The Development of CRISPR Based Medicines for the Treatment of Hematological Diseases

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Editas Medicine

Medicines that Aim to Repair Any Broken Gene

Potential to create the next major category of transformative medicines

Pipeline Strategy to Enable Successful Medicines

Medical Need

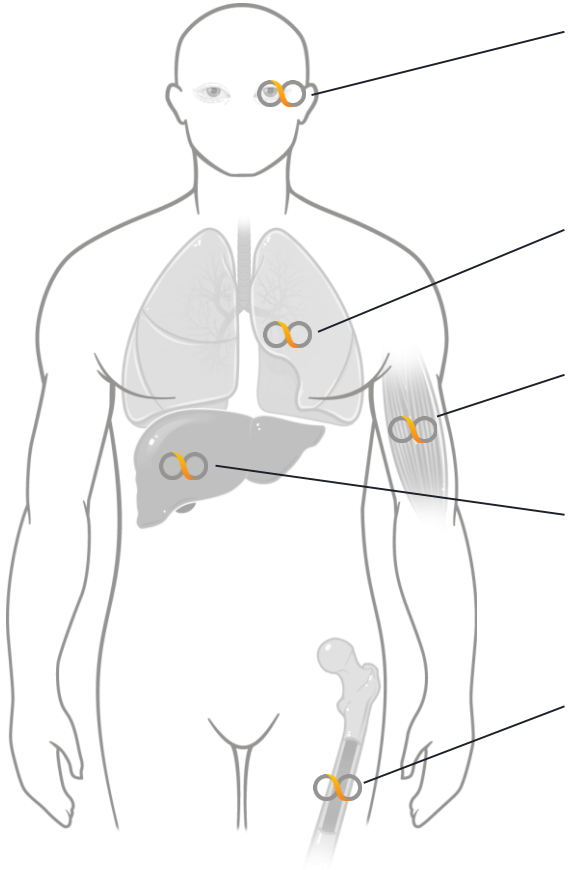
- Severe diseases where current treatments, if any, are poor
- Potential for durable therapies to provide unique benefit

Biology & Clinical

- Clear biological hypothesis for genomic intervention
- Favorable clinical and regulatory path

Technical

- Validated delivery approaches
- Mutation feasibly corrected



Eye

- Leber Congenital Amaurosis 10
- Ocular HSV
- Additional ocular indications

Lung

- Cystic Fibrosis

Muscle

- Duchenne Muscular Dystrophy

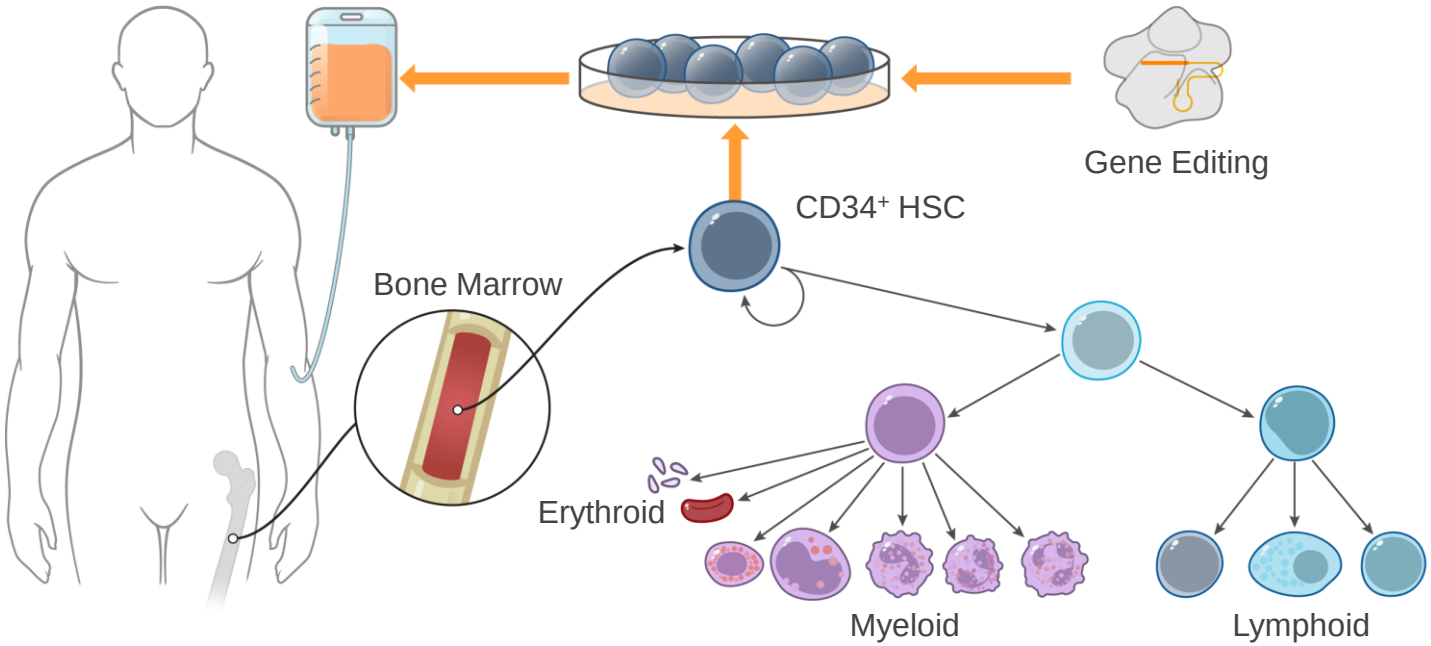
Liver

- Alpha-1 Antitrypsin Deficiency
- Infectious diseases of liver

Bone Marrow & Blood

- Hemoglobinopathies
- Engineered T cells for cancer
- Additional bone marrow and blood indications

CRISPR Unlocking the Promise of Cell Therapy



Hematopoietic stem cells have the potential to yield multiple medicines for **blood diseases** including sickle cell disease and beta thalassemia

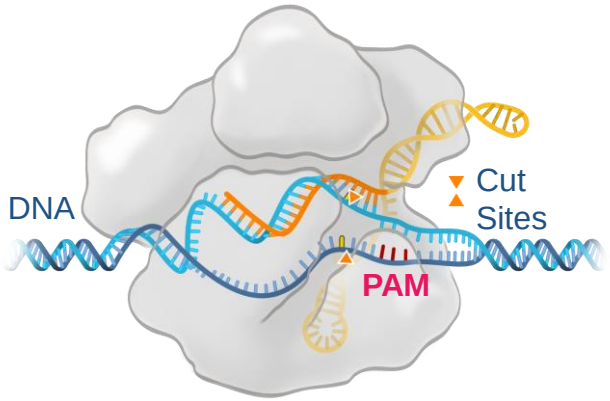
T cells are therapeutic platform for **cancer, autoimmune, and infectious diseases**

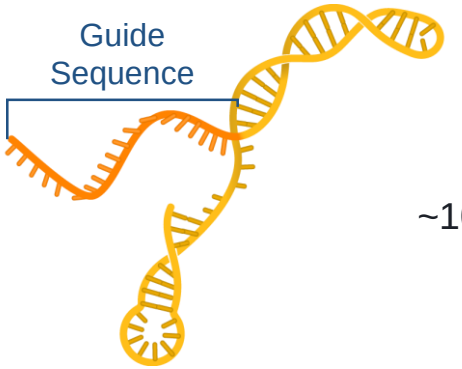
Recent approval of first CAR-T product demonstrated **rapid development and approval of a transformative cell therapy**

HSC: Hematopoietic Stem Cell; CAR-T: Chimeric Antigen Receptor T cell

CRISPR is a RNA-Guided Nuclease

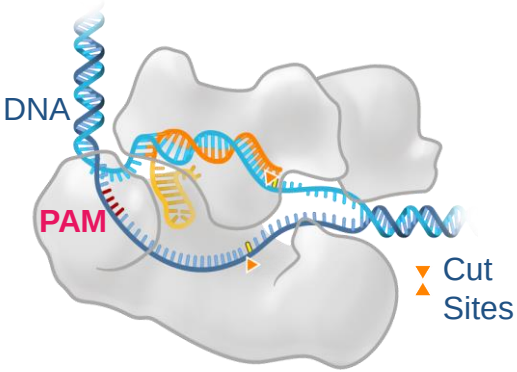
Cas9

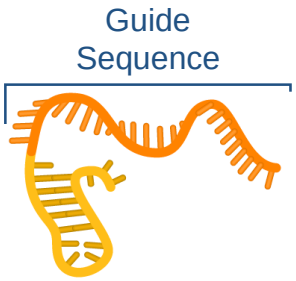




~100 nt

Cpf1





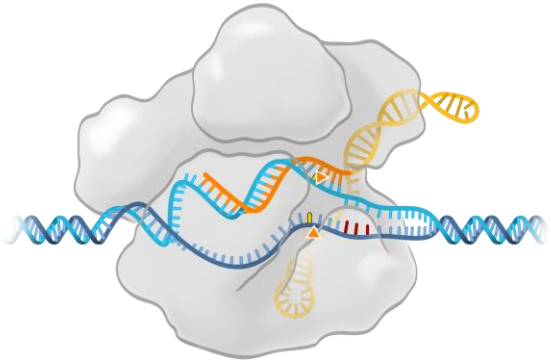
42 nt

Nuclease **Guide RNA**

Editing machinery can be engineered to target nearly any genomic location

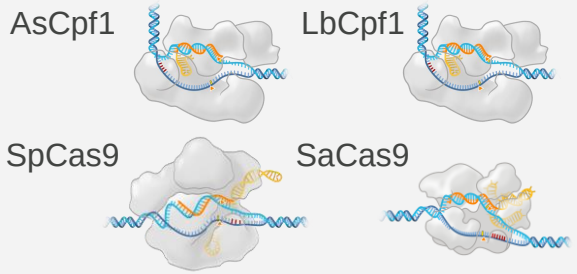
Broad Toolkit of CRISPR Nucleases

Cas9

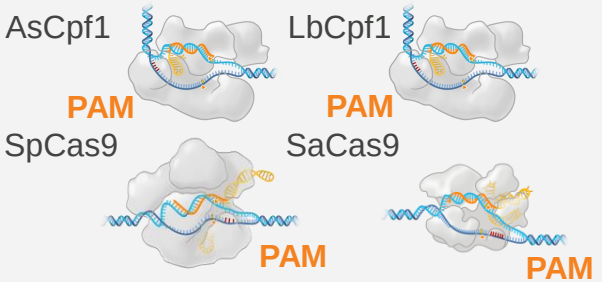


Cpf1

MULTIPLE EDITING SYSTEMS



ADVANCED FORMS FOR FLEXIBLE TARGETING

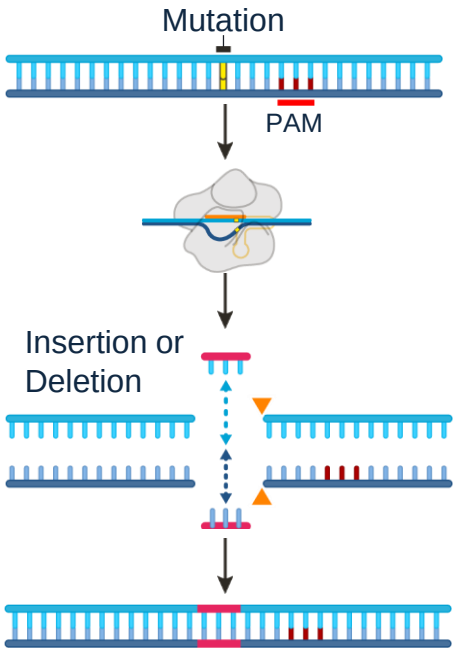


ADVANCED FORMS WITH INCREASED SPECIFICITY

AsCpf1: *Acidaminococcus* species Cpf1; LbCpf1: *Lachnospiraceae* bacterium Cpf1; PAM: Protospacer Adjacent Motif; HiFi: High Fidelity; eS: enhanced Specificity

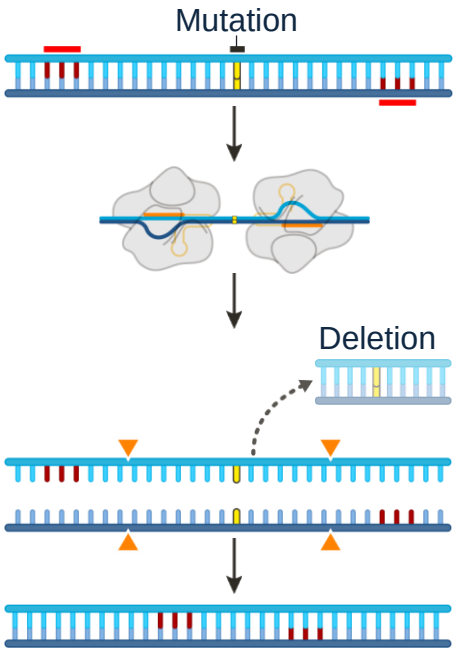
CRISPR Flexibility Addresses Diverse Mutations

Cut and **Revise**



e.g., Engineered T cells

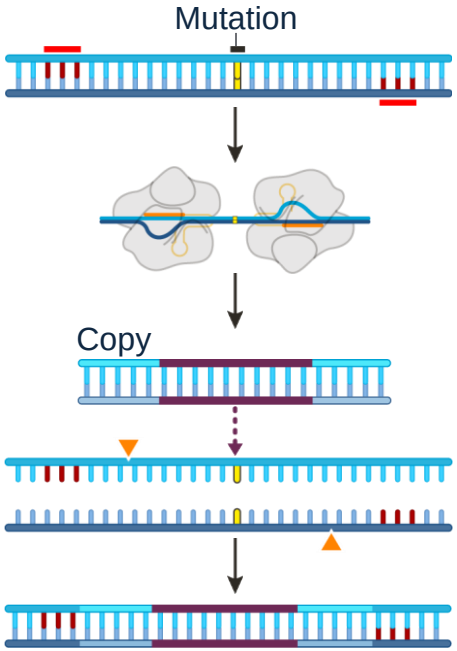
Cut and **Remove**



e.g., LCA10

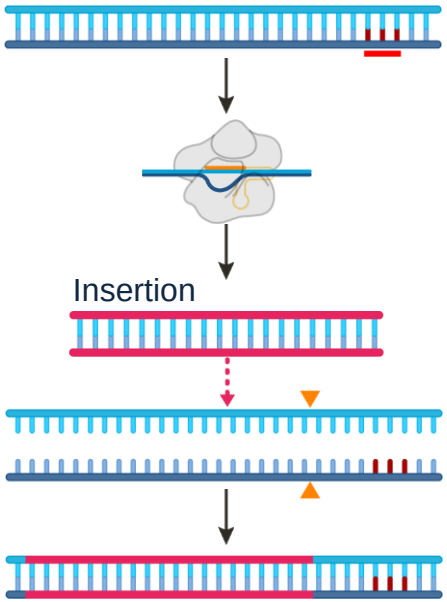
Non-homologous end joining typically **disrupts a gene or eliminates a disease-causing mutation**

Cut and **Replace**



e.g., Hemoglobin Beta

Cut and **Insert**



e.g., Safe harbor

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

LCA10: Leber Congenital Amaurosis 10

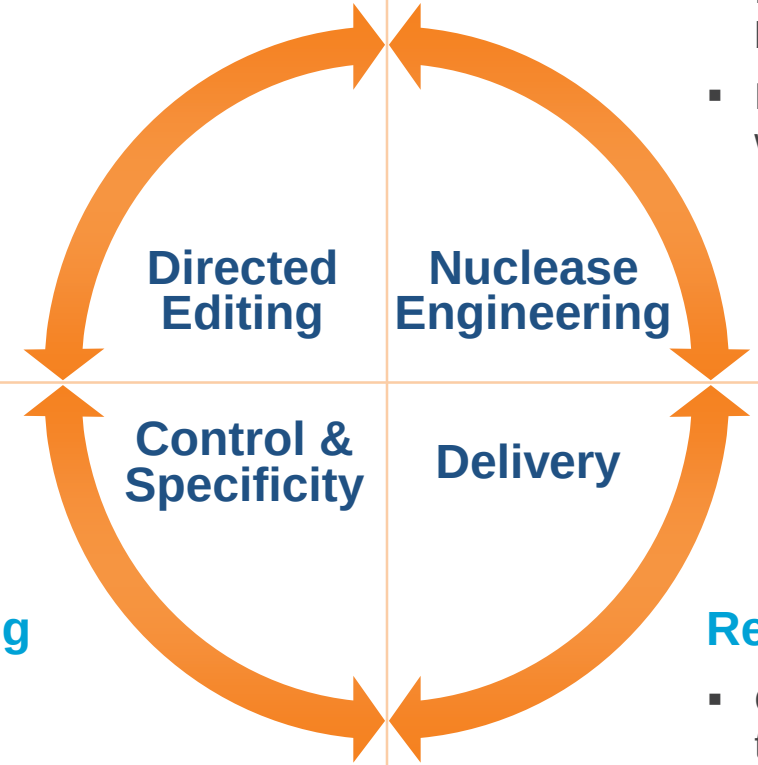
Platform to Create CRISPR Medicines

Achieve Right Repair

- Engineer and deliver correct template
- Use cut orientation to drive template utilization

Efficiently Edit Wide Range of Mutations

- Expand opportunity with broader PAM recognition
- Enhance safety and efficacy with modifications to nuclease



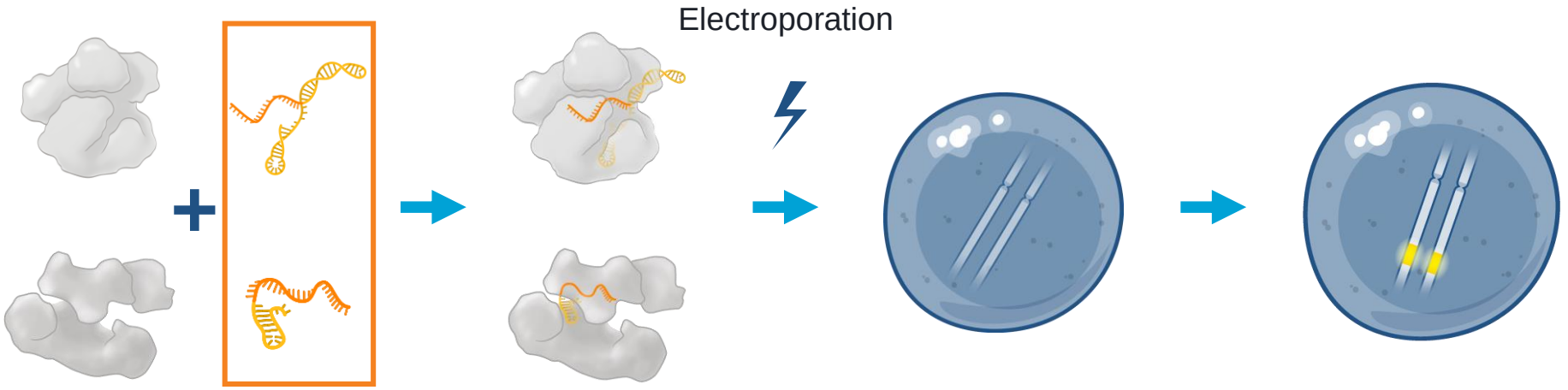
Tightly Control Cutting

- Comprehensive, unbiased and empirical approach
- Self-regulating systems to control expression

Reach Site of Disease

- Optimize modality for each tissue and cell type
- AAV, LNP, electroporation

Scalable, Consistent Engineered Cell Therapies

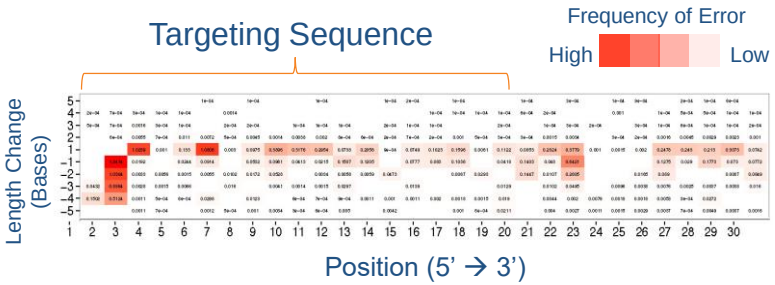


Optimized Delivery of RNP to Primary T cells Via Electroporation

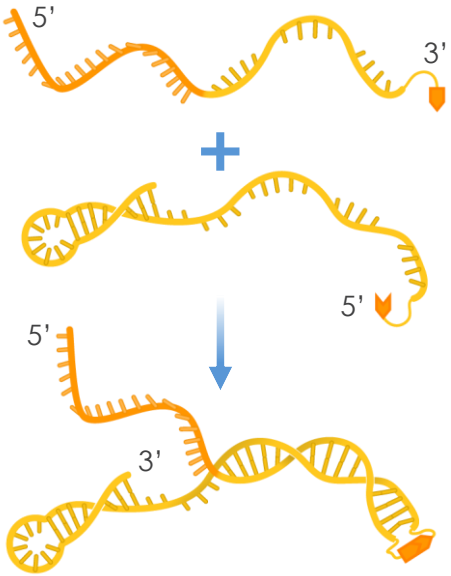
Proprietary Approaches to Guide RNAs

Single gRNA

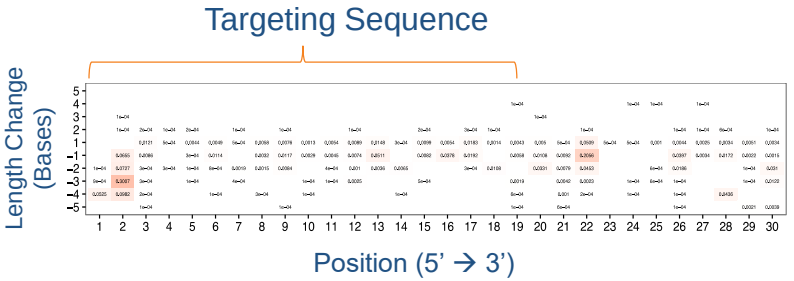
Heterogeneous product
(full-length, truncated, errors)



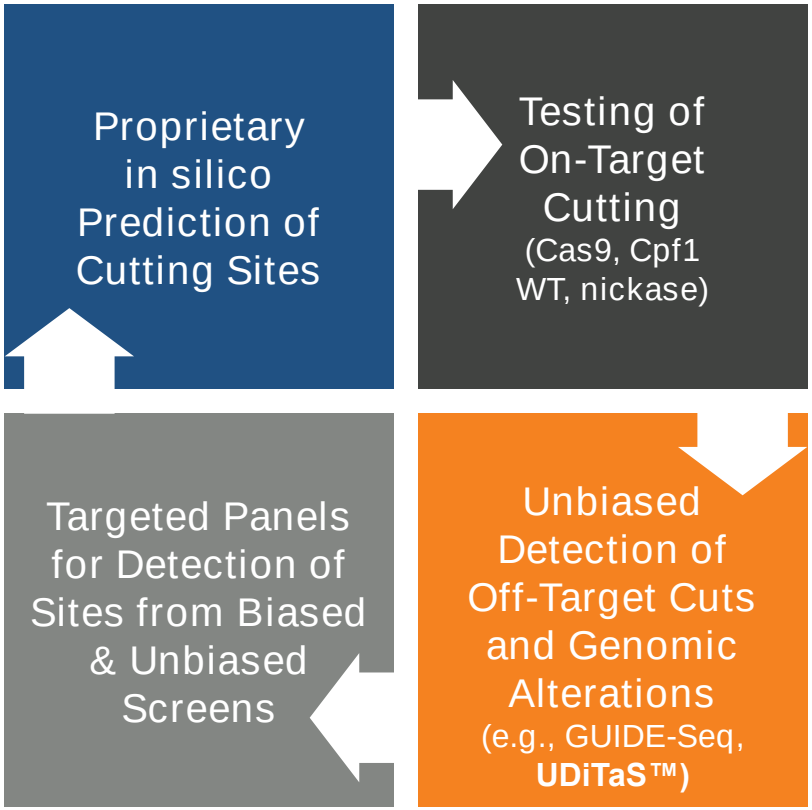
Covalently-Coupled Dual gRNA



Well-defined product
(full-length only)



Orthogonal Specificity Approaches for Best gRNAs



Combine computational with unbiased empirical cell-based methods to accurately and thoroughly identify potential off-target sites and select best gRNAs

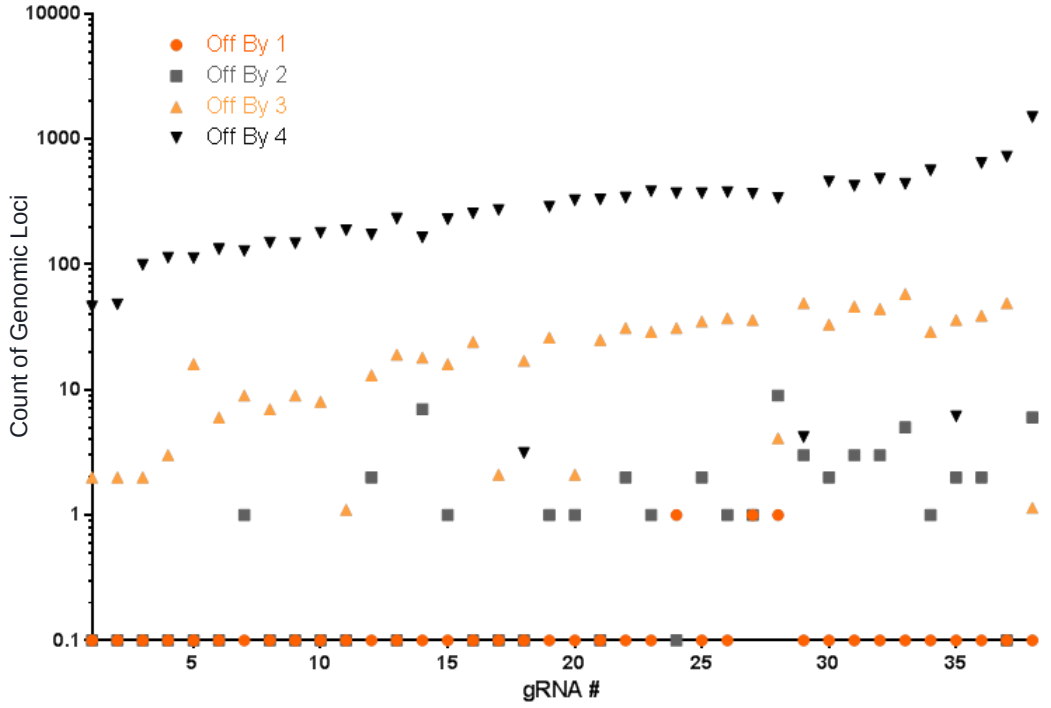
Lead Finding and Specificity to Select gRNA

In silico selection eliminates 50%+ of gRNAs

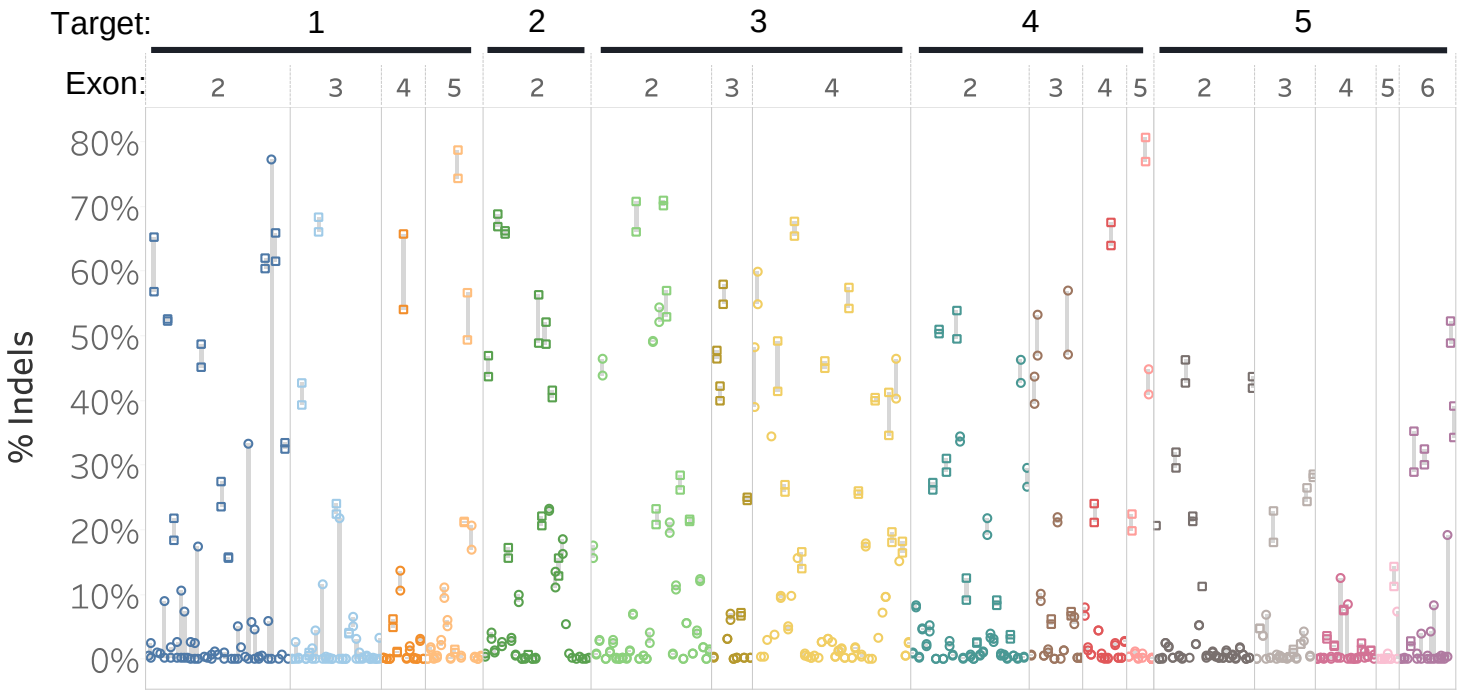
Example *in silico* filtering

Gene	Total gRNAs	Tested gRNAs
1	198	100
2	100	37
3	181	95
4	147	72
5	283	90

In silico Selection



Scale Up Powers Lead Finding & Optimization



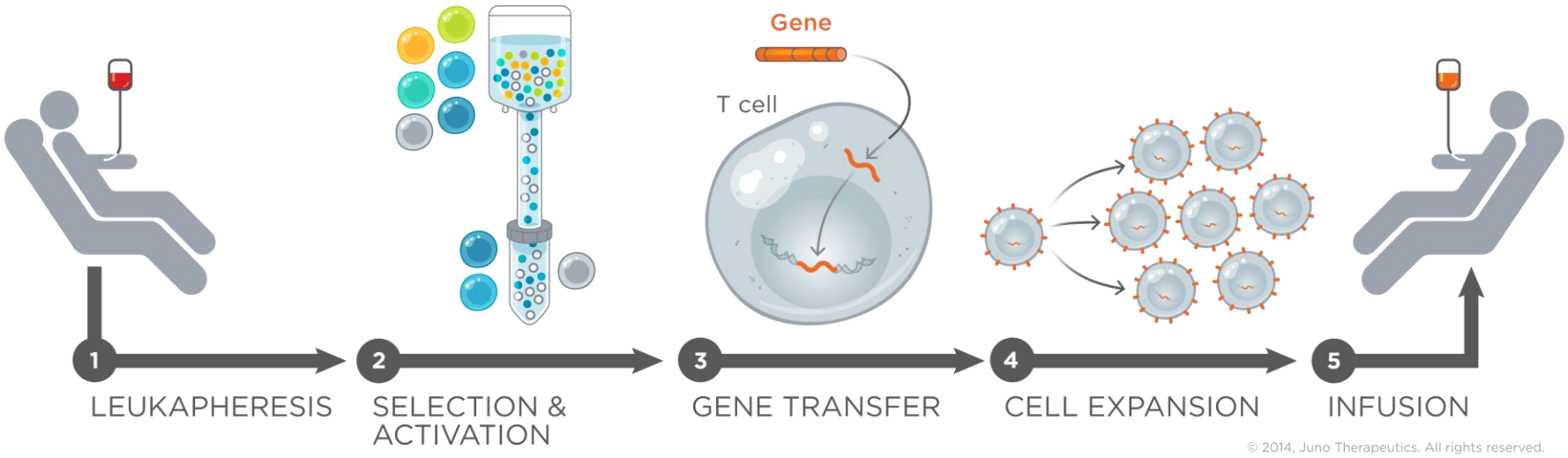
Primary screening of 5 targets with 2 enzymes in primary human T cells demonstrating high activity and reproducibility

- Fully tracked and automated process
- RNP and target agnostic (any variant or enzyme with a sequencing readout)
- Flexible format for single point screening, dose response or any combination

- Performed in primary T cells
- Synthetic gRNAs unhinge initiating G requirement
- Thousands of gRNAs per year

Gene Editing for Next-Gen CARs/TCRs

Juno Therapeutics collaboration expands and accelerates ex vivo products



- CAR and TCR engineered T cell therapies have the potential to be transformative additions to immuno-oncology landscape
- Alliance with Juno seeks to address key goals for engineered T cells
  - Improving T cell persistence
  - Overcoming the tumor microenvironment
- Learnings from Juno collaboration are applicable to any T cell based therapeutic

Gene Editing for Next-Gen CARs/TCRs

Targeting of T cell checkpoint pathways => PD-1

- T cell reactivity against “self” is controlled by a series of checkpoint pathways downstream of cell surface receptors such as PD-1 and CTLA-4.
- In cancer patients, T cells recognize tumor cells that express neo antigens (“foreign”) but are often prevented from being fully activated by the expression of cognate receptors for PD-1 and CTLA-4 on tumor cells.
- Successful immunotherapy targets these interactions in order to “release” the tumor specific T cells.

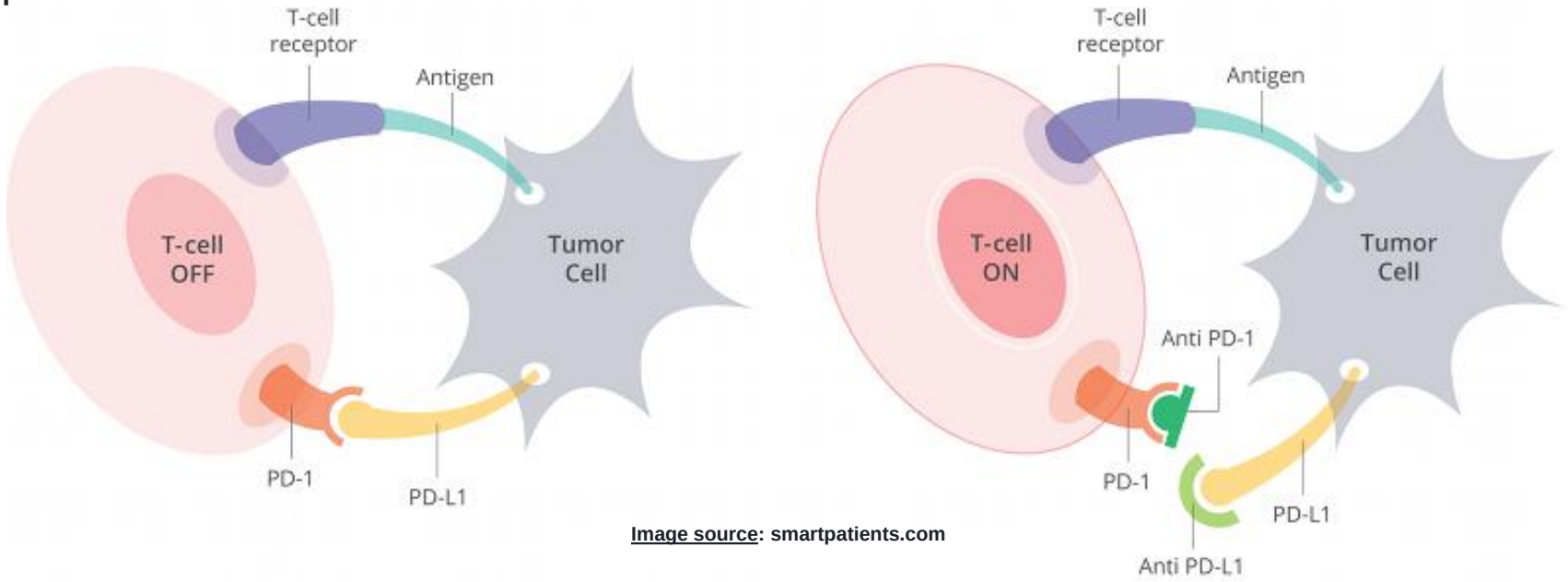


Image source: smartpatients.com

Targeting of T cell checkpoint pathways – PD-1

Can We Target PD-1 in Engineered T cells By Gene Editing?

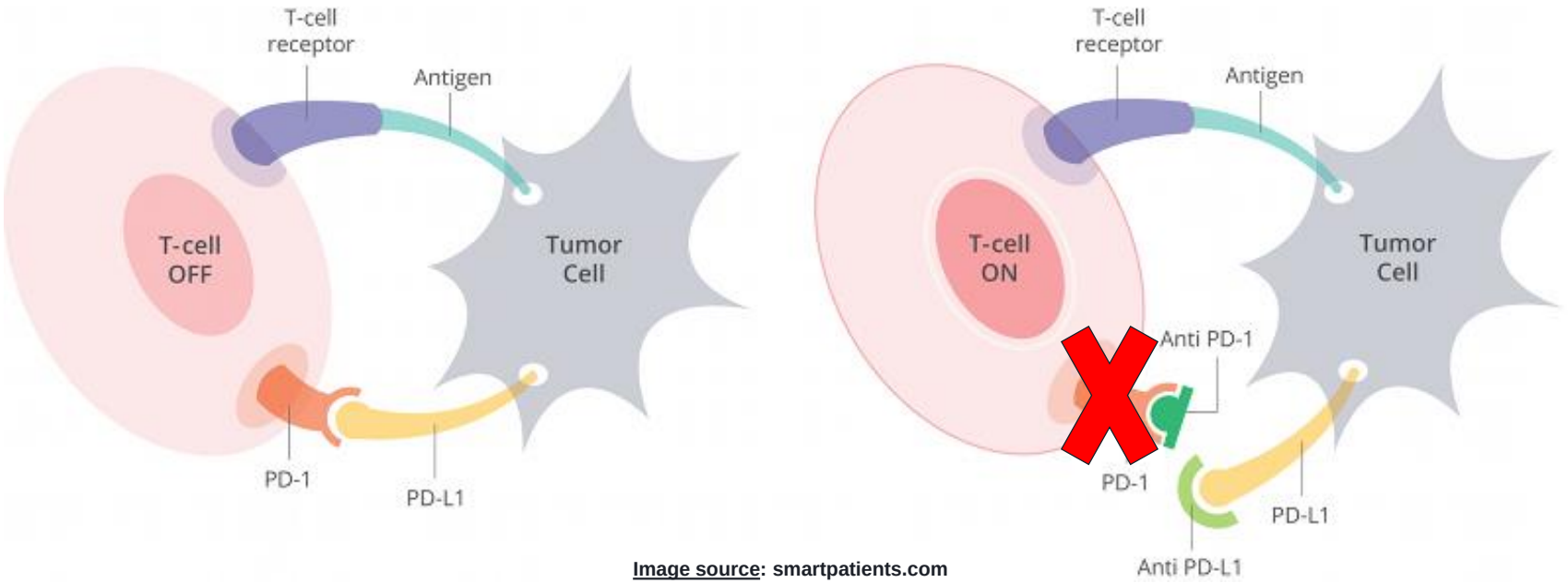
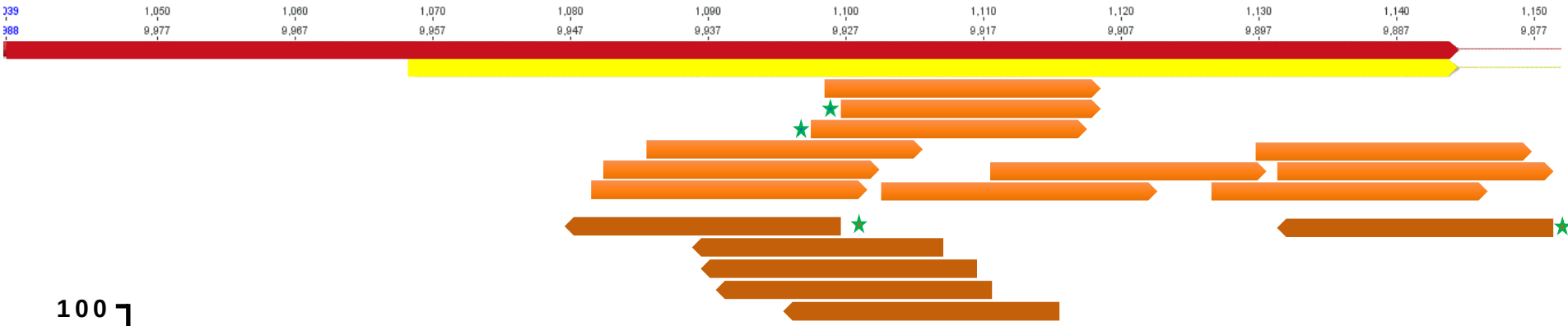
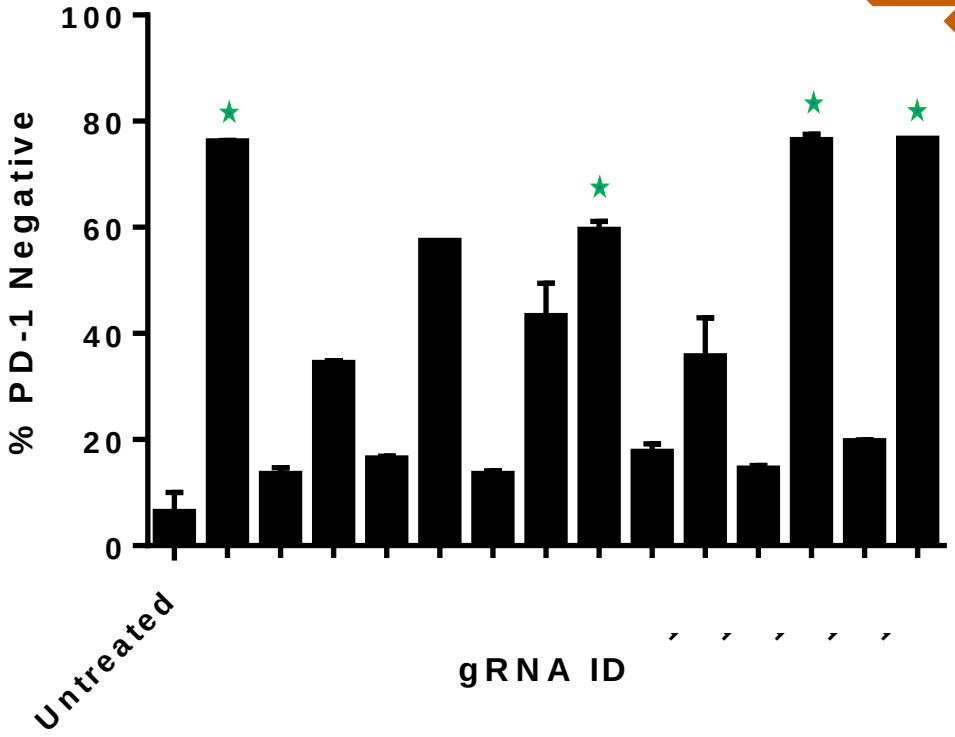


Image source: smartpatients.com

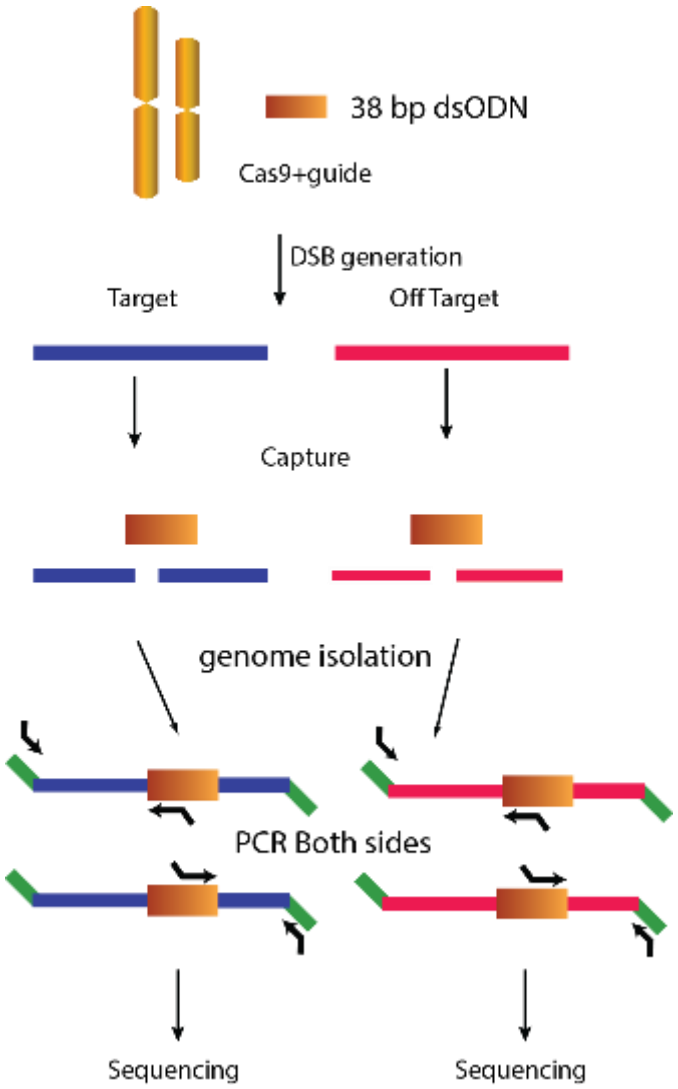
Identification of Robust Guide RNA Leads

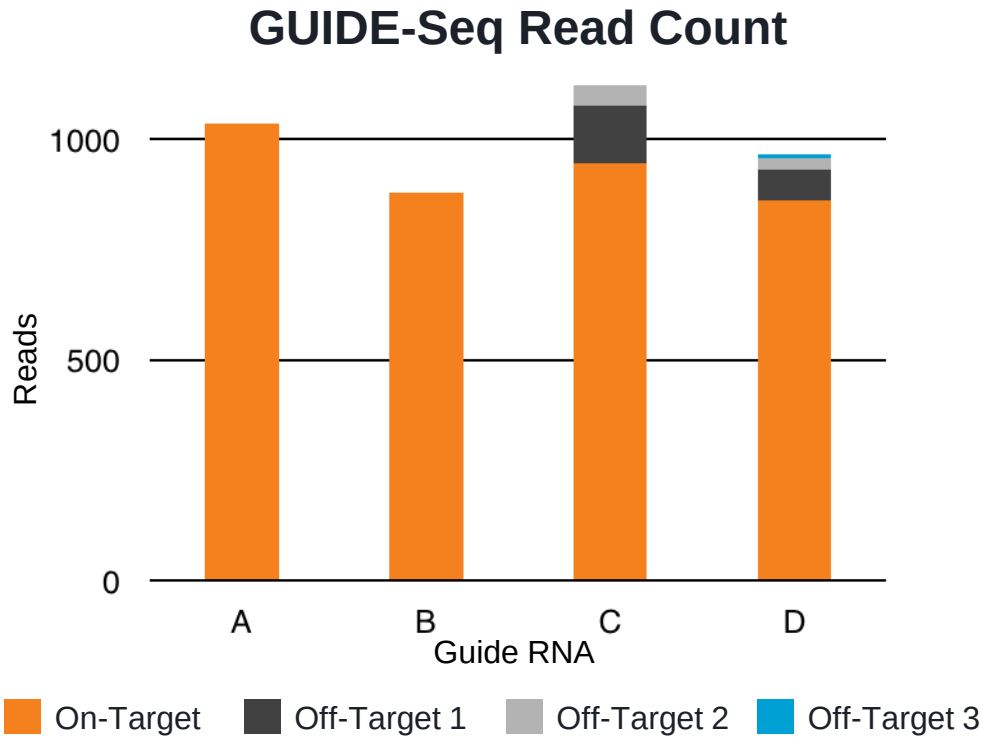




- S. pyogenes Cas9 RNPs in primary human T cells to knock-out PD-1
- Several gRNAs performed well as assessed by FACS
- Sequencing confirmed indels
- gRNAs analyzed by GUIDE-Seq to identify off targets

Control and Specificity to Drive Precision

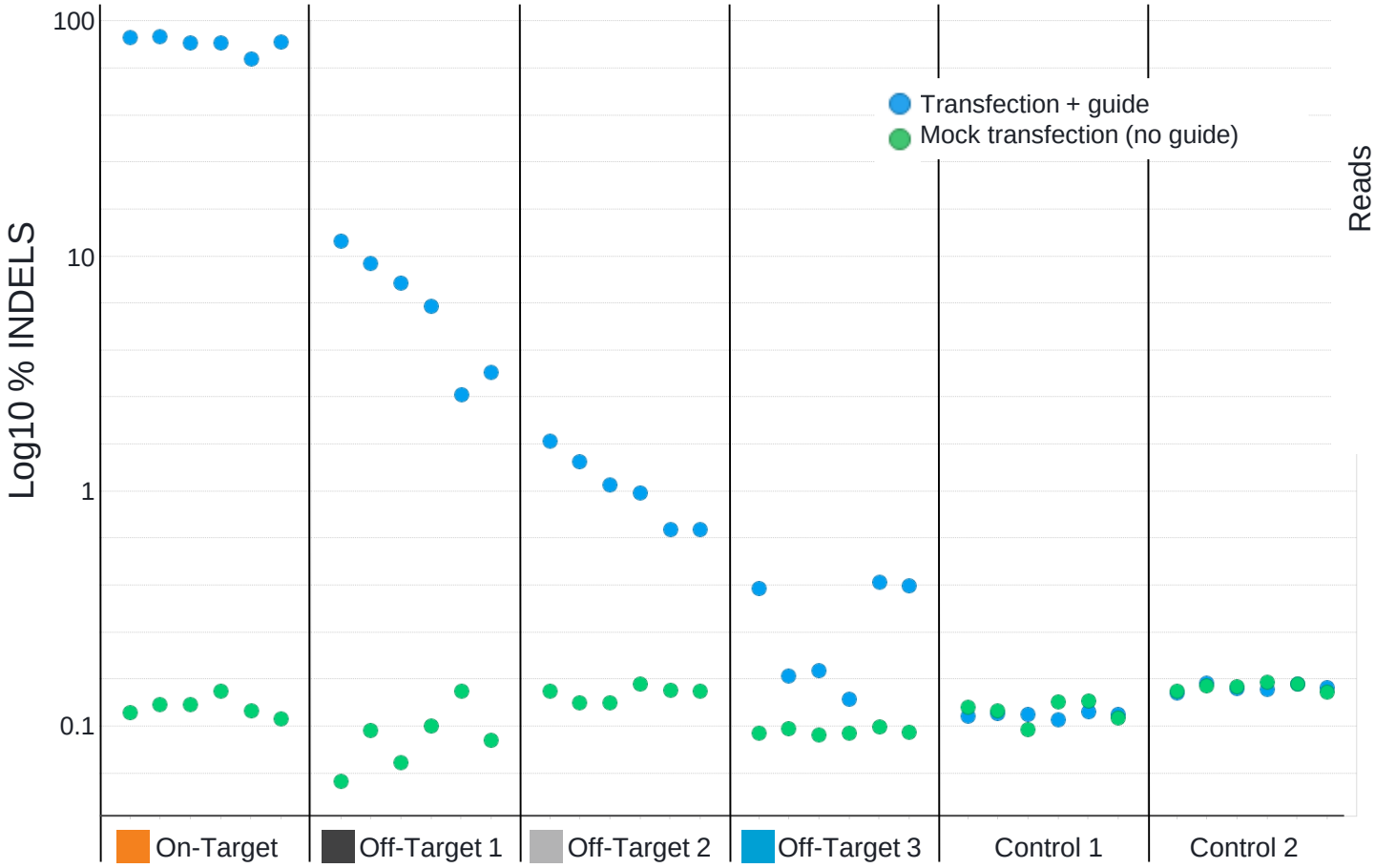


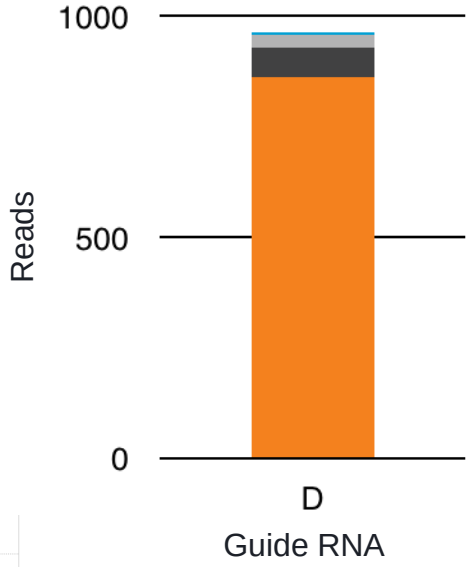


- GUIDE-Seq drives empirical demonstration of selectivity of product candidates
- Off-targets identified by GUIDE-Seq would not be accurately predicted by *in silico* methods alone

GUIDE-Seq Confirmation by Targeted Sequencing

Rank order confirmed





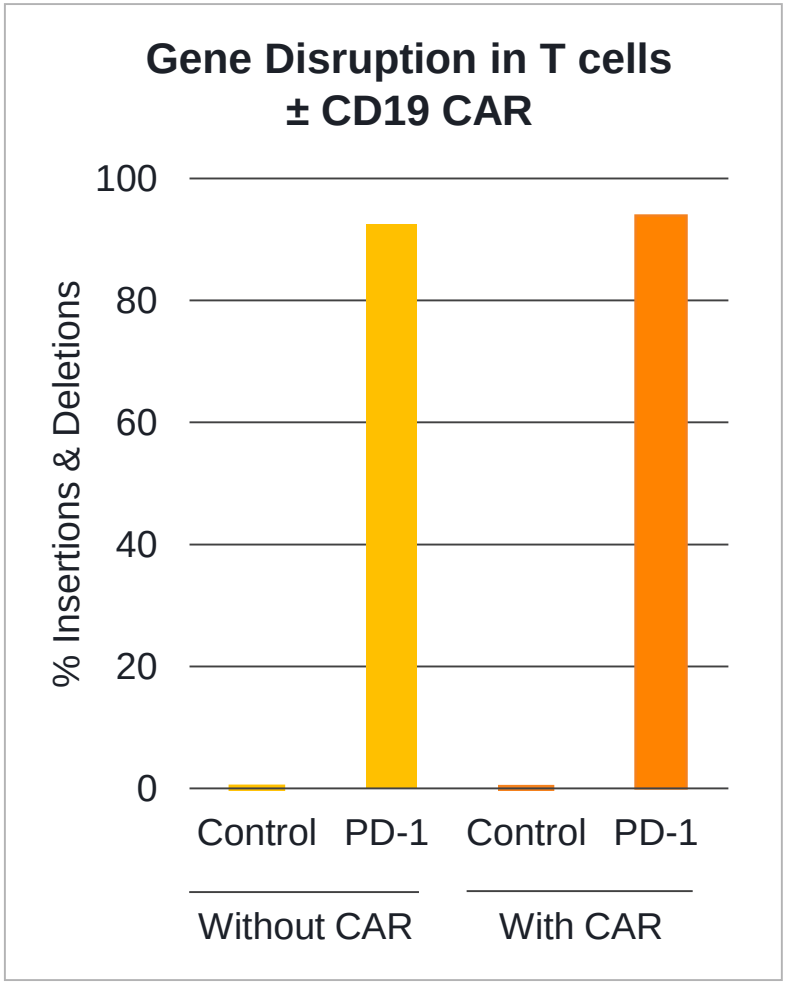
Note: background sensitivity is ~0.15- 0.1%

High Efficiency Editing in CAR-T Cells

Highly efficient gene knockout with >90% PD-1 KO in T cells also carrying Juno CAR

Guides with no detected off-target edits using multiple orthogonal methodologies

Success on many targets and multiplexing both in collaboration and wholly-owned



KO: knock-out or elimination of targeted gene

Summary

Robust guide screening platform in T cells for multiple enzymes

Highly efficient gene knockout for many T cells targets with >90% KO efficiency

Guides with no detected off-target edits using multiple orthogonal methodologies

Building internal manufacturing expertise for multiple ex vivo programs

KO: knock-out or elimination of targeted gene

Thank You