CRISPR-mediated Editing of Hematopoietic Stem Cells for the Treatment of β-Hemoglobinopathies

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American Society of Gene & Cell Therapy
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Highlights

- Developed high-throughput CRISPR-based screening method in human hematopoietic stem cells (HSCs) to identify sites that increase fetal hemoglobin (HbF) protein
- Novel editing approach at β-globin locus supports potent induction of HbF protein in erythroid progeny of healthy adult human HSCs
- CRISPR-edited HSCs engraft efficiently in bone marrow and reconstitute blood production long term *in vivo* in mice

Edited HSCs have the potential to provide a durable therapy for patients with β-hemoglobinopathies

Presentation Overview

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

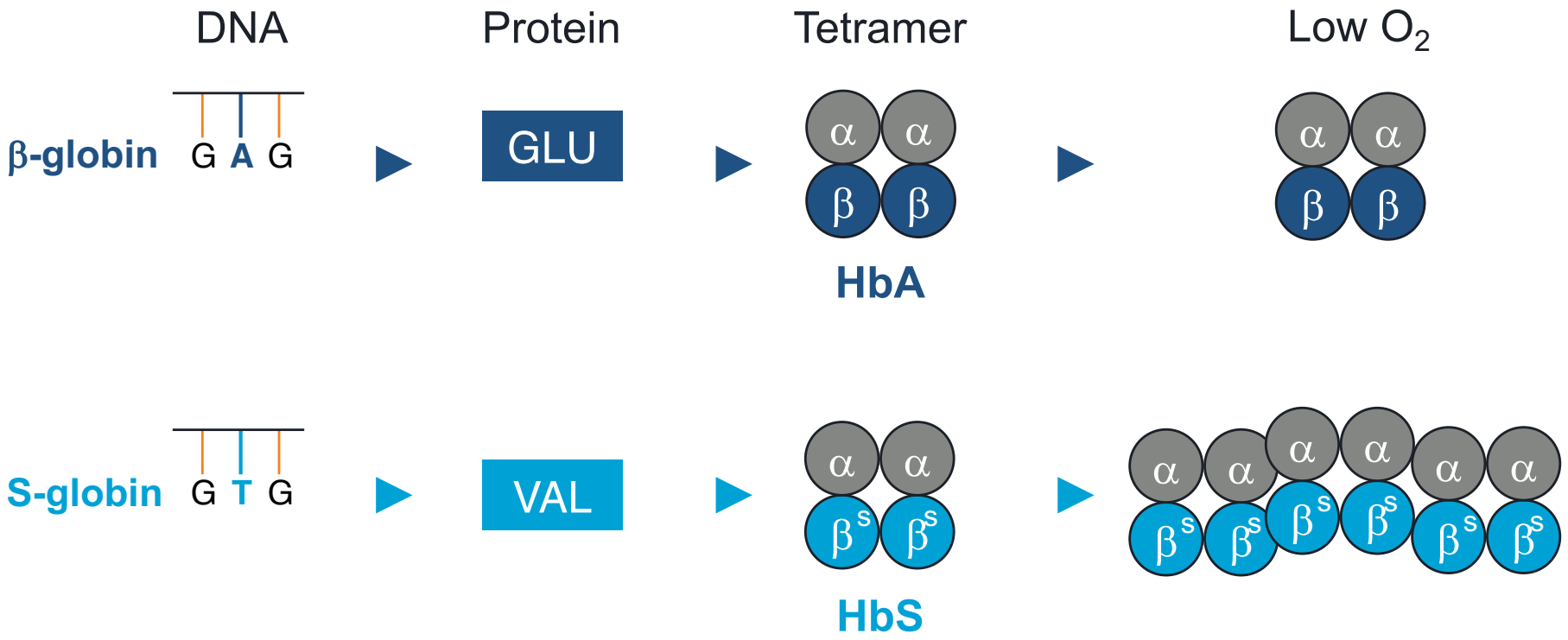
Platform for CRISPR Medicines

Editing of Adult Human HSCs to Increase HbF Protein

Repopulating the Blood System with Edited HSCs

Mutation in β-Hemoglobin Gene Causes Sickling

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy



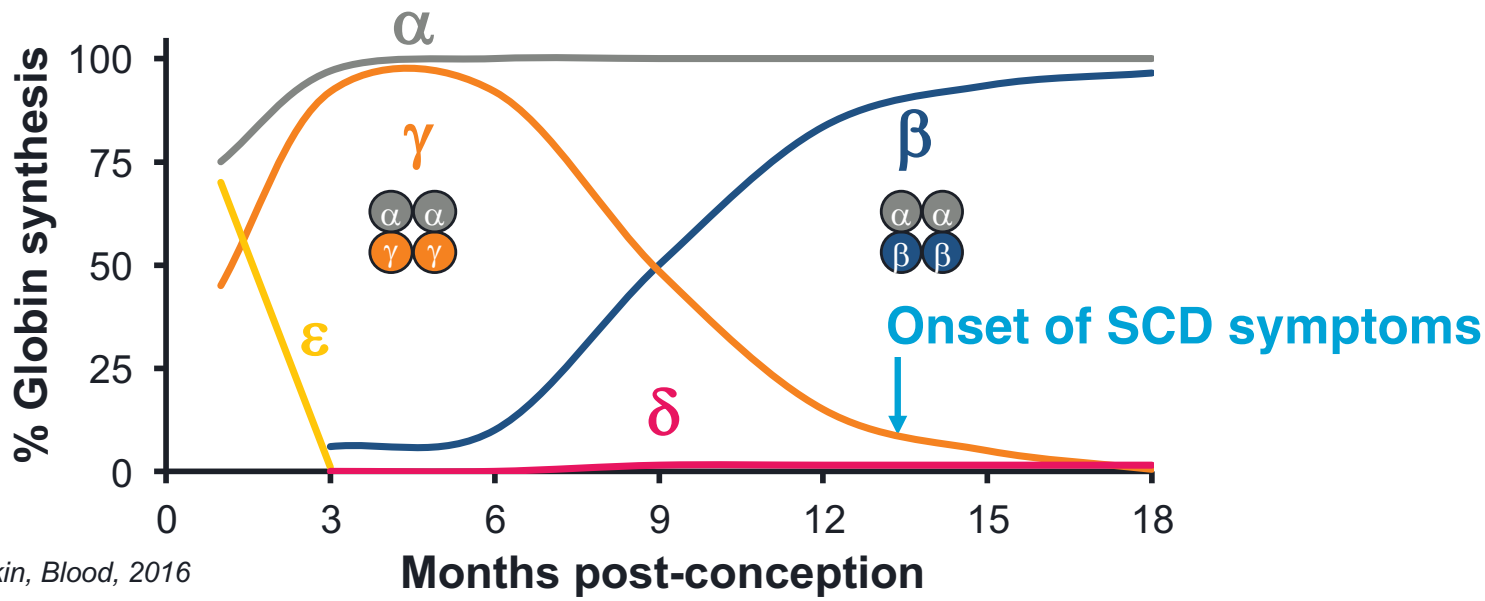
Disease Symptoms and Complications

Vaso-occlusion, pain crises, acute chest syndrome, stroke, embolism, hypertension, organ damage, anemia, premature mortality

Human β-globin Locus: Regulation of Gene Expression

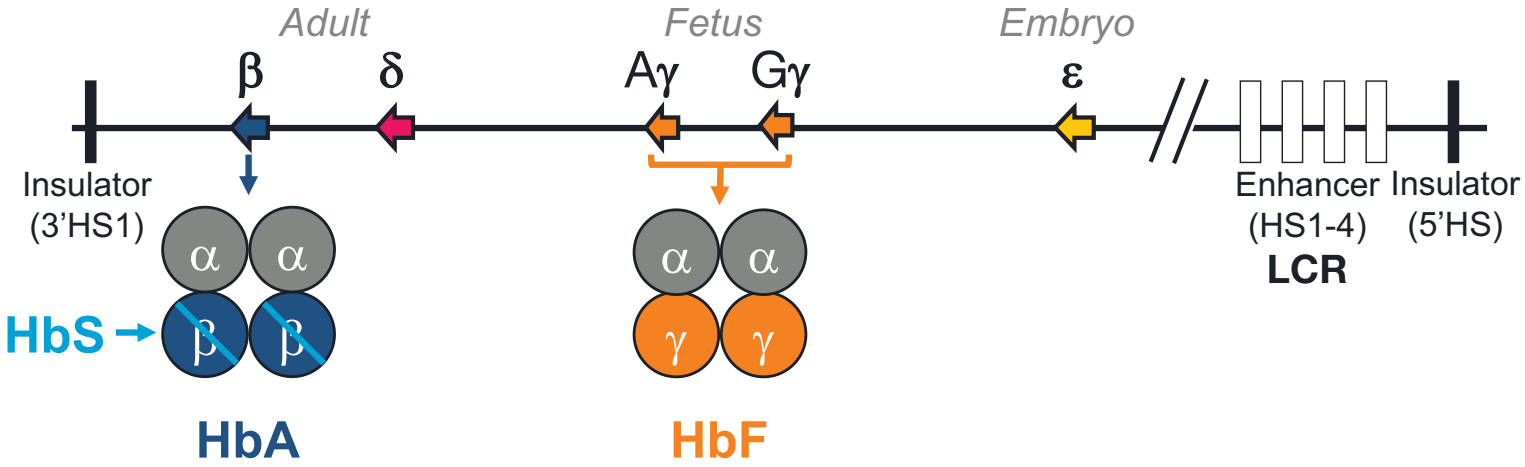
Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

Globin Switch



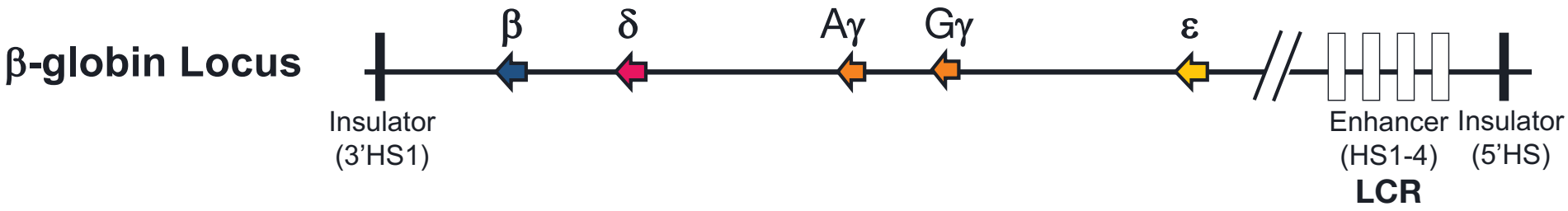
Adapted from Canver and Orkin, Blood, 2016

β-globin Locus

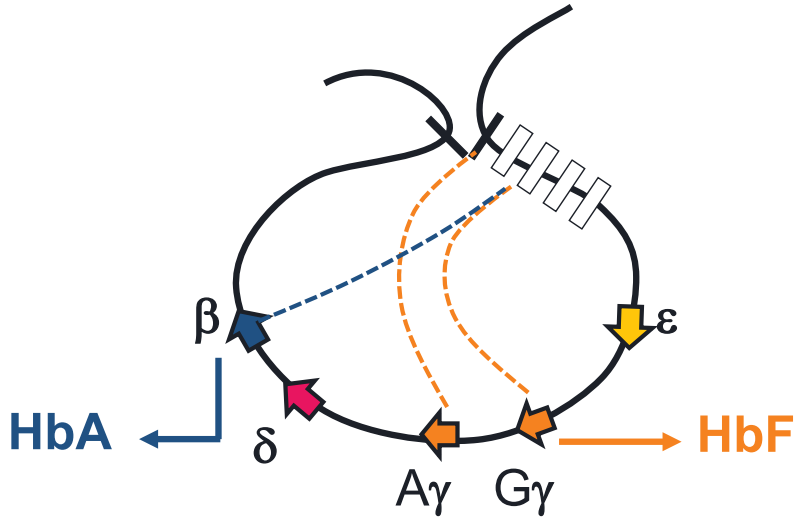


Globin Switching Model is Based on Chromatin Looping and LCR Interaction with Globin Promoters

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy



Chromatin Looping Model of β-globin Gene Transcription



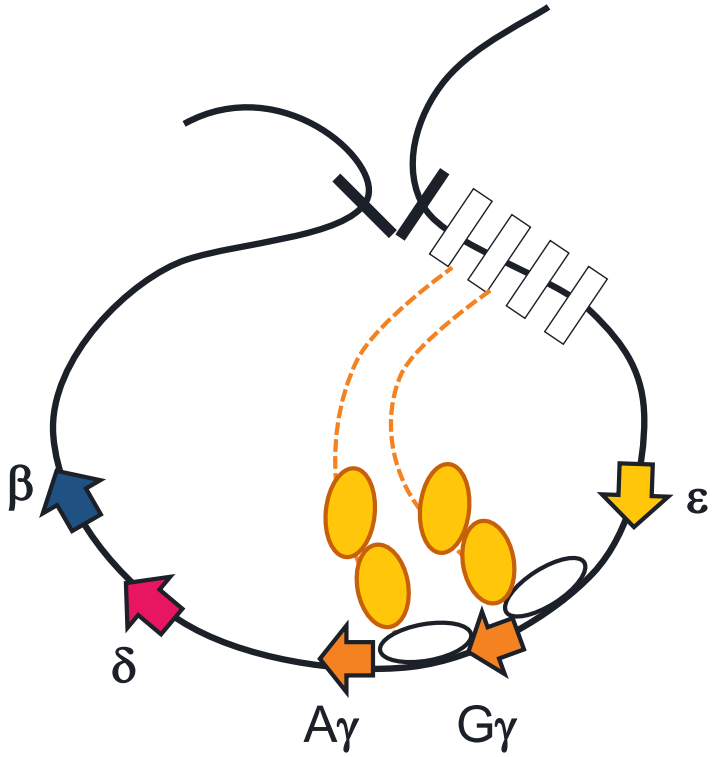
Adapted from Kim & Dean, *Mol Cell,* 2012 and Wilber et al, *Blood*, 2011

Locus control region (LCR) regulates β-like globin genes

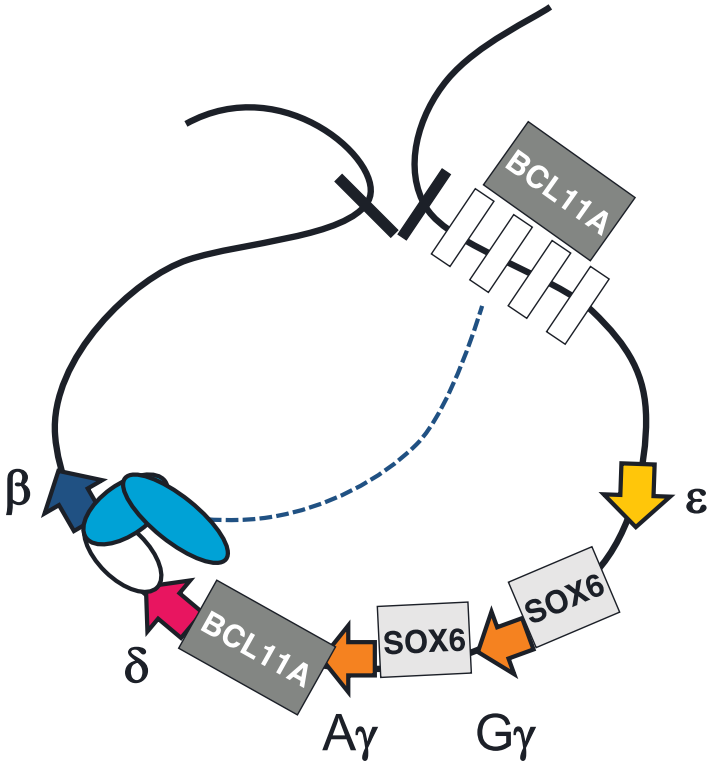
Several Proteins Involved in Globin Switch Regulation

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

Gamma Globin Expression → HbF



Beta Globin Expression → HbA

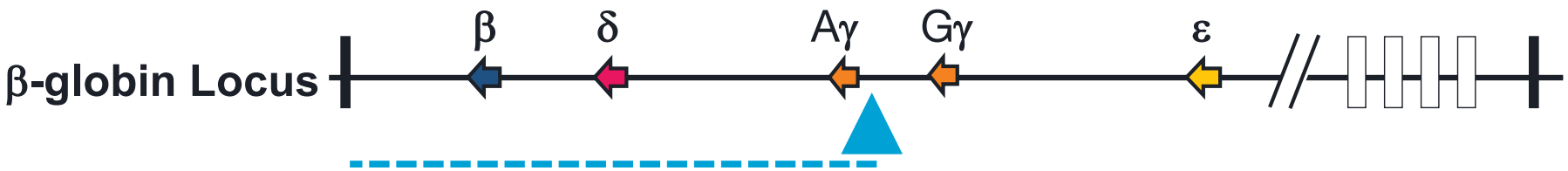


Adapted from Kim & Dean, *Mol Cell,* 2012 and Wilber et al, *Blood*, 2011

Complex process regulated by transcriptional activation and repression (e.g., *BCL11A, SOX6)*

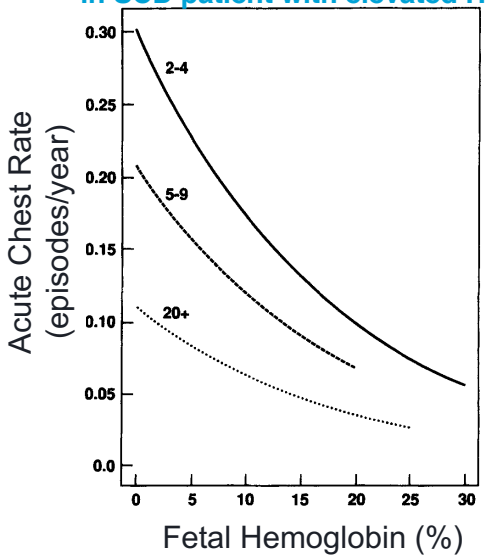
Hereditary Persistence of Fetal Hemoglobin (HPFH): Inherited Mutations that Increase Fetal Hemoglobin

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

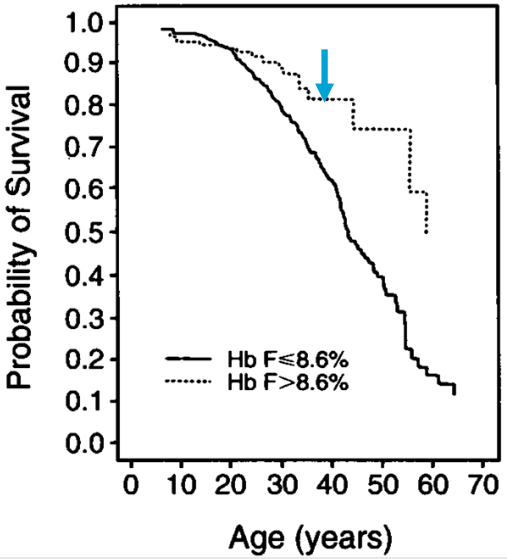


Adapted from Higgs, Engel and Stamatoyannopoulos (Review) Lancet, 2012

Lower Rate of Acute Chest Syndrome† in SCD patient with elevated HbF



Enhanced Survival‡ of SCD patients with HbF > ~8.6%

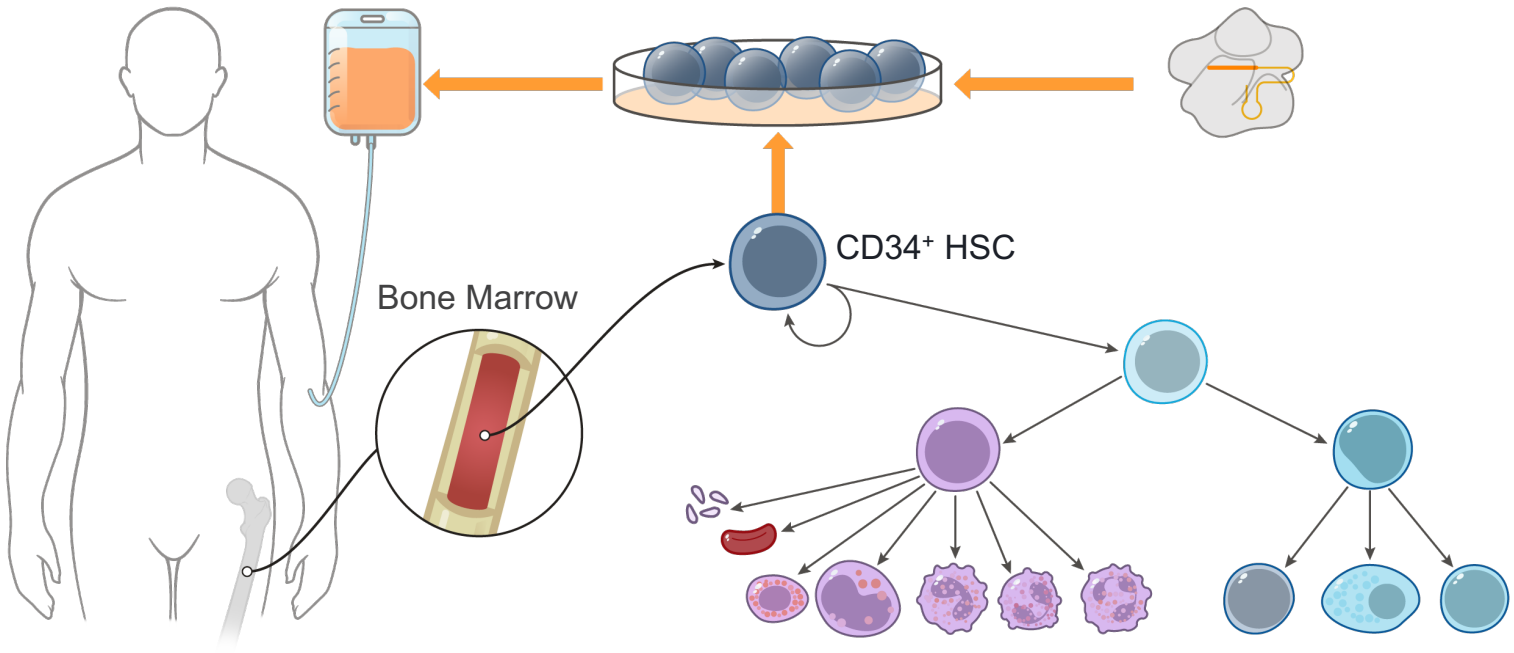


Patients with elevated HbF have reduced disease severity†, enhanced survival‡

†Castro et al., Blood, 1994, ‡Platt et al., *NEJM*, 1994, §Powars et al., *Blood*, 1984

CRISPR Editing to Induce Fetal Hemoglobin

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy



Genome Editing Strategy

Disrupt regulatory elements in β-globin locus that repress HbF

Presentation Overview

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

Platform for CRISPR Medicines

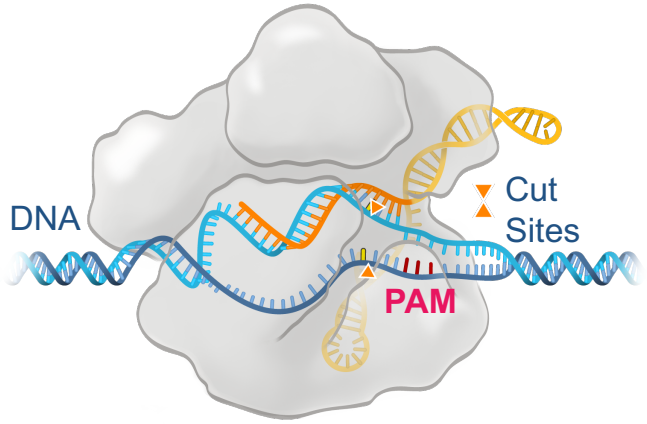
Editing of Adult Human HSCs to Increase HbF Protein

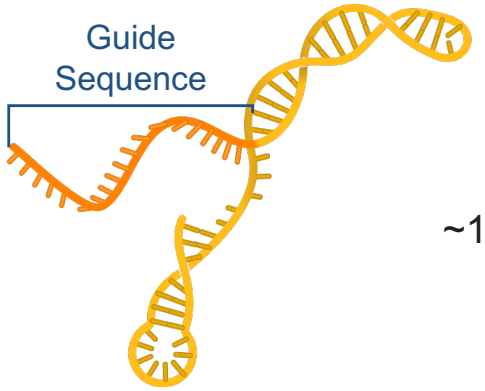
Repopulating the Blood System with Edited HSCs

CRISPR Unlocks Genome Editing

Platform for CRISPR Medicines

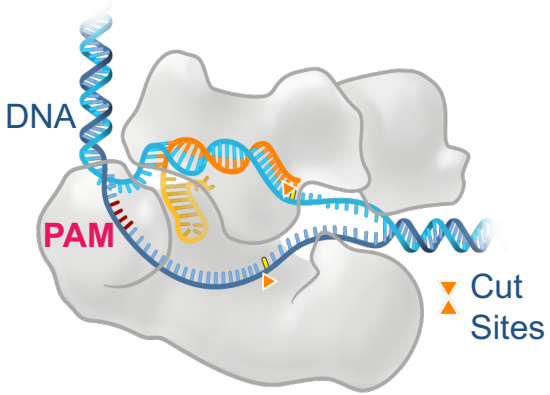
Cas9

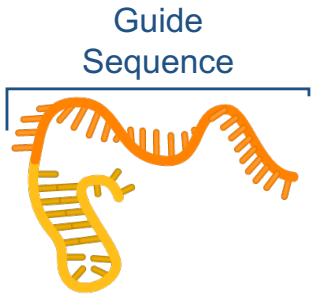




~100 nt

Cpf1





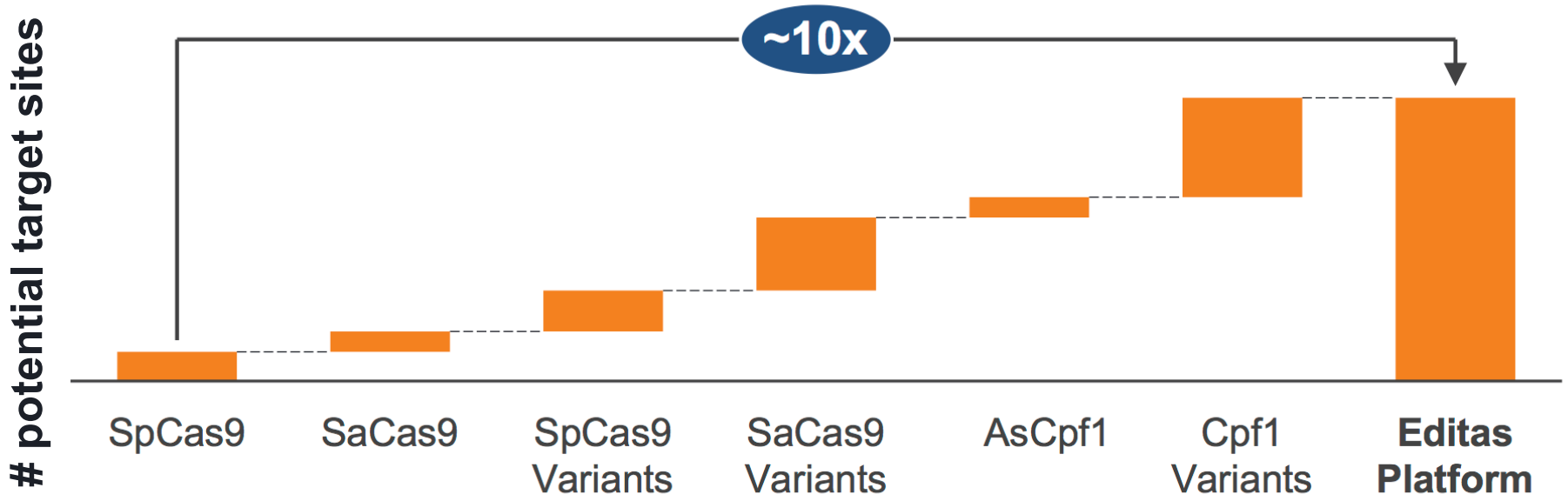
42 nt

Nuclease **Guide RNA**

Editing machinery can be engineered to target many genomic locations

Unparalleled Platforms for CRISPR Medicines

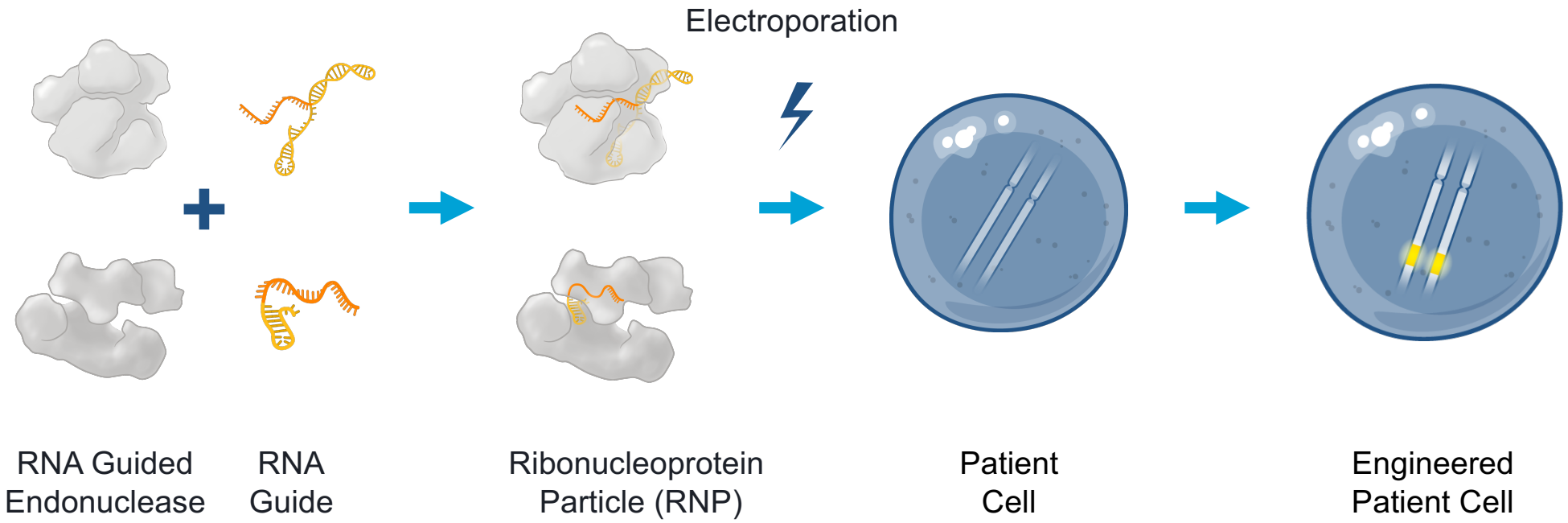
Platform for CRISPR Medicines



Access to Cas9 and Cpf1 systems, species, and variants supports targeting of sites that are hard to target with most commonly used version of Cas9

Scalable, Consistent Engineered Cell Therapies

Platform for CRISPR Medicines

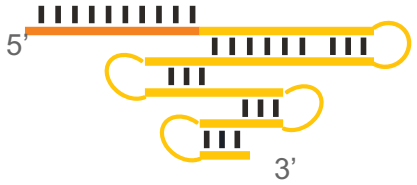


Engineer multiple components to HSC sensitivity (maintain cell viability, potency)

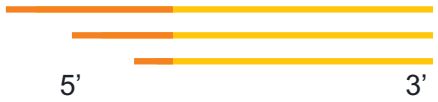
Covalently-Coupled Dual gRNA Create Opportunities

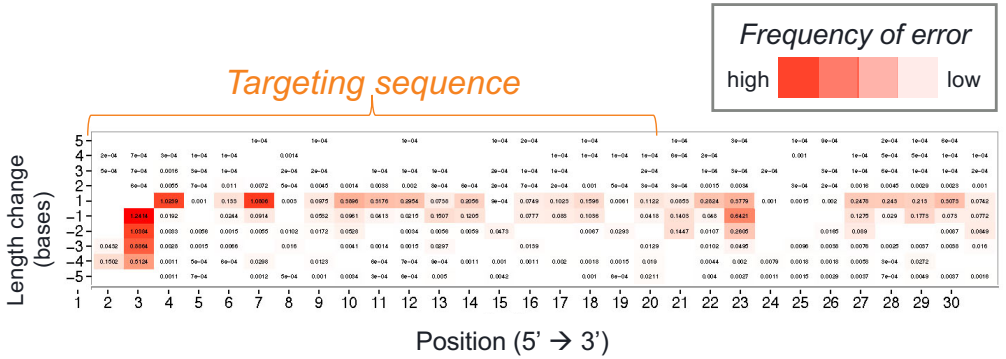
Platform for CRISPR Medicines

Single gRNA

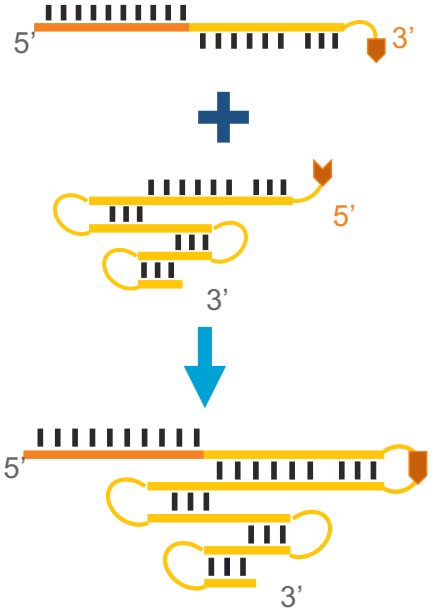


Heterogeneous product
(full-length, truncated, errors)

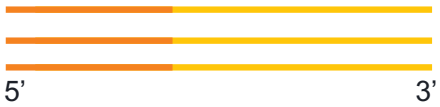


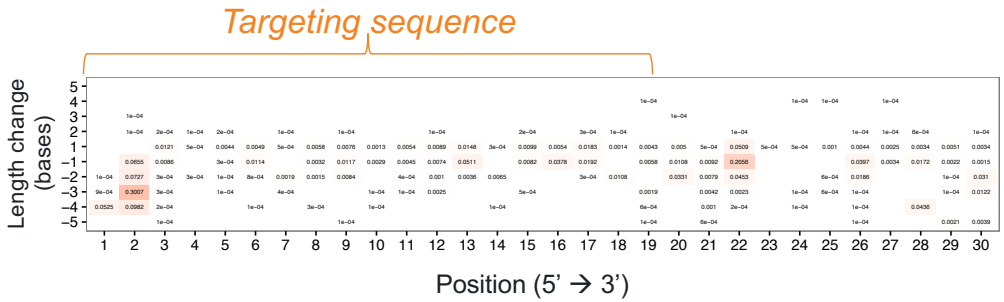


Covalently-Coupled Dual gRNA (dgRNA)



Well-defined product
(full-length only)



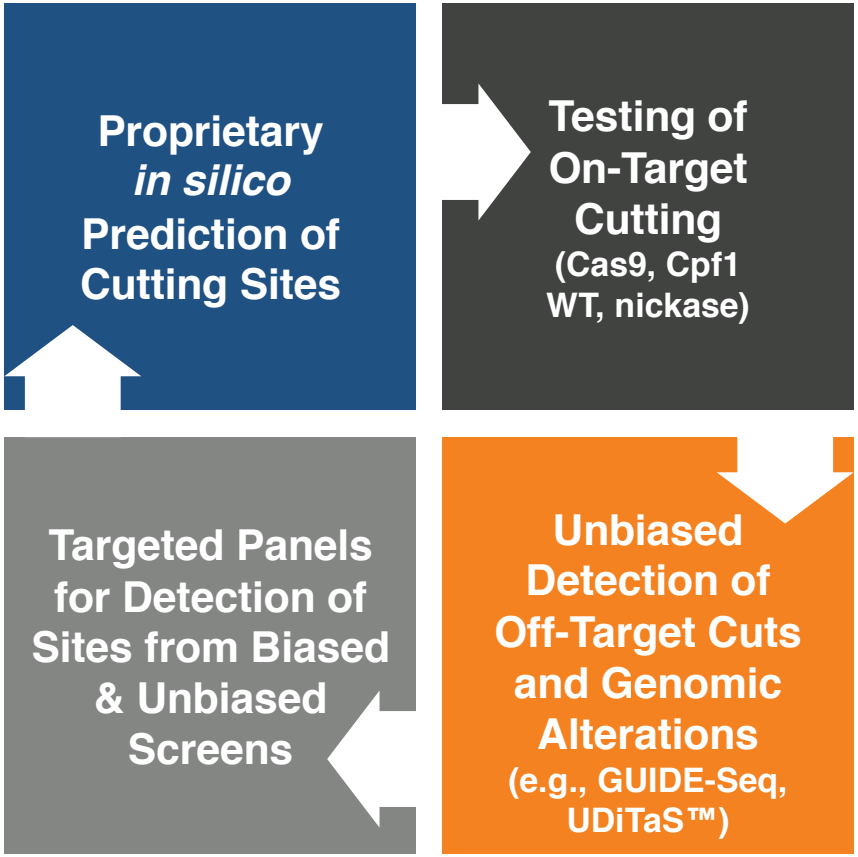


Increased flexibility to positional and end modifications

Fidelity, scale, and purity are potentially superior for making medicines

Orthogonal Specificity Approaches for Best gRNAs

Platform for CRISPR Medicines



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

Presentation Overview

Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy

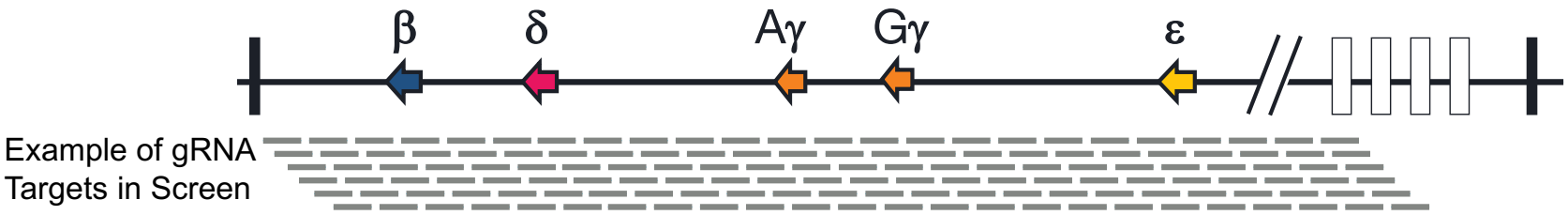
Platform for CRISPR Medicines

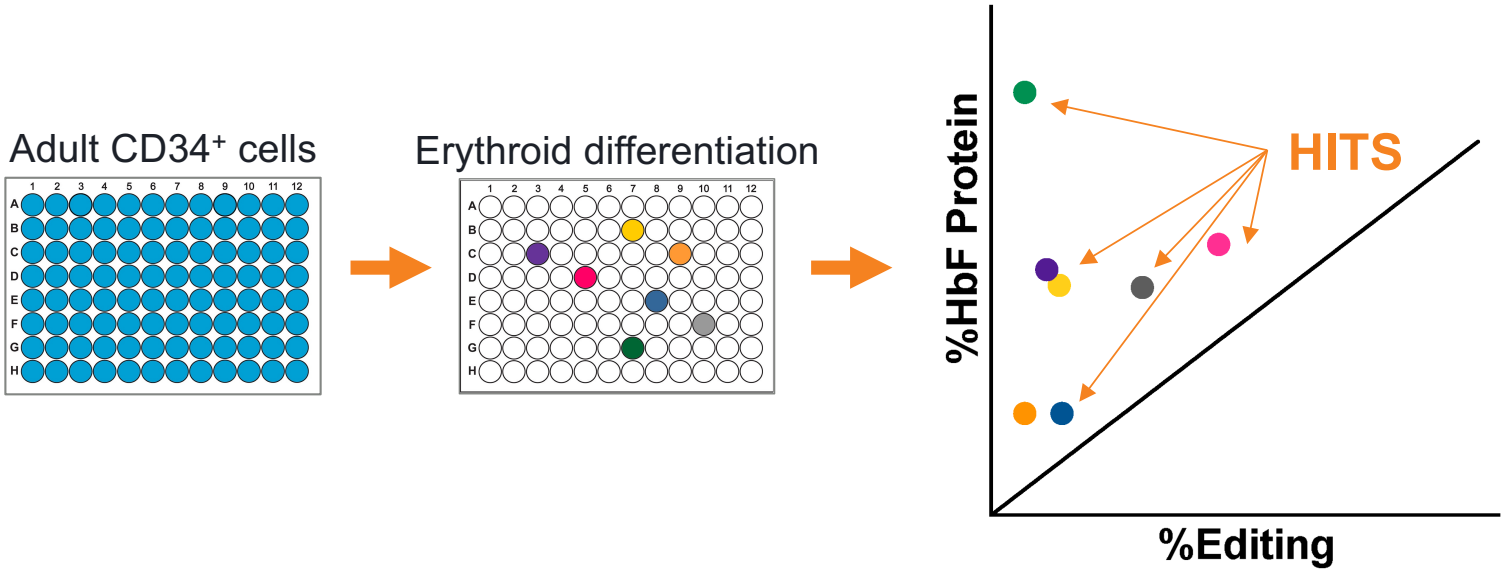
Editing of Adult Human HSCs to Increase HbF Protein

Repopulating the Blood System with Edited HSCs

Guide Screening in CD34+ Cells Supports Target Identification Based on HbF Protein Induction

Editing of Adult Human HSCs to Increase HbF



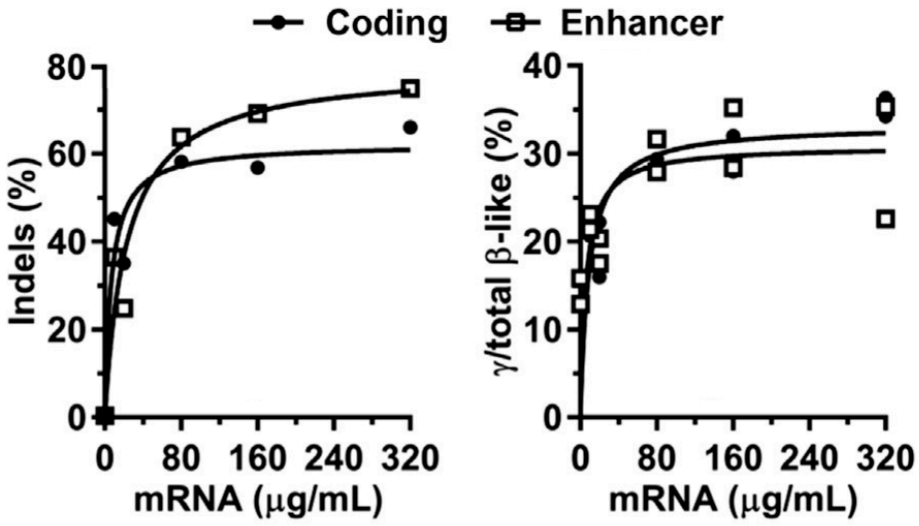


Hits with more potent HbF/edit ratio advance to optimization and validation studies

Published Results Show Potential of *BCL11A* Editing

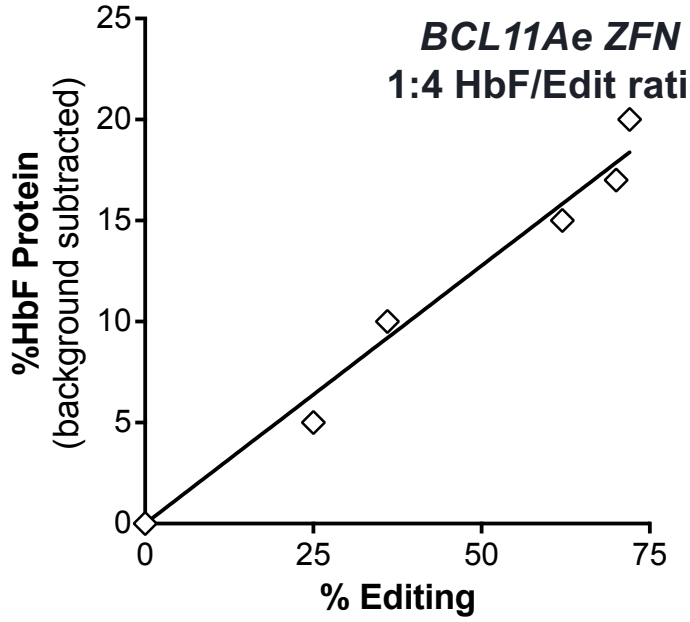
Editing of Adult Human HSCs to Increase HbF

BCL11A ZFN mRNA dose response: Editing and HbF induction



Adapted from Chang et al., Molecular Therapy, 2016

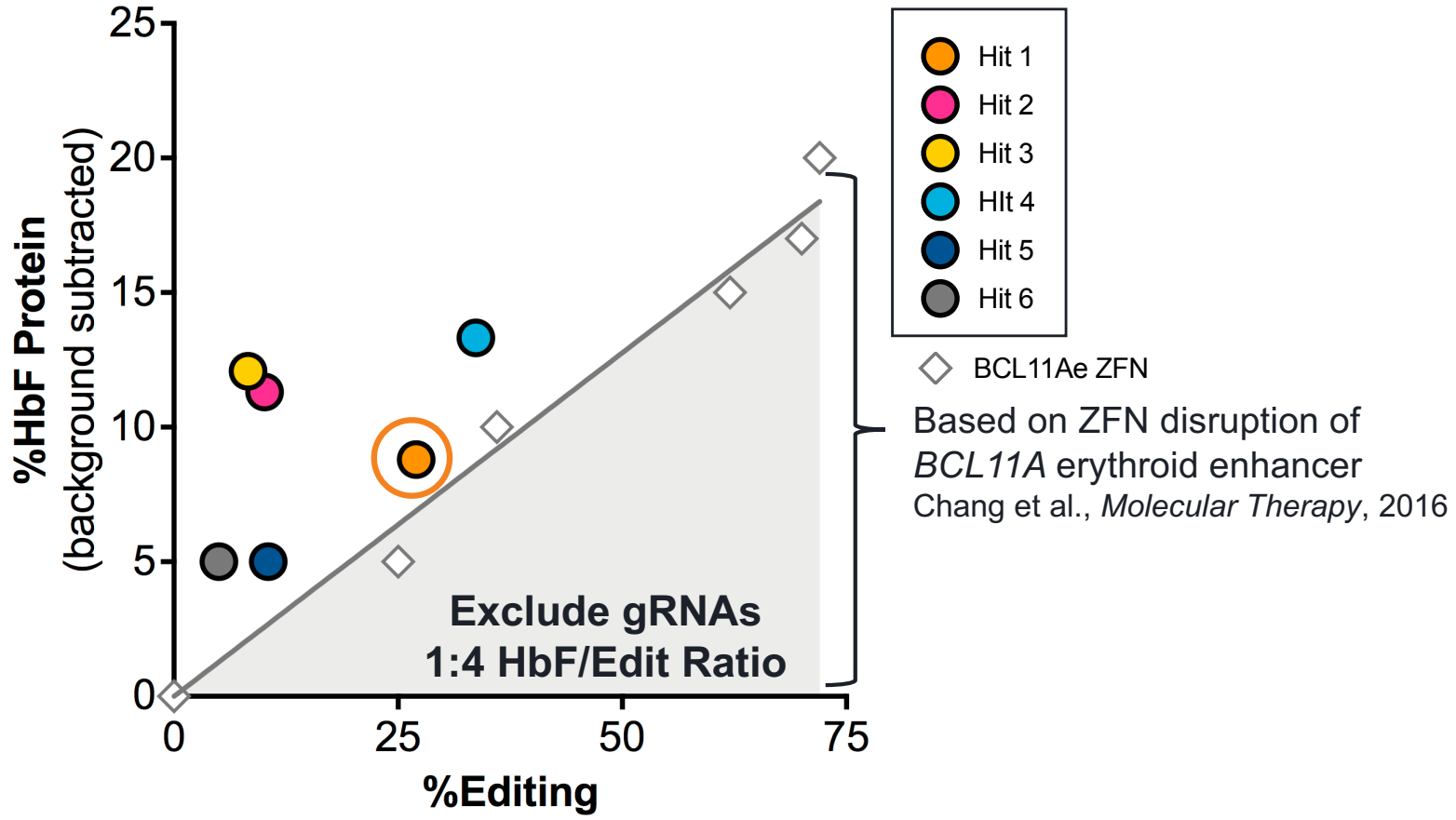
Estimation of correlation between HbF and editing provides benchmark



ZFN editing of *BCL11A* erythroid enhancer yields HbF induction with 1:4 HbF/edit ratio

Screening gRNAs in CD34+ Cells Identifies Potent Hits

Editing of Adult Human HSCs to Increase HbF

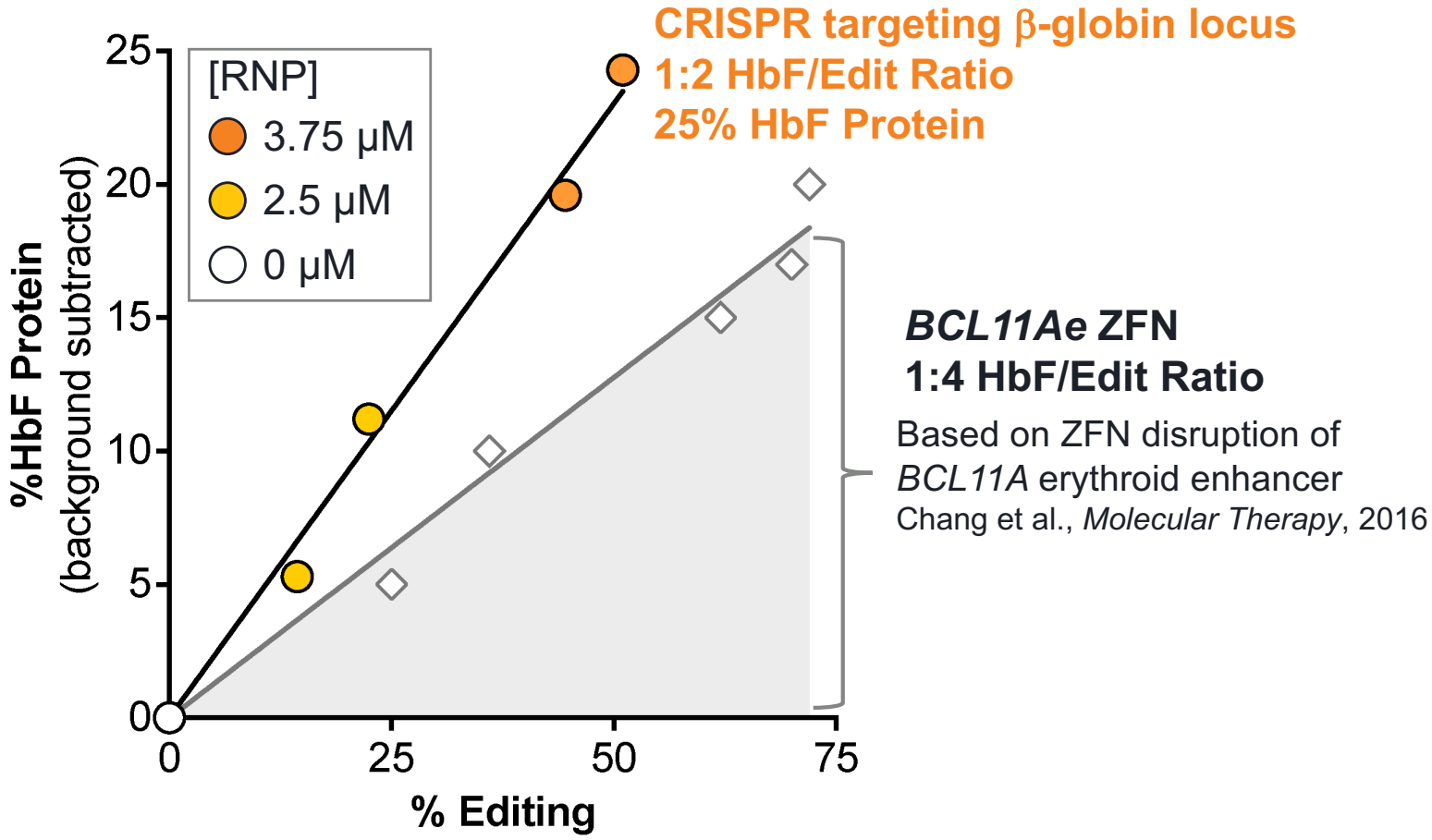


Hits with more potent HbF/edit ratio advance to optimization and validation studies

† HbF Protein Analysis by HPLC = Fetal hemoglobin /Total β–like hemoglobin
Background subtraction - For edited samples, HbF levels detected in donor matched untreated controls were subtracted

Potent HbF Protein Induction by New Editing Approach

Editing of Adult Human HSCs to Increase HbF



Low-dose RNP editing increases HbF protein to ~25% in erythroid progeny of healthy HSCs

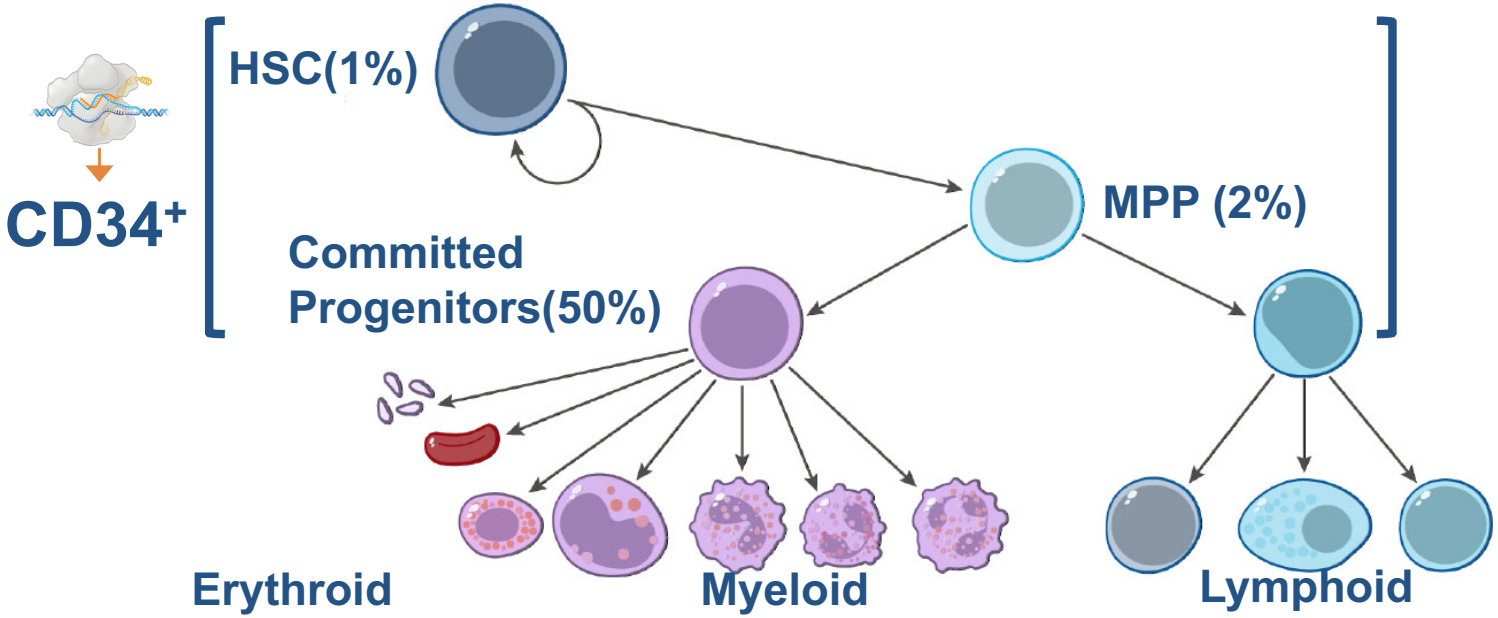
0, 2.5, 3.75 µM RNP tested, 2 different HSC donors

Presentation Overview

- Sickle Cell Disease, β-globin Gene Regulation, and Editing Strategy
- Platform for CRISPR Medicines
- Editing of Adult Human HSCs to Increase HbF Protein
- **Repopulating the Blood System with Edited HSCs**

Long-term Engrafted Hematopoietic Stem Cell Analysis is Required for Target Validation

Repopulating the Blood System with Edited HSCs

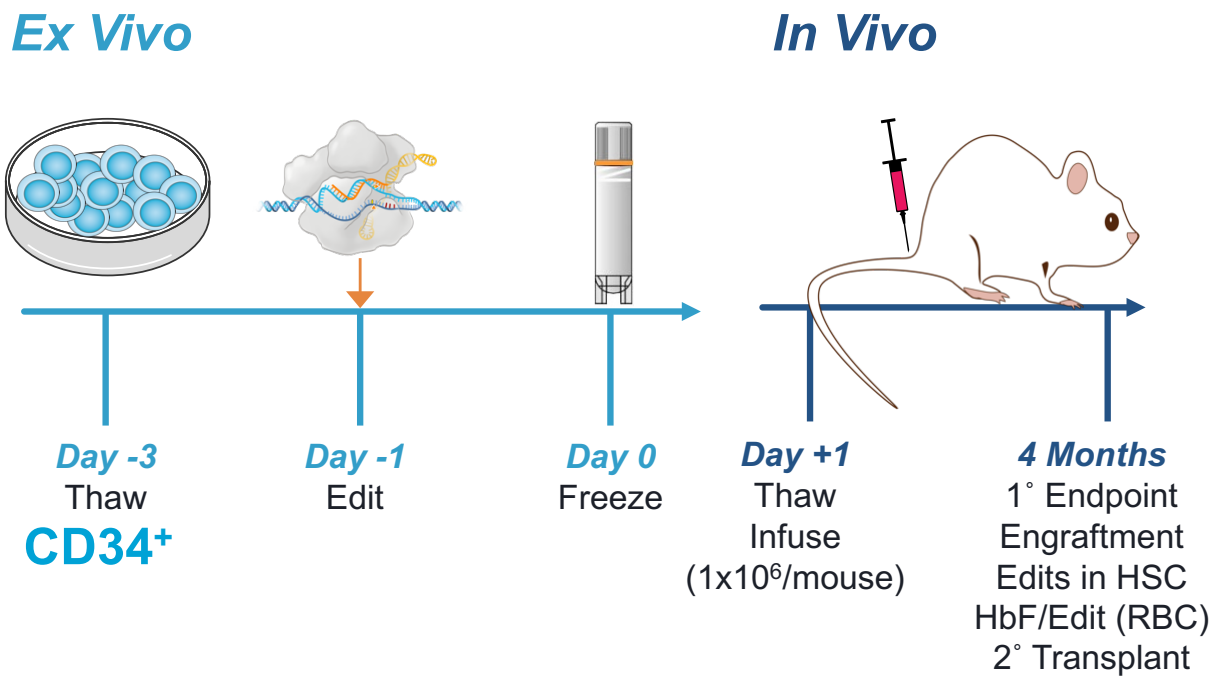


<1% of bulk CD34+ cells are true HSCs (self-renewing and multipotent)

Edits in bulk CD34+ cells may not represent edits in long-term functional HSCs

Long-Term Engraftment Analysis of Edited Human Hematopoietic Stem Cells

Repopulating the Blood System with Edited HSCs



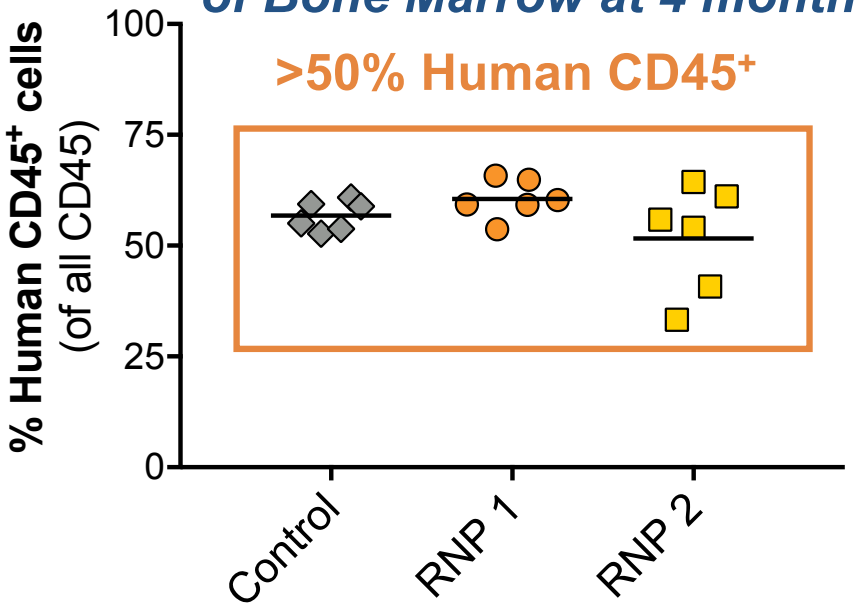
Analyze long-term reconstitution of human hematopoiesis *in vivo* (4+ months)

Evaluate editing in marrow, spleen, and blood (human subsets)

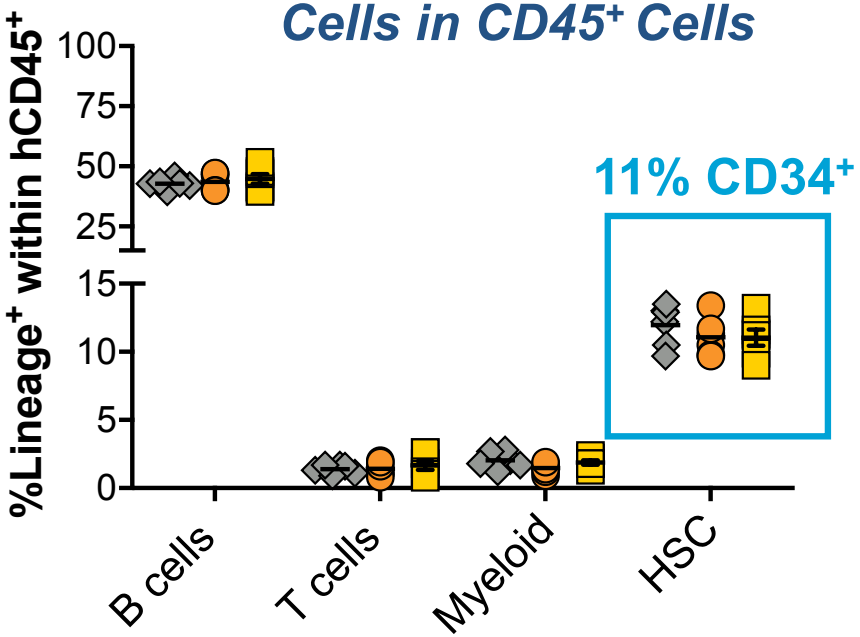
Adult Human Hematopoietic Stem Cells Edited at β-Globin Locus Repopulate Bone Marrow

Repopulating the Blood System with Edited HSCs

Human Blood Cell Repopulation of Bone Marrow at 4 months



Human HSCs, Lymphoid, Myeloid Cells in CD45+ Cells



Edited LT-HSCs engraft, maintain multipotency (4 months post-transplant)

Do multiple edited HSCs contribute hematopoiesis in vivo?

- Use indel diversity as a marker for HSC polyclonality

Lineage Tracking of HSC Progeny *in vivo*

Repopulating the Blood System with Edited HSCs

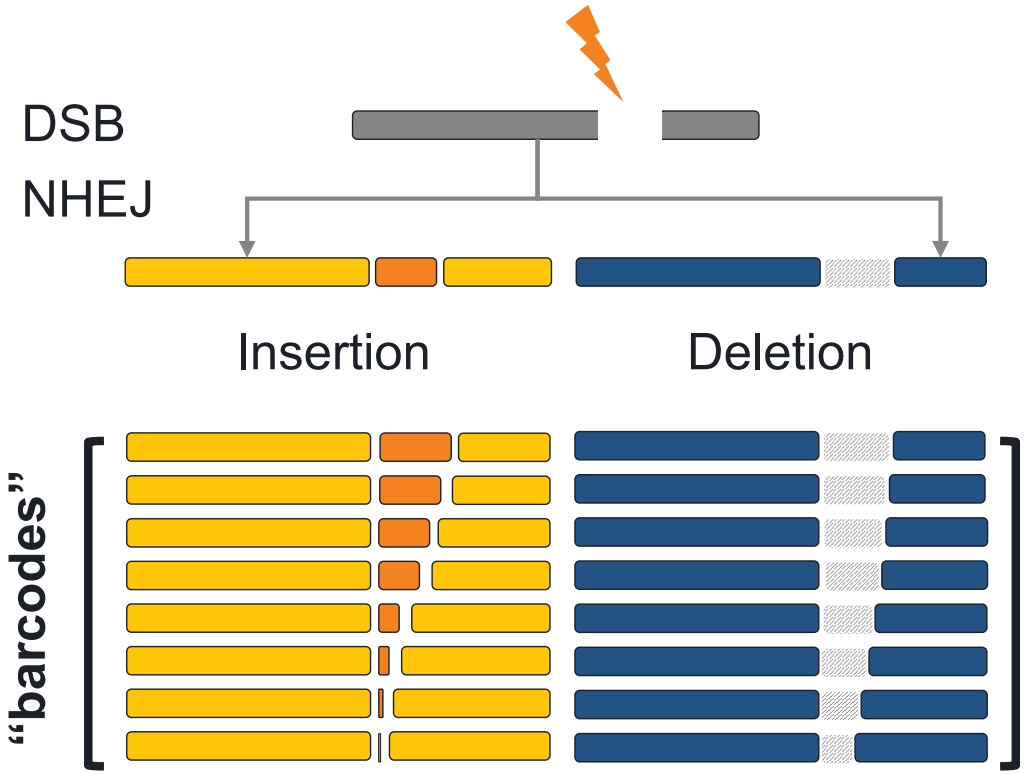
- Gene therapy results in multiple sites that support tracking HSC contribution based on the unique and common integration sites detected

- Gene editing should only result in one site modified

Unique alleles that occur from differences in DNA repair at the target site are used to survey for edited HSC diversity and differentiation potential

Tracking Hematopoiesis Based on Edited Alleles

Repopulating the Blood System with Edited HSCs



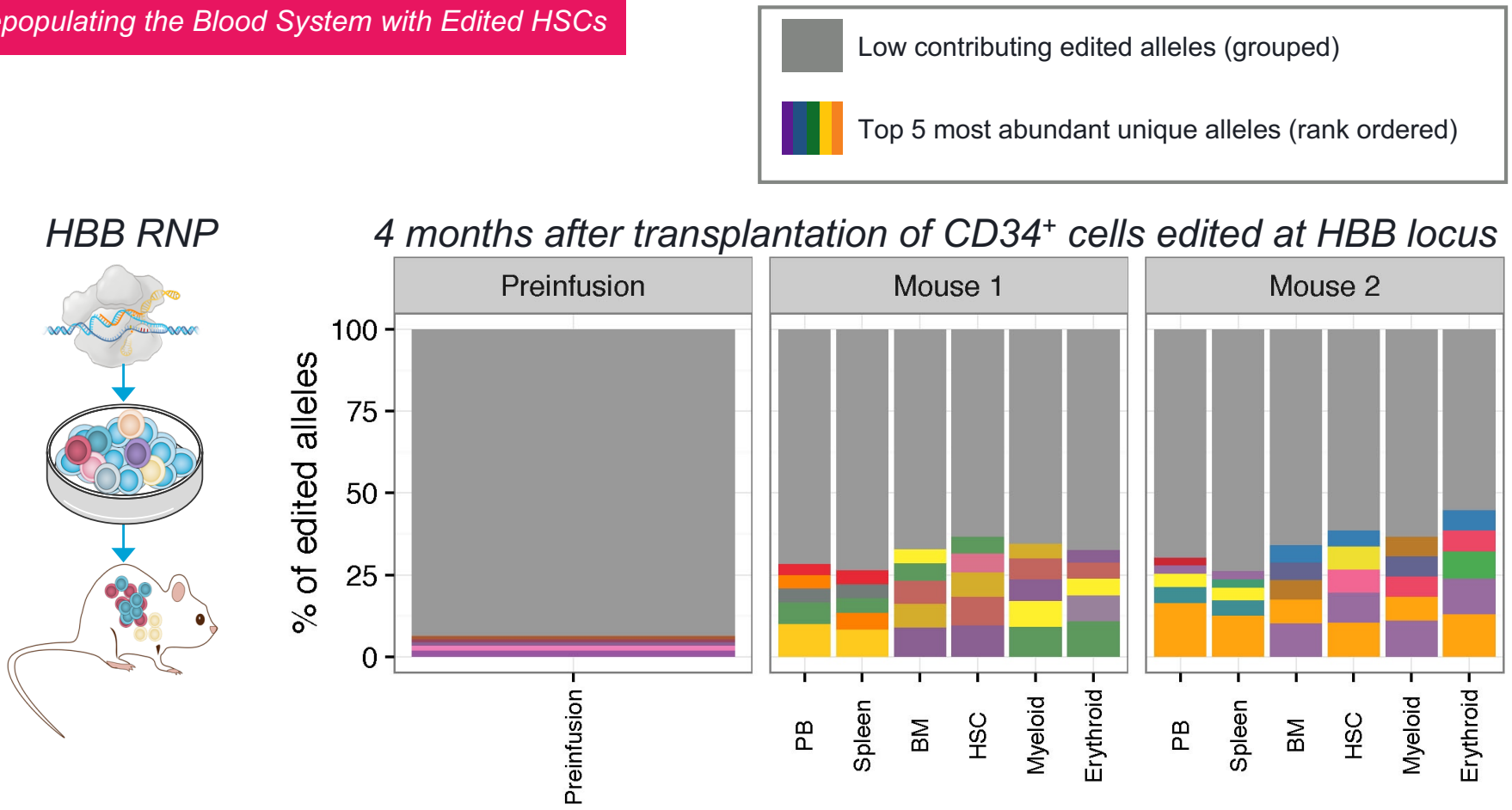
Each unique edit provides barcode based on indel characteristics and position

Multiple unique indels in HSCs/progeny suggests **edited HSC diversity maintained**

Polyclonal Hematopoiesis from CRISPR-Edited HSCs

Evaluating CRISPR-edited HSC polyclonality: *HBB* as model locus for method development

Repopulating the Blood System with Edited HSCs



Multiple edited HSCs are contributing to blood lineages with no dominant edited allele detected

Summary and Conclusions

- **Developed a high throughput screening platform in HSCs** to evaluate efficiency and potency of CRISPR nucleases and gRNAs in HSCs targeting HbF protein induction
- **Identified potent hits that increase HbF protein ~25%** in erythroid progeny of healthy donor $CD34^+$ cells treated with low dose RNP
- **Edited $CD34^+$ cells reconstitute hematopoiesis** *in vivo* and engraft long-term **(>50% chimerism**)

CRISPR-edited HSCs for the treatment of β-hemoglobinopathies have potential to provide superior clinical benefit to patients