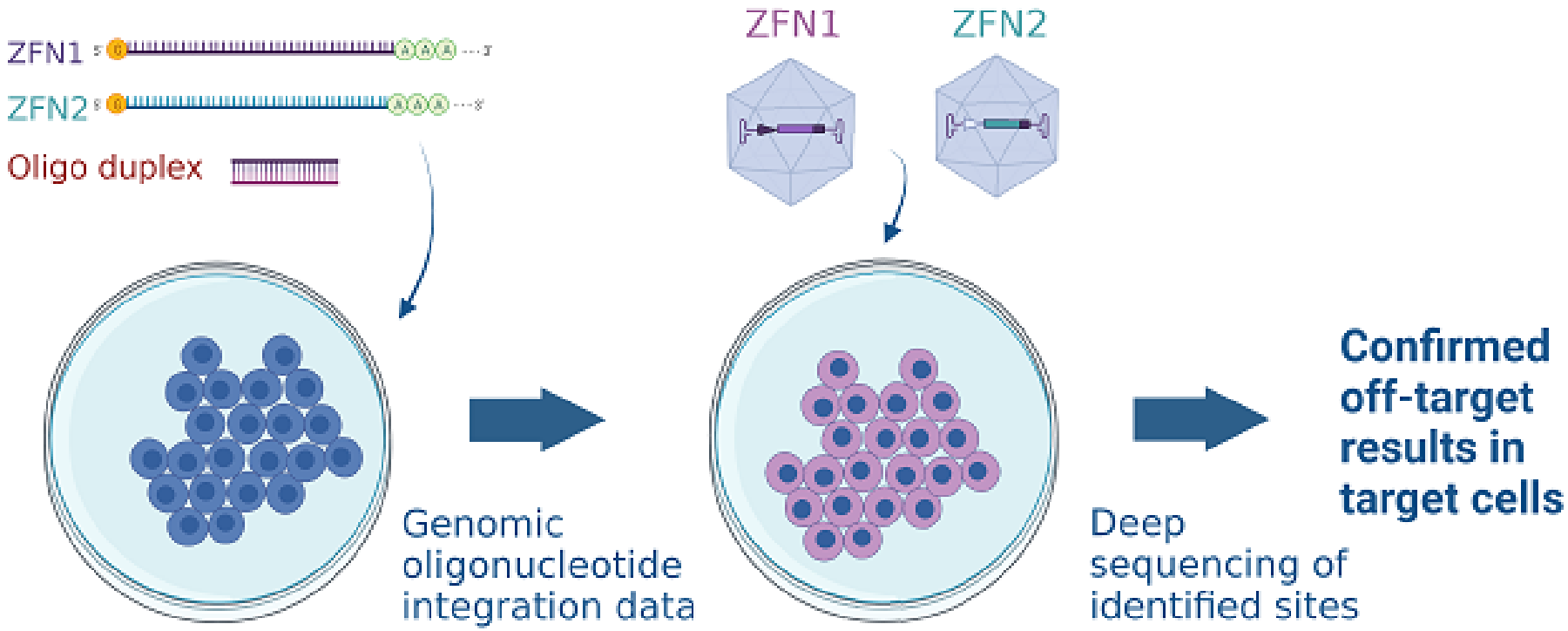
Off-Target Activity: Unbiased Approach - Oligonucleotide Capture

Step 1: Oligo capture unbiased approach to off-target detection

Step 2: Confirmation of nuclease activity at identified off-target sites in target cells



Surrogate cell type

Target cell type

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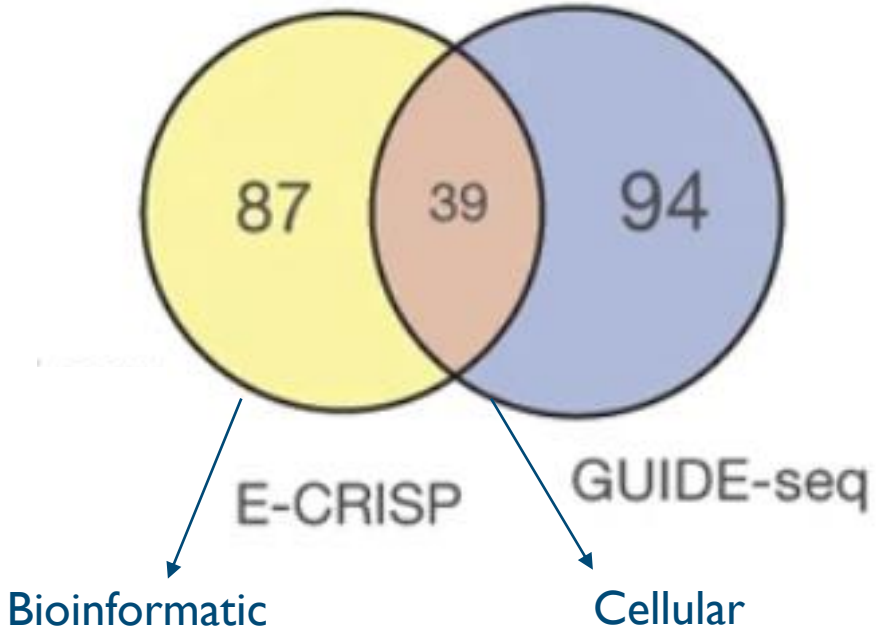
How Do Biased and Unbiased Off-Target Identification Methods Compare?



Bioinformatics alone cannot predict all off-target risks.
Unbiased cellular methods are favored as a source of truth.

GUIDESeq overview by Tsai, S.Q., et al. *Nature Biotechnology* 33.2 (2015): 187-197.

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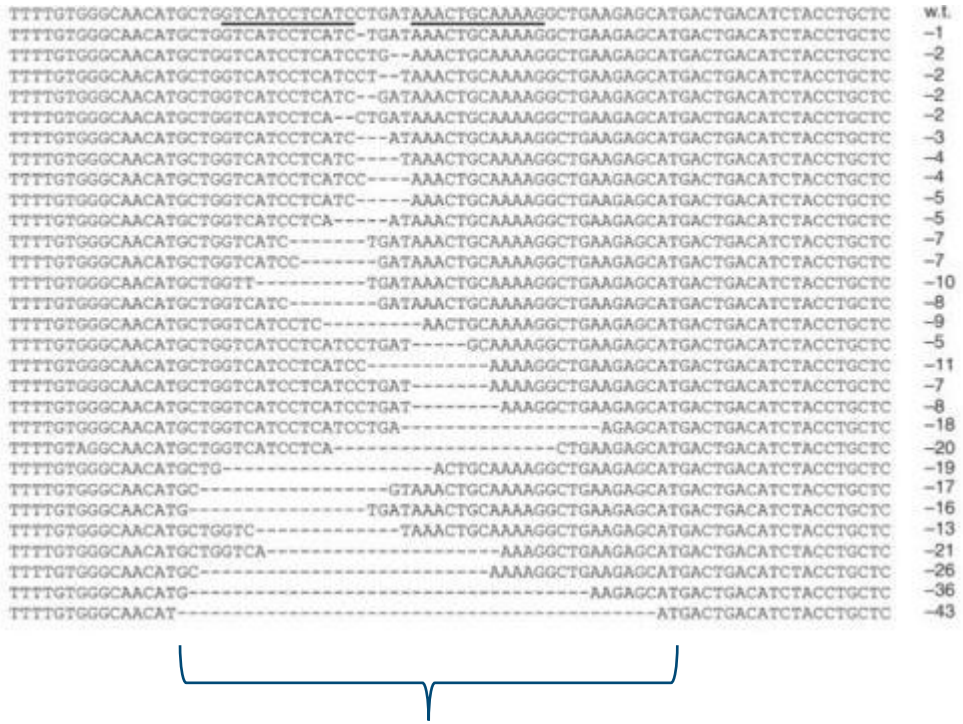
Off-Target Quantification

ZFN cut in cell culture



Deletion profile produced in a cell population

Perez, E.E., et al. *Nature Biotechnology* 26.7 (2008): 808-816.

Now that we know where the off-targets are located, how do we quantify them?

Isolate genomic DNA from cell population

- Ex vivo: confirm early off-target list with clinical candidate cell (as close to final manufacturing process as possible)
- In vivo: confirm early list with target cell type (e.g., primary human hepatocytes for AAV genome editing targeting liver)

PCR amplify and nextgen sequence (MiSeq, NextSeq)

Count frequency of mutations at cut site

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Off-Target Risk Assessment

- What is the location of off-target sites relative to known genes?
  - Are they within an exon? (high risk of impact)
  - Are they within an intron or intergenic? (less risk of impact)
- Consult literature to assess the biological risk of off-target genes
- Employ cellular assays, if available (example, soft-agar assay)

Goal = no detectable off-targets
OR
low level off-targets at low-risk locations

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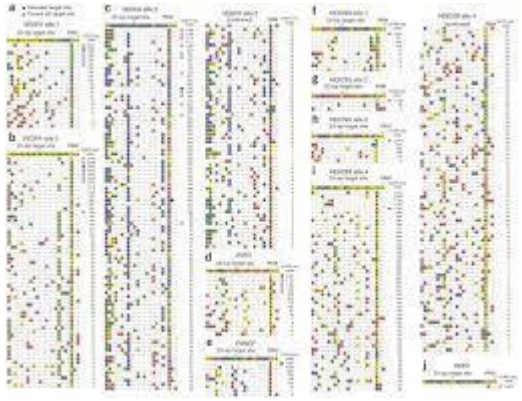
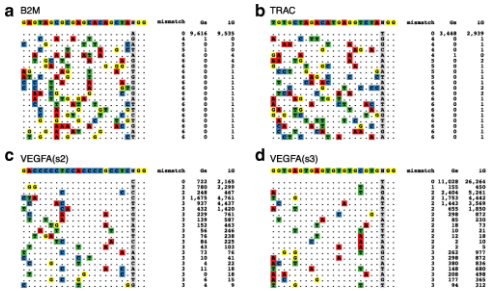
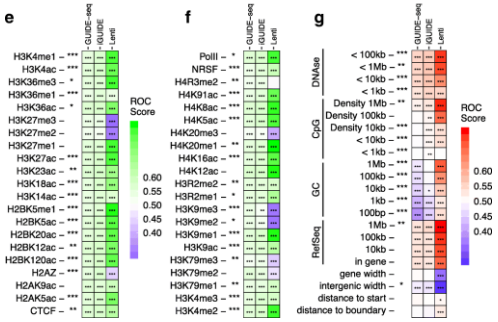
Investment in analytics

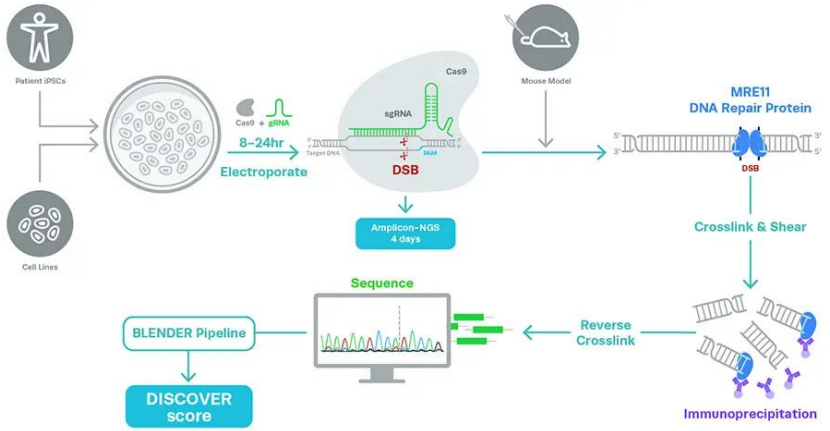
- Proper molecular biology techniques for identification
- In-vitro detection
- Sequencing
- Bioinformatics & Pipeline
- Activity model





Next Generation Sequencing





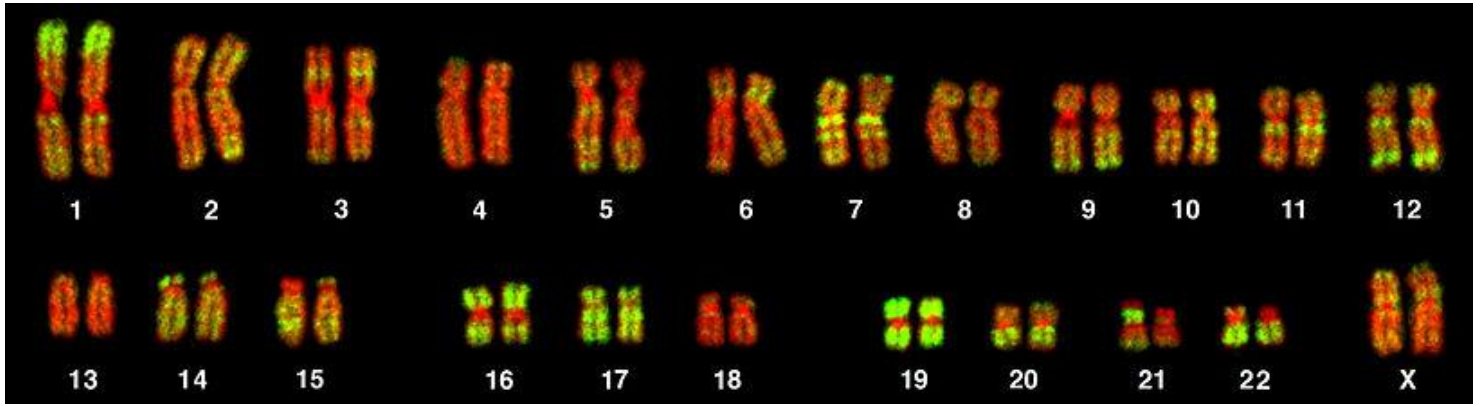
Avoiding Off-Targets By Good Design Practices

Chance of sequence variation causing increased off-target activity can be mitigated by targeting highly unique locations in the genome

During nuclease design, avoid on-target locations with known high frequency variation (always query dbSNP)

Bioinformatic safeguards - analysis of the target gene vs. the entire genome

The human Alu sequence (green) is repeated >1 million times.



Bolzer, A., et al. *PLoS biology* 3.5 (2005): e157.

Avoid picking ZFN, TALEN, or CRISPR target sites with exact genomic repeats or highly similar sequences

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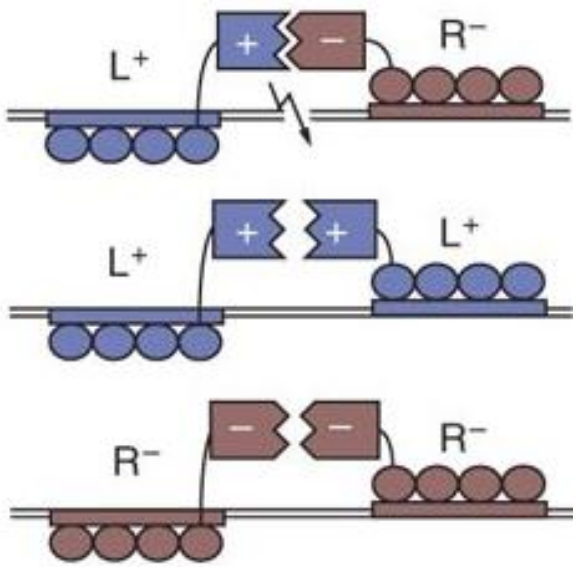
Avoiding Off-Targets by Good Design Practices

Molecular safeguards - the FokI (+/-) heterodimer of Sangamo ZFNs

ZFNs



Miller, J.C., et al. *Nature Biotechnology* 25.7 (2007): 778-785.

Thoughtful molecular design of nucleases can universally minimize off-target activity

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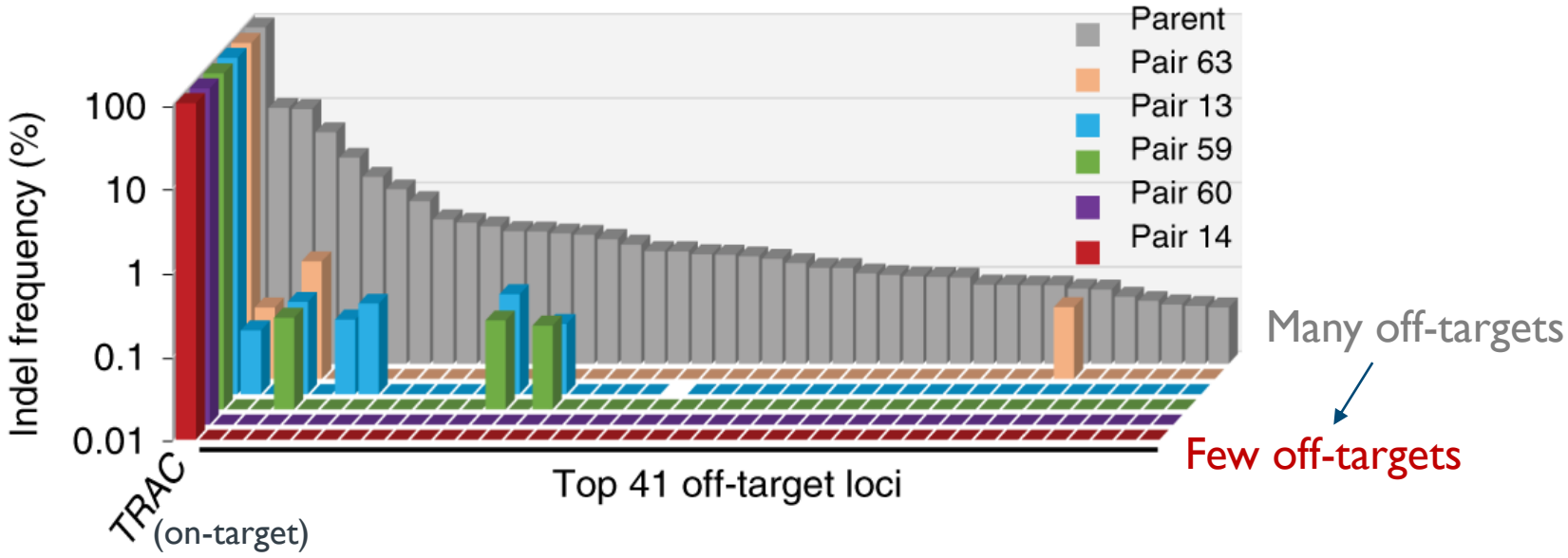
Avoiding Off-Targets by Good Design Practices

Molecular safeguards - ZF and FokI variants that reduce off-targets



Miller, J.C. et al. *Nature Biotechnology* 37 (8), 945-952 (2019)

Advanced molecular design can fine tune ZFNs to suppress off-targets while maintaining on-target efficacy

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Conclusions

- Gene therapy/genome editing products offer great hope for improving patient health and quality of life
- Gene therapy/genome editing are still new fields and risks are not fully characterized
- FDA new genome editing guidance document provides high level road map
- Genome editing platforms continue to evolve and enhance specificity
- Nonclinical safety assessment is based on tailored case-by-case and weight of evidence approach to inform risk/benefit, safety profile and clinical dose selection
- Invest in analytics and bioinformatics pipeline
- Minimize generation of off-targets by employing good design practices

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Thank you for your attention

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