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Genome Editing In the Clinic

Fyodor D. Urnov, PhD

Project Leader and Senior Scientist,
Sangamo BioSciences, Inc.

NAS International Summit on Human Gene Editing

December 3, 2015

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Genome editing has expanded the definition of the term **“druggable target”**:

If it’s in the DNA, it’s a druggable target

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Bedside to Bench to Bedside

The use of somatic cell genome editing to create a disease-protective genetic state:

1. (T cells and HSPCs) CCR5 editing → HIV
2. (HSPCs) BCL11A enhancer editing → β-thalassemia / SCD
3. (Liver) *In vivo* targeted gene addition → monogenic disease

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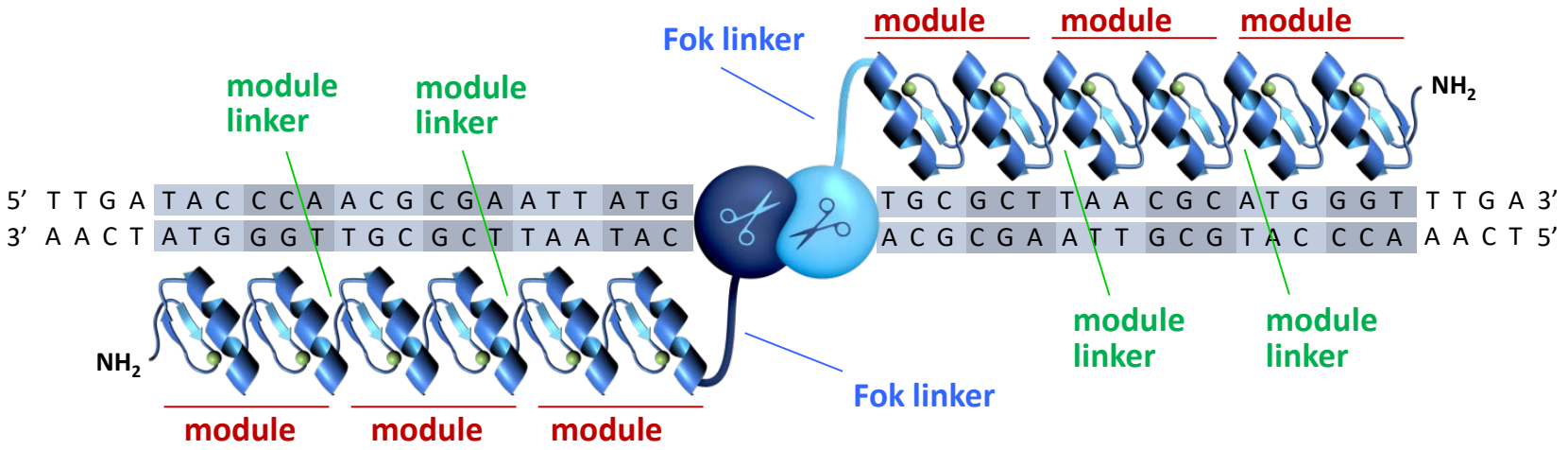
Pharmaceutical Drug Development Applied to Genome Editing

- Generate therapeutic reagents maximized for potency / specificity (the “medicinal chemistry” of genome editing)



- Using established regulatory frameworks to characterize the product appropriately for safety:
  - Tailored to the nature of the technology
  - Appropriate for the intended product and indication (risk-benefit)
  - *In vitro* (using multiple distinct assays / readouts)
  - *In vivo* (using appropriate models)

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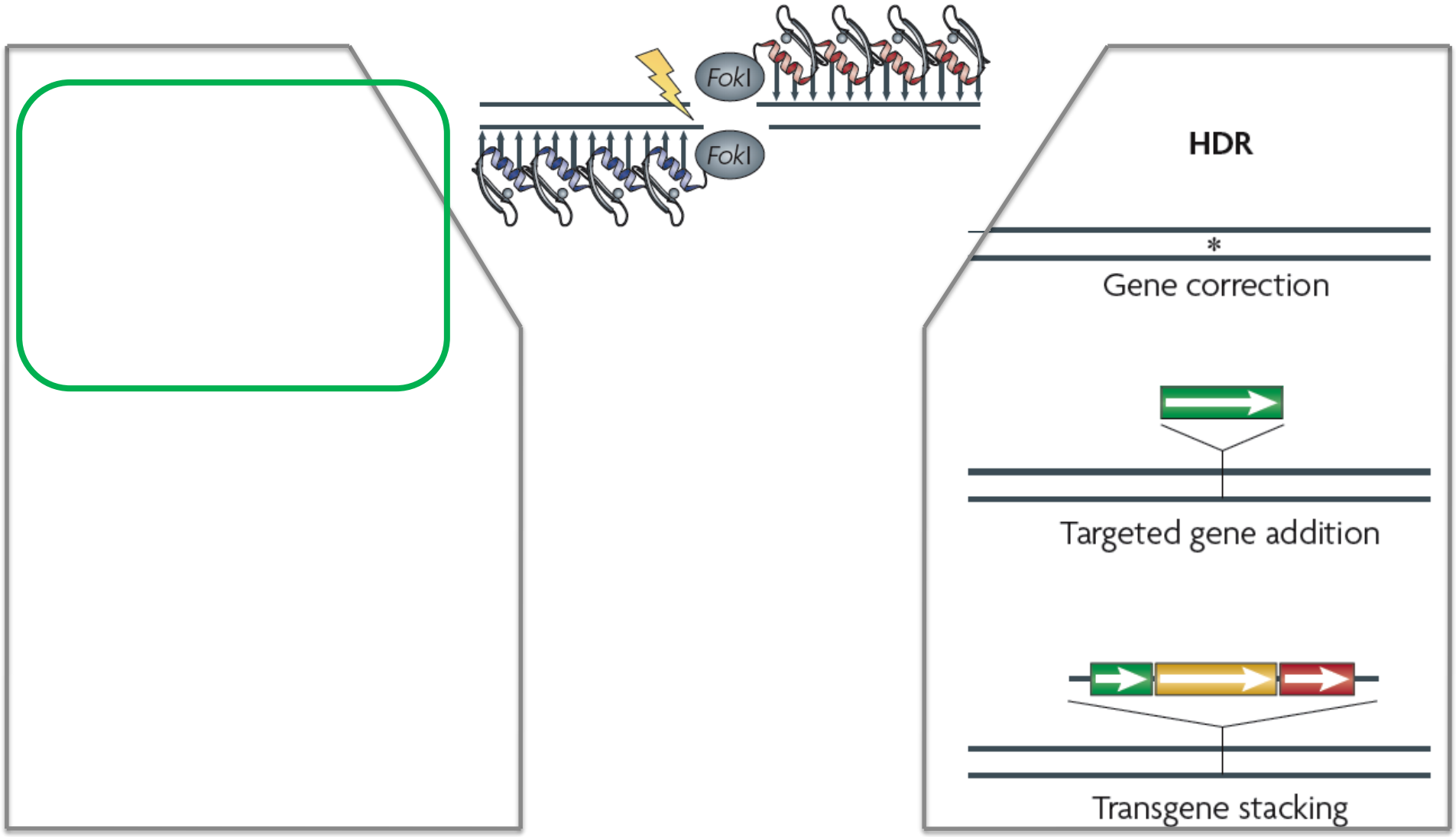
Human Genome Editing With Engineered ZFNs: Targeted Disruption



Urnov, Rebar, Holmes, Gregory *Nature Reviews Genetics* 2010

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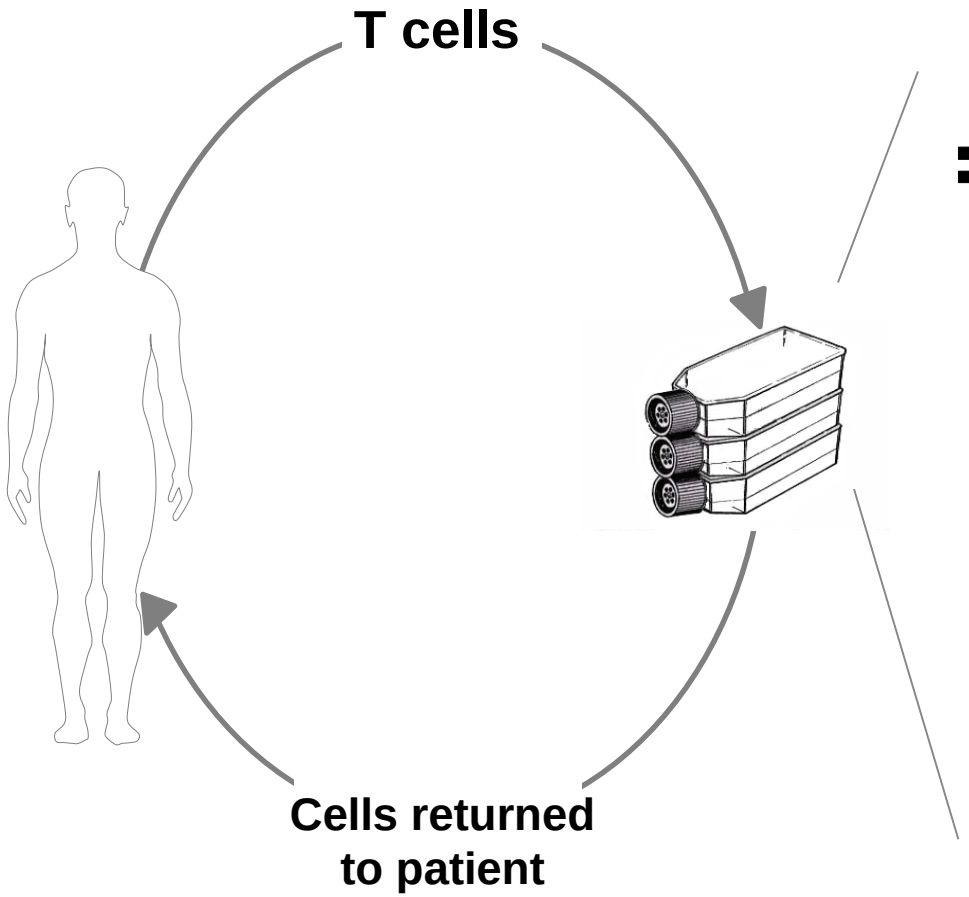
Cell, Vol. 86, 367–377, August 9, 1996, Copyright ©1996 by Cell Press

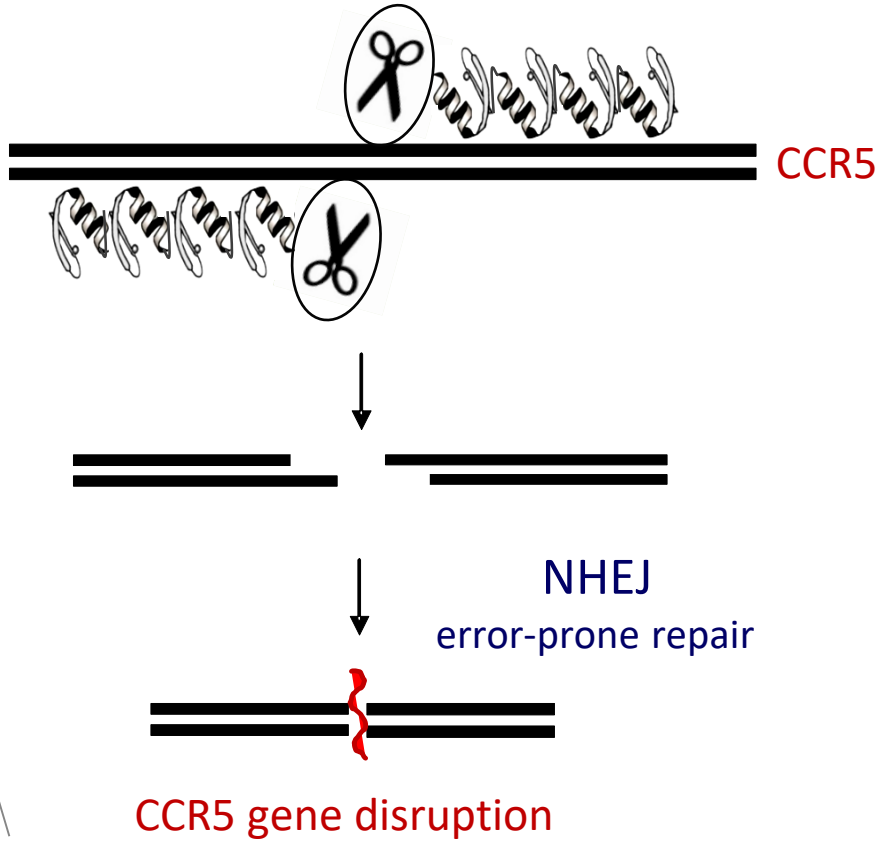
Homozygous Defect in HIV-1 Coreceptor Accounts for Resistance of Some Multiply-Exposed Individuals to HIV-1 Infection

Rong Liu,* William A. Paxton,* Sunny Choe,* Daniel Ceradini,* Scott R. Martin,* Richard Horuk,† Marcy E. MacDonald,‡ Heidi Stuhlmann,§ Richard A. Koup,* and Nathaniel R. Landau*

designated EU2 and EU3, required about 1000-fold more virus to establish infection than contol cells from unexposed donors. While a small fraction of the cells did become infected with this high inoculum, the virus failed

Genome Editing for HIV





Preclinical efficacy: Perez et al Nature Biotechnology 2008
Manufacturing: Maier et al Human Gene Therapy 2013
Clinical data from first cohort: Tebas et al NEJM 2014

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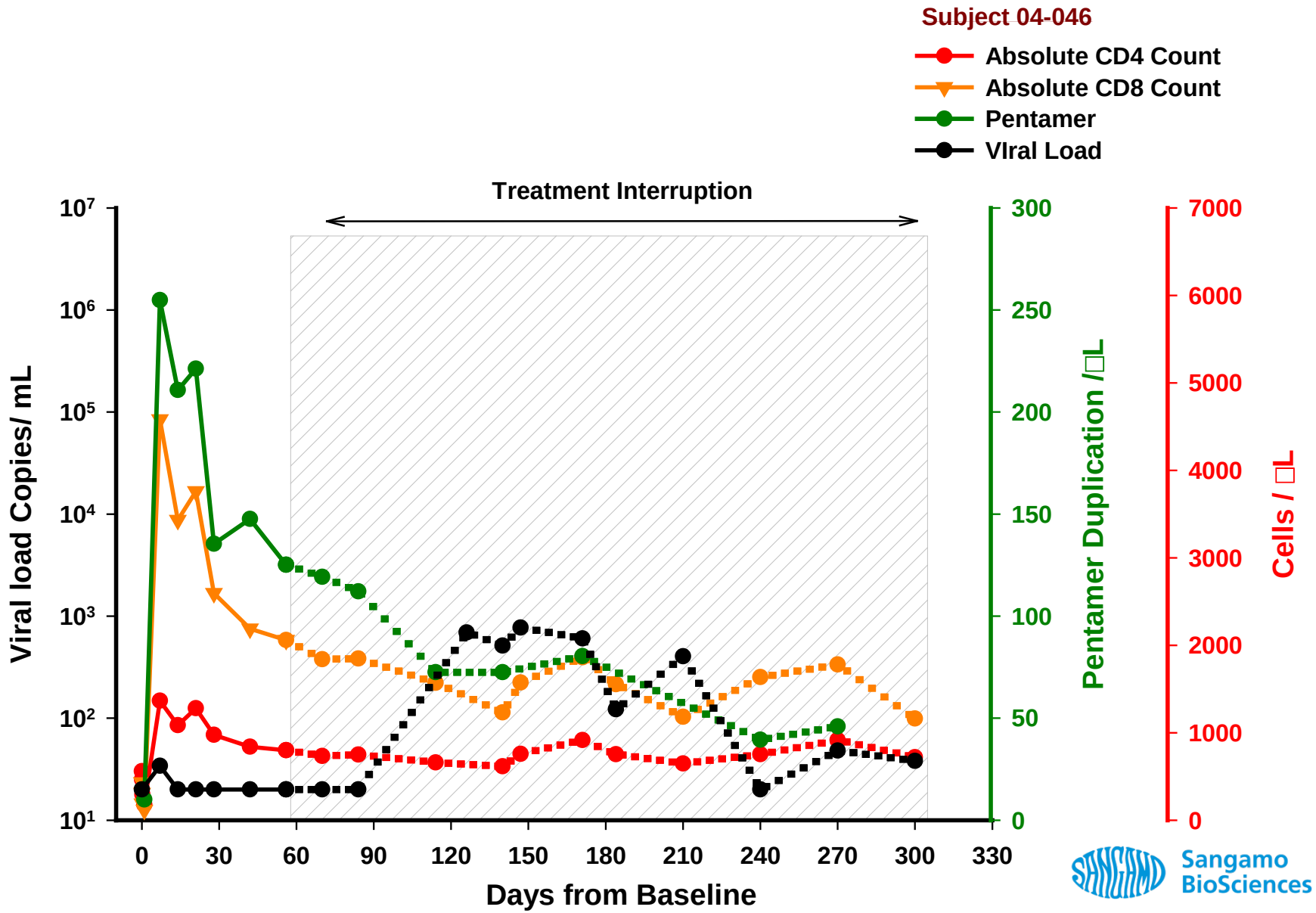
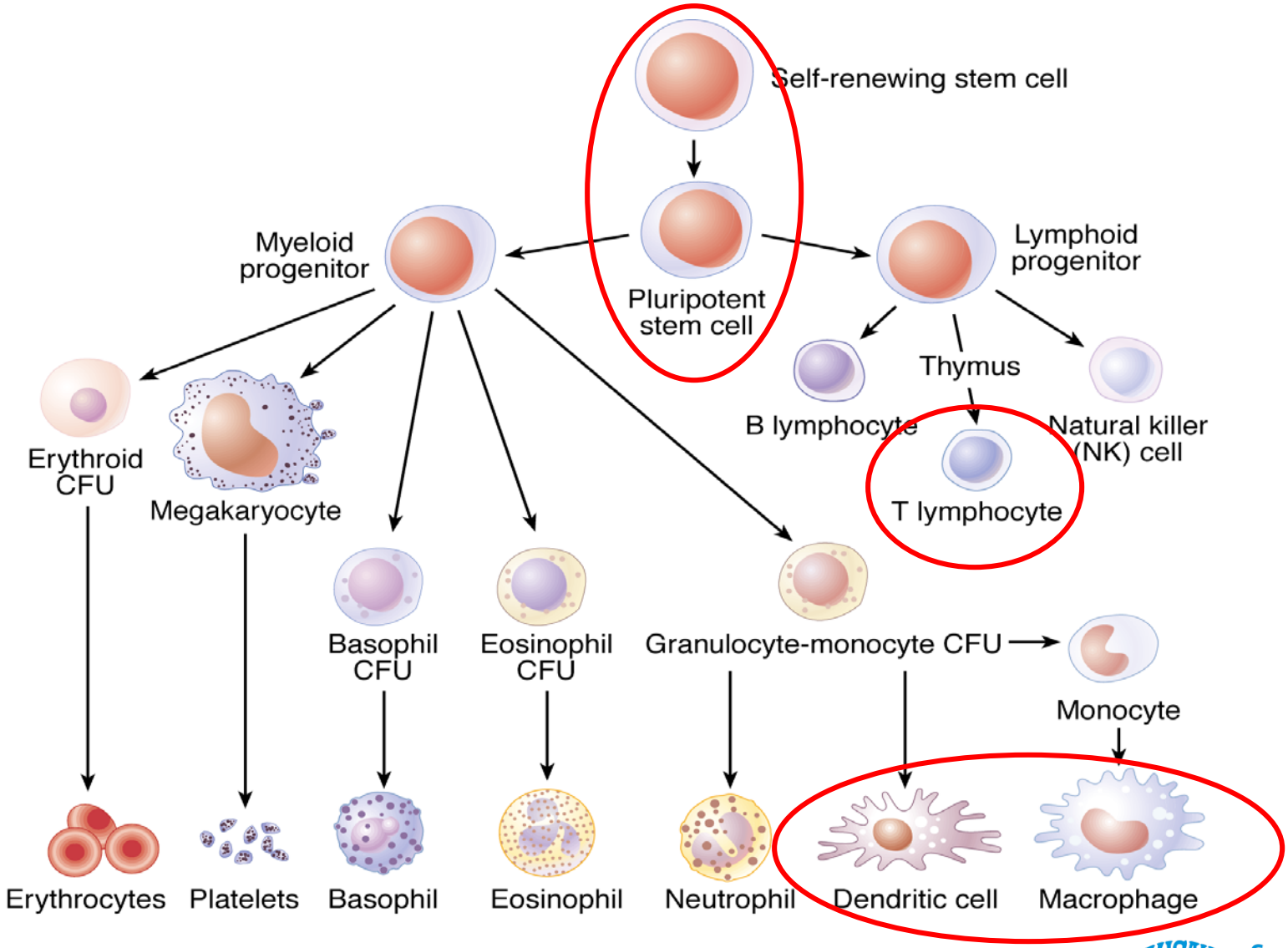
In-clinic Efficacy Readouts: Genome Modification, CD4/8 Count, Viral Load



Editing Hematopoietic Stem Cells Endows All Their Progeny With the Desired Genomic Signature



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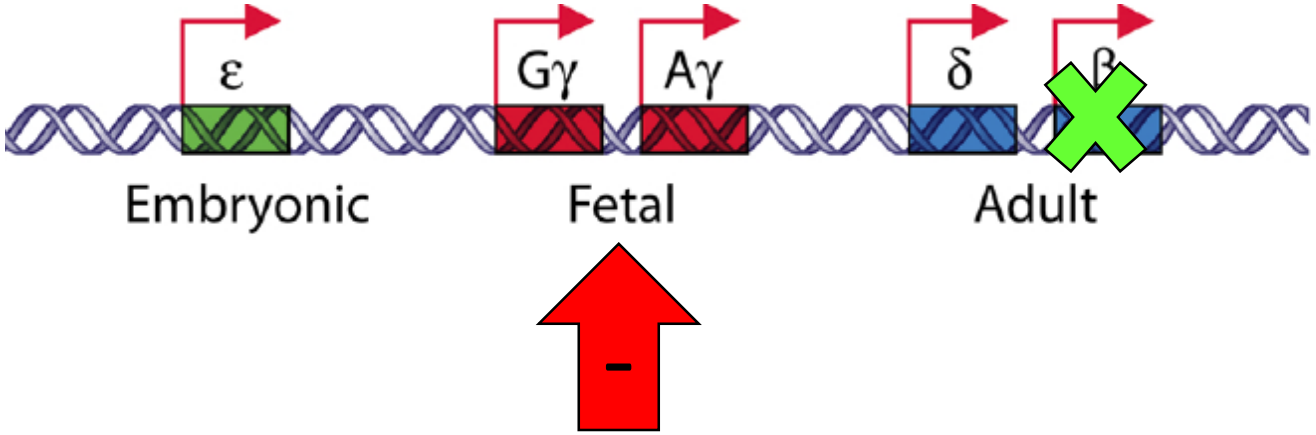
Disease-Protective Genetic Variation in β-thalassemia



BCL11A

2008:

PNAS

Genome-wide association study shows *BCL11A* associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia

Manuela Uda*, Renzo Galanello†, Serena Sanna*, Guillaume Lettre‡§, Vijay G. Sankaran‡¶, Weimin Chen‖, Gianluca Usala*, Fabio Busonero*, Andrea Maschio*, Giuseppe Albai*, Maria Grazia Piras*, Natascia Sestu*, Sandra Lai*, Mariano Dei*, Antonella Mulas*, Laura Crisponi*, Silvia Naitza*, Isadora Asunis*, Manila Deiana*, Ramaiah Nagaraja**, Lucia Perseu*, Stefania Satta†, Maria Dolores Cipollina†, Carla Sollaino†, Paolo Moi†, Joel N. Hirschhorn‡§, Stuart H. Orkin‡¶††, Gonçalo R. Abecasis‖, David Schlessinger**, and Antonio Cao*††

BCL11A ↓ = fetal globin ↑ = disease ameliorated or eliminated

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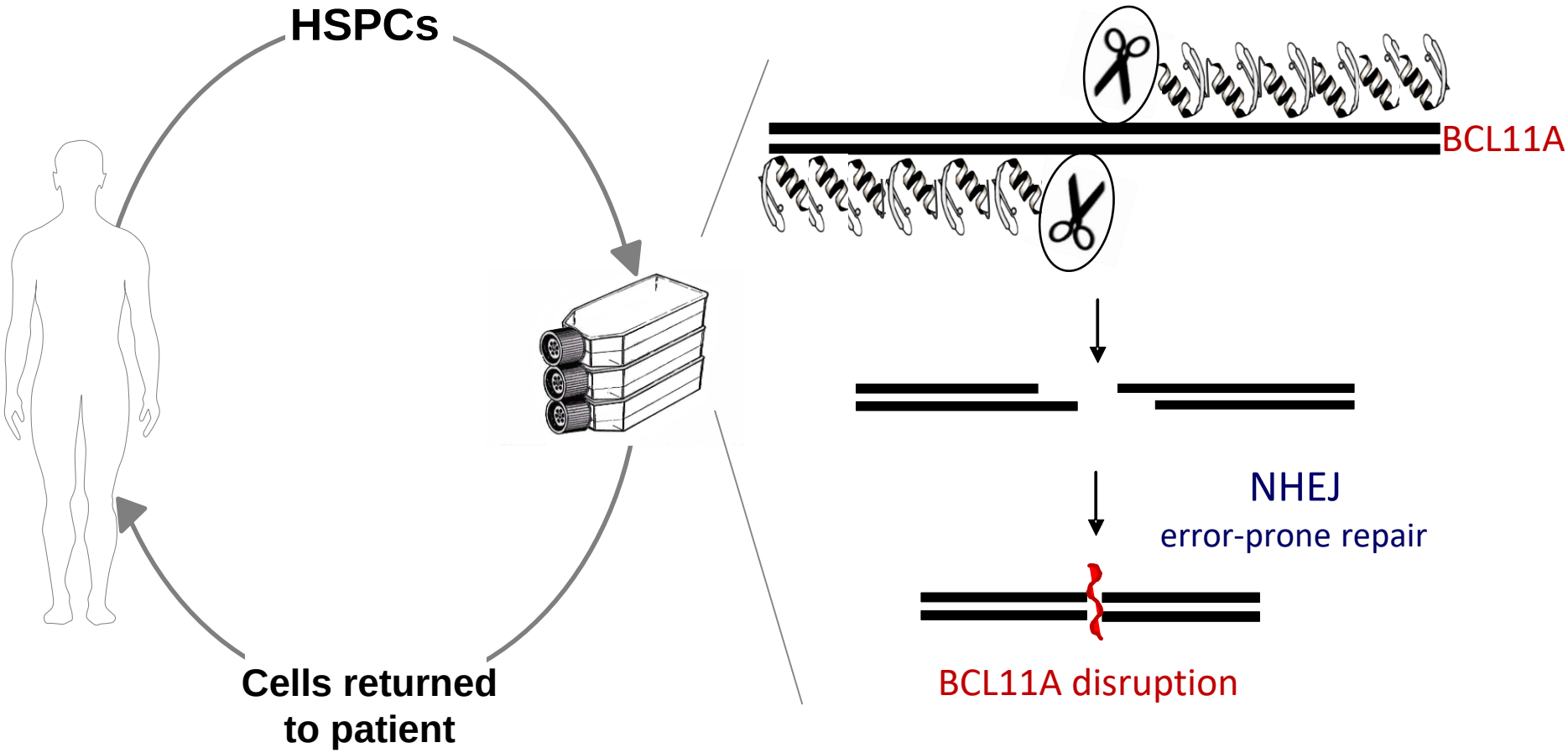
Genome Editing for β-thalassemia and Sickle Cell Disease: a "Simple" Plan



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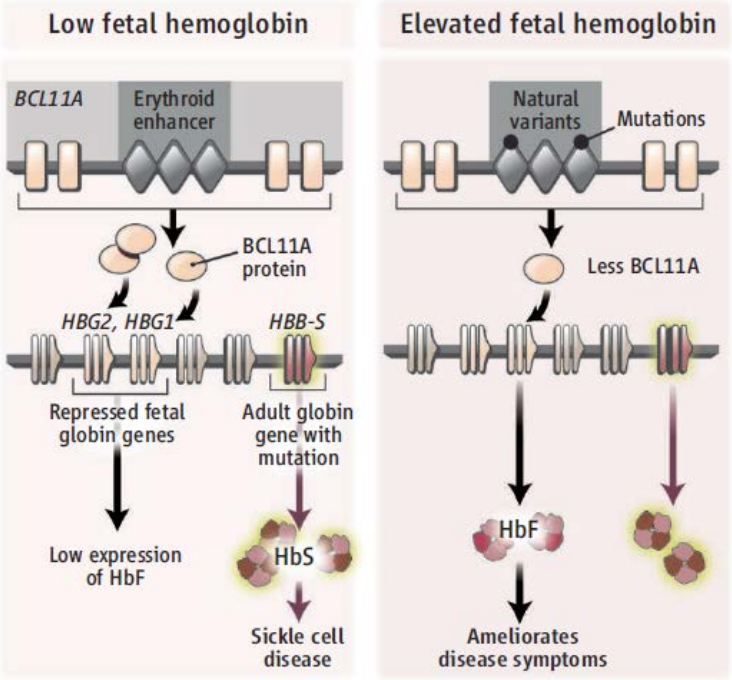
The Achilles Heel of the BCL11A Erythroid Enhancer

Bauer/Orkin – *Science* Oct 2013

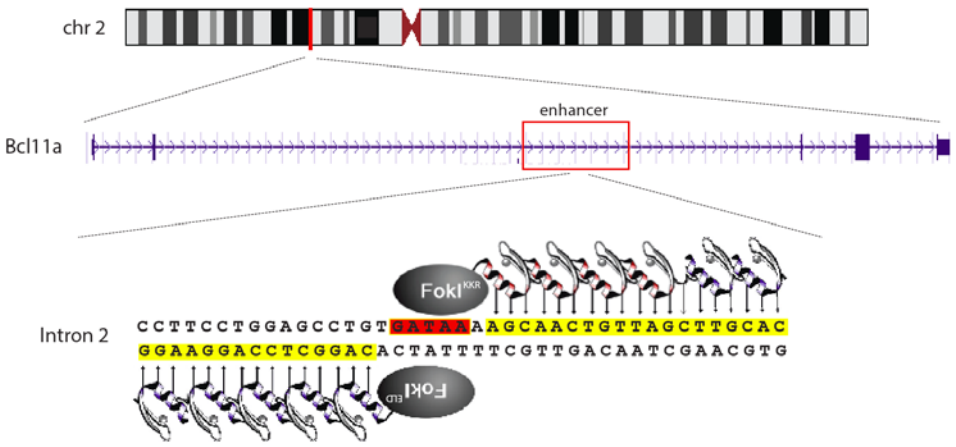
"We propose the GWAS-identified enhancer of BCL11A as a particularly promising therapeutic target for genome engineering in the β-hemoglobinopathies."



Vierstra et al *Nat Meth* 2015

A genome editing-based "walk" across the enhancer using two orthogonal nuclease platforms illuminated a single, specific binding site for GATA1 as key to enhancer function in erythroid-specific BCL11A activation and fetal globin suppression.

(also see Canver et al Nature 2015)



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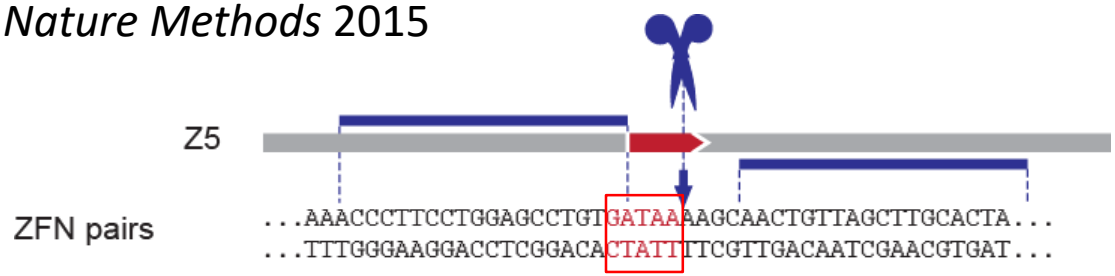
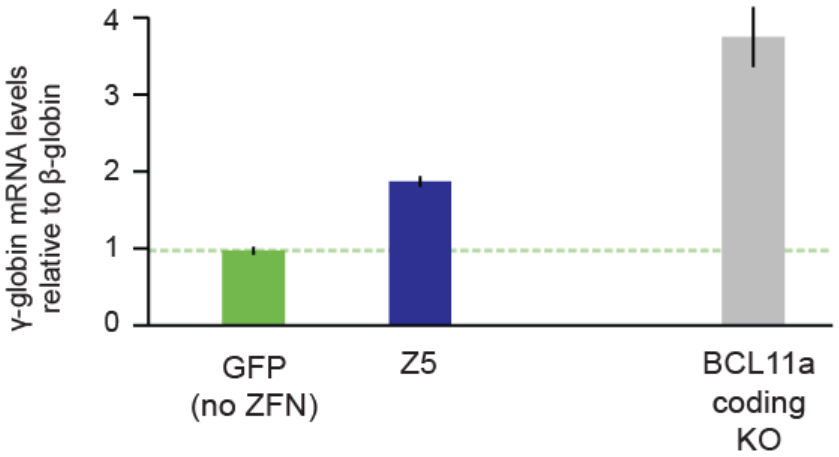
Initial Hit Yielded Enhanced γ-globin Levels

Vierstra et al *Nature Methods* 2015





Transfected ZFN pair

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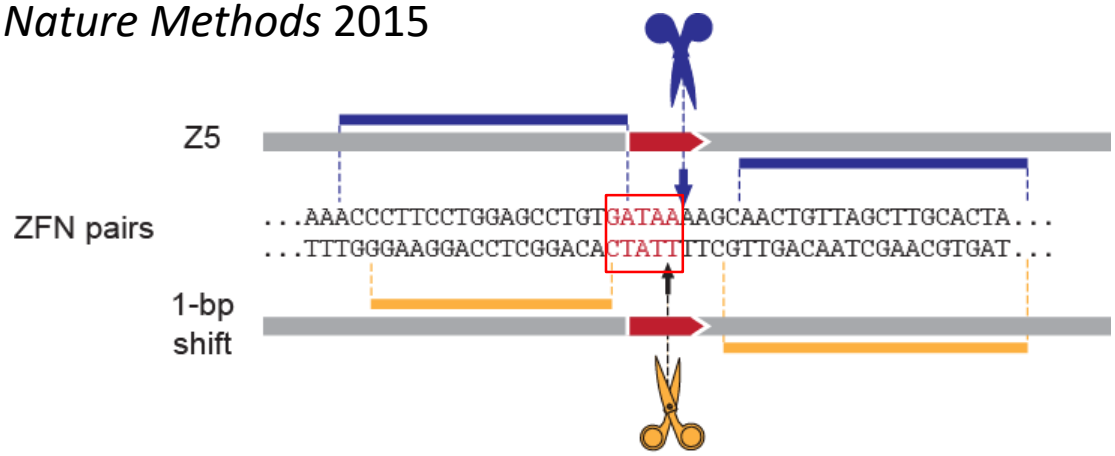
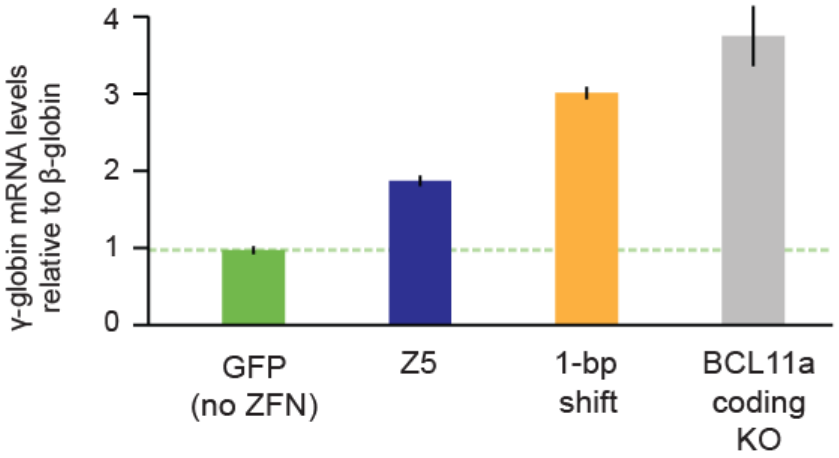
Fine-tuning Cut Site Further Enhances γ-globin Levels

Vierstra et al *Nature Methods* 2015





Transfected ZFN pair

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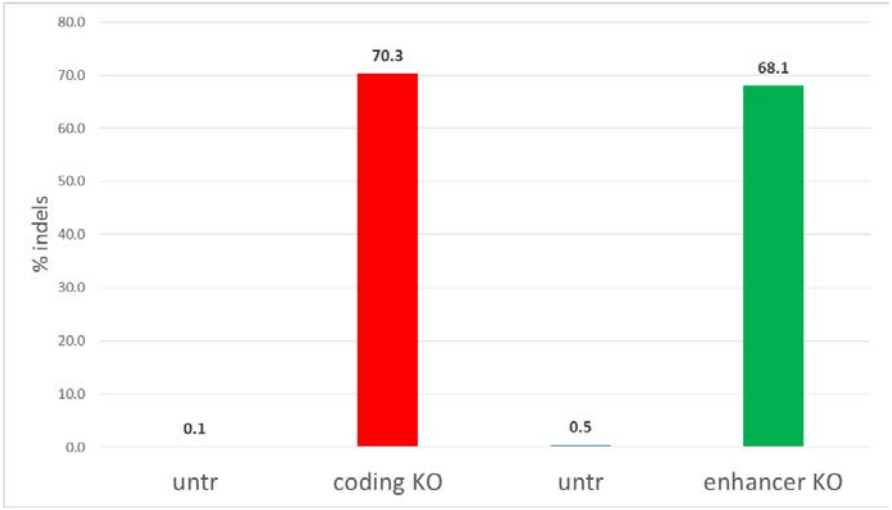
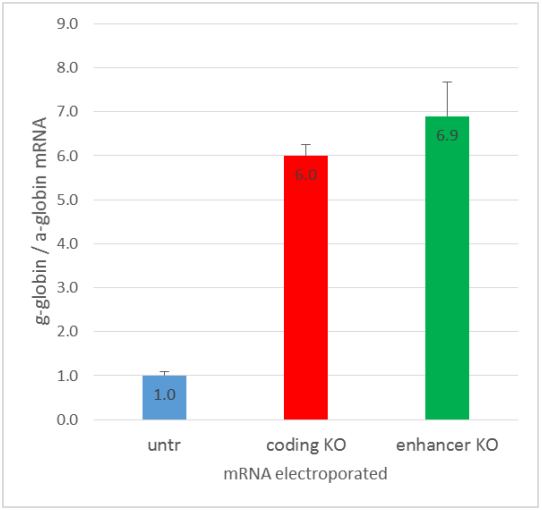
BCL11A Knockout vs Enhancer Disruption: Equivalent Effects on Fetal Globin in PB-derived HSPCs

- erythropoiesis proceeds indistinguishably to control cells (Vierstra et al 2015)
- equivalent elevation of gamma globin in RBCs obtained from PB-derived HSPCs

Editing



Level of fetal globin mRNA



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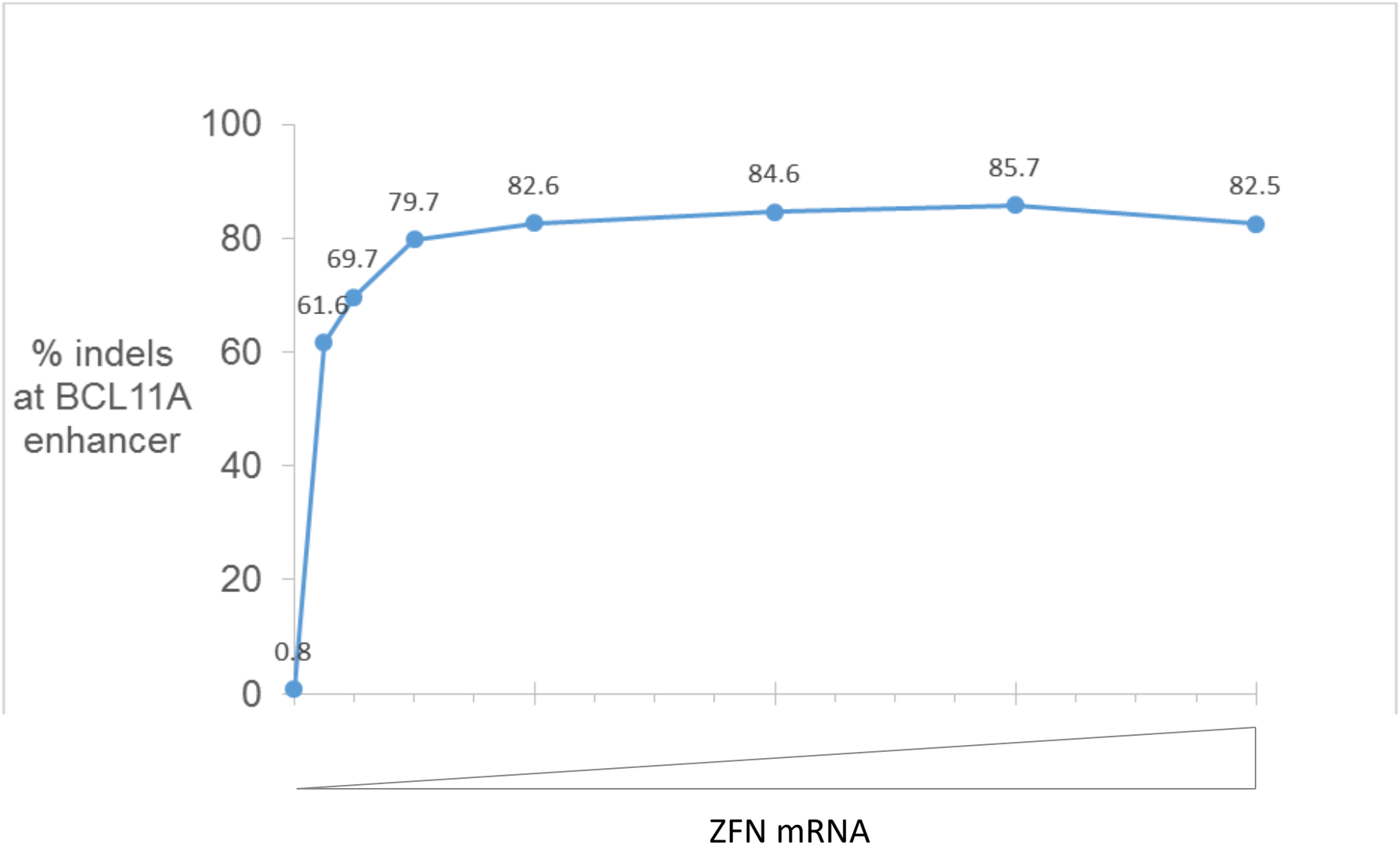
85% On-Target Editing in HSPCs Using Clinical Lead ZFNs



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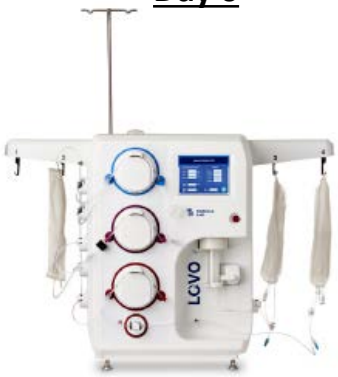
Genome Editing En Route to the Clinic: CD34 GMP Cell Manufacturing Process

Day -2 & -1

Apheresis from G-CSF/Plerixafor mobilized subjects

Day 0



Platelet depletion

Day 0

CliniMACS CD34 enrichment

Day 0-1

Rest cells in culture

Day 2

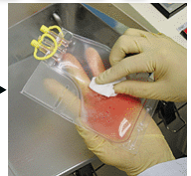
Maxcyte ZFN mRNA electroporation

Day 2-4

Static culture step to allow ZFN gene expression

Day 4

Formulate CD34 cells



Gene modified CD34 cells
Final Product after QC testing and release

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From GWAS to the Clinic

Proposal (Bauer et al (2013)):

"We propose the GWAS-identified enhancer of BCL11A as a particularly promising therapeutic target for genome engineering in the β-hemoglobinopathies"

Findings:

- An editable "Achilles Heel" in the enhancer
- Enhancer KO elevates disease-protective fetal globin
- Efficient knockout at clinical scale
- **On-track for IND filing H1 2016 for our beta-thalassemia program**

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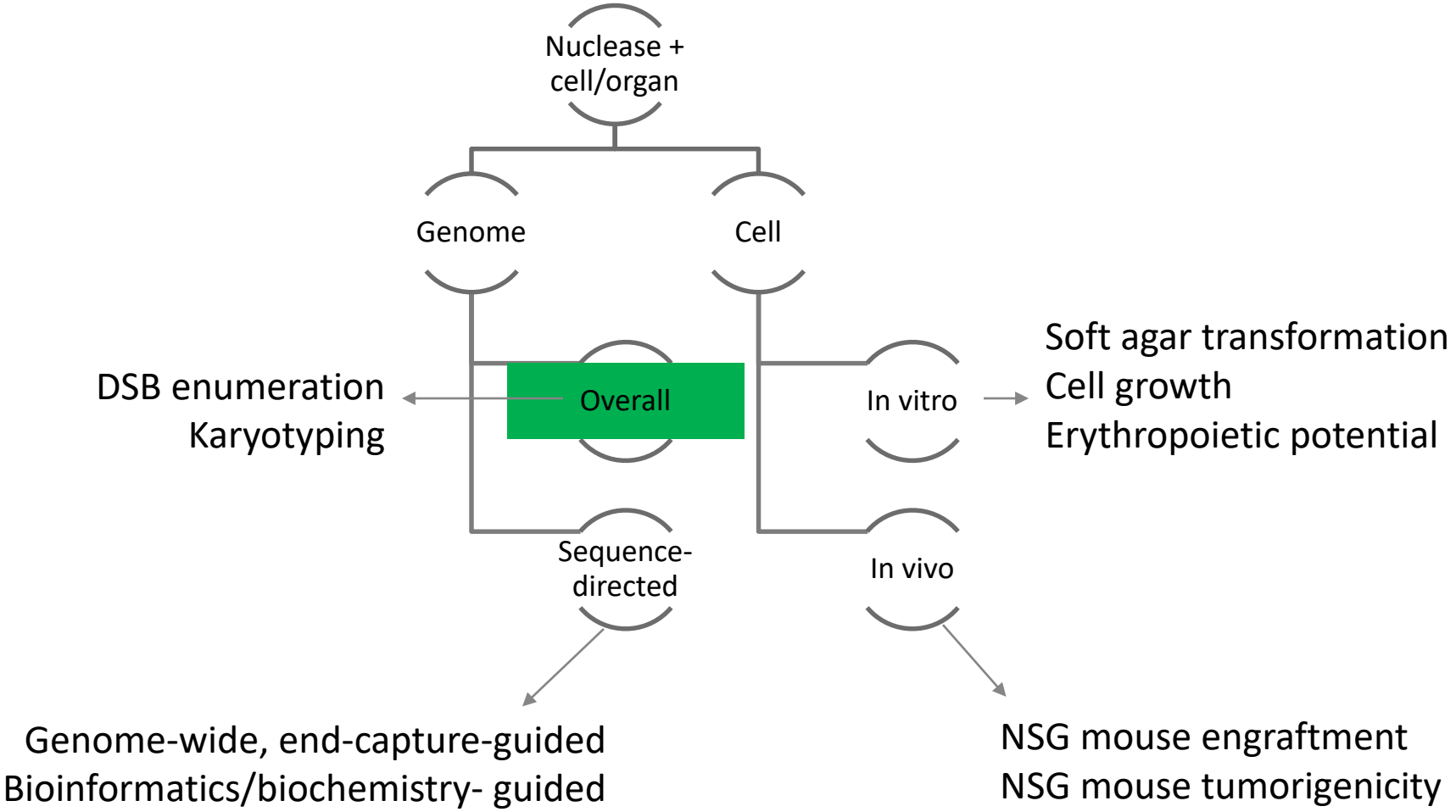
Readouts of Genome Editing Safety: Charted Path Based on Two Open INDs For ZFN-Driven HSPC Editing



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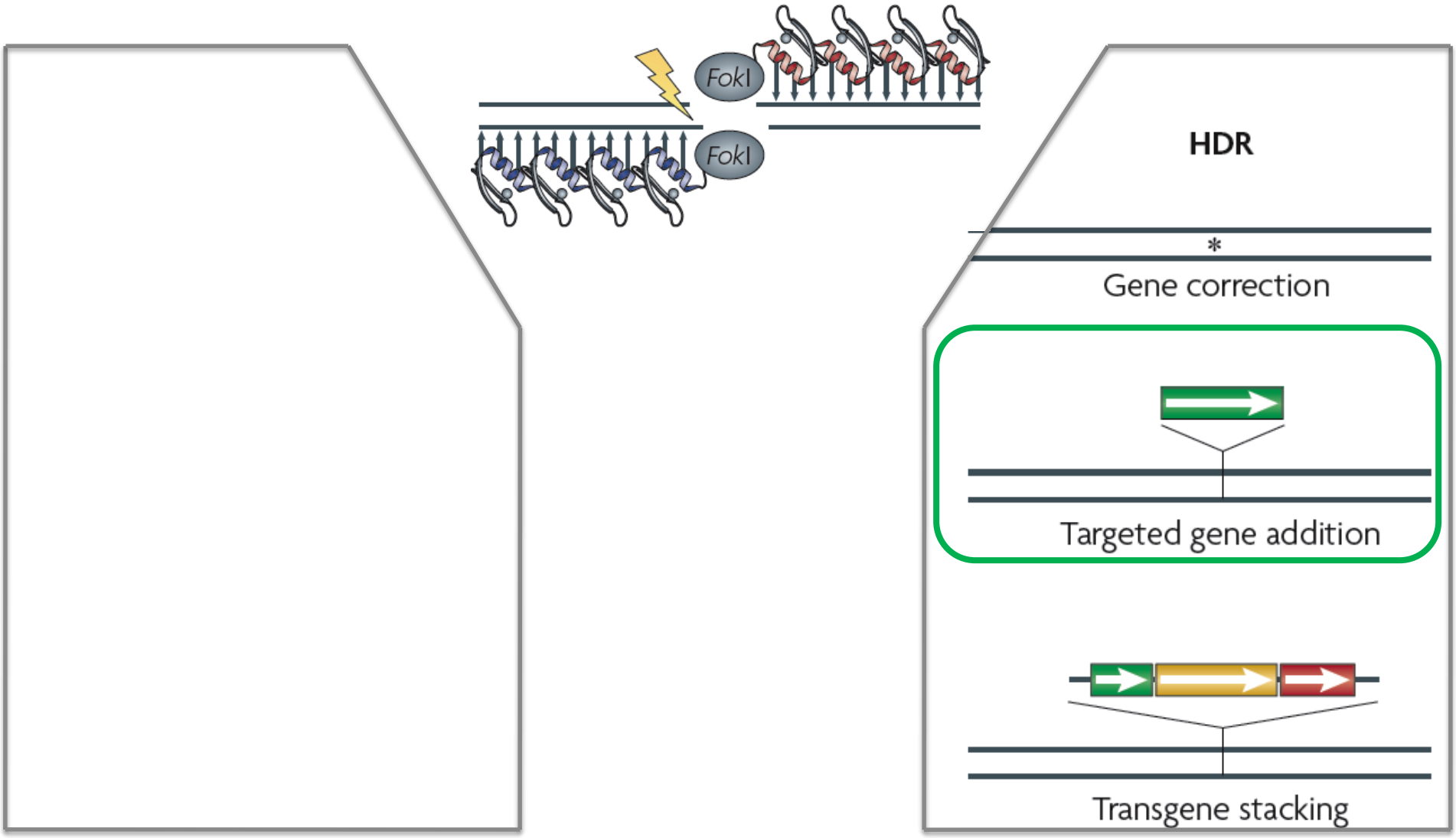
Human Genome Editing With Engineered ZFNs: Targeted Gene Addition



Urnov, Rebar, Holmes, Gregory *Nature Reviews Genetics* 2010

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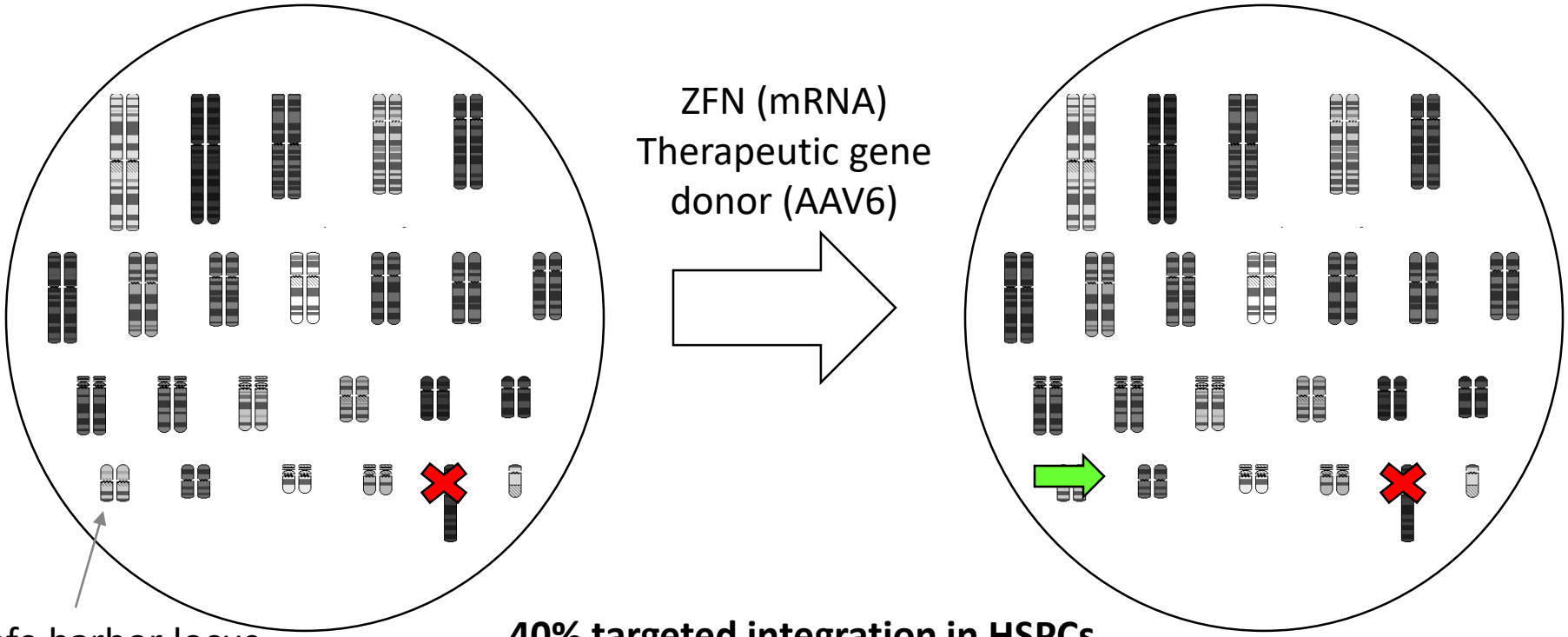
Targeted Addition of Therapeutic Gene to a “Safe Harbor”



40% targeted integration in HSPCs
50% targeted integration in T cells

Hockemeyer et al *Nature Biotechnology* 2009
DeKelver et al *Genome Research* 2010
Lombardo et al *Nature Methods* 2011
Wang et al *Nature Biotechnology* 2015; *NAR* 015

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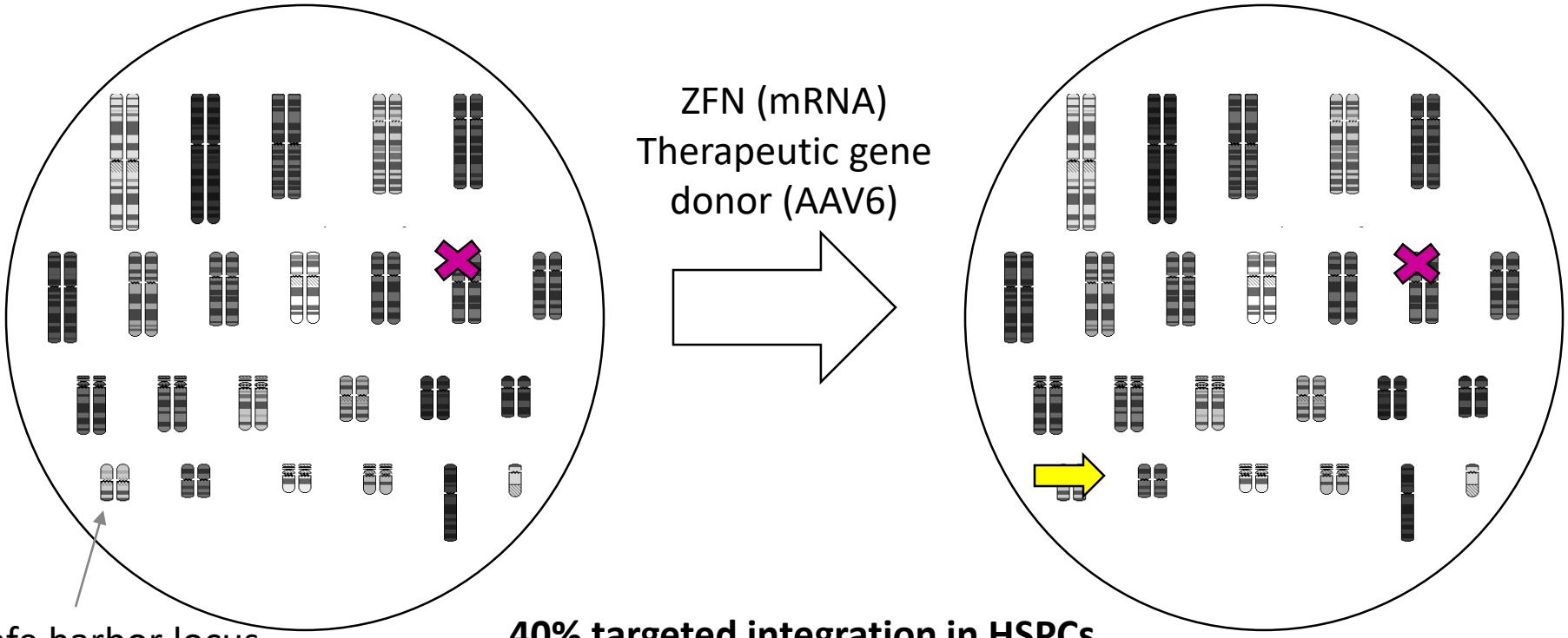
Targeted Addition of Therapeutic Gene to a "Safe Harbor"



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Hockemeyer et al *Nature Biotechnology* 2009
DeKelver et al *Genome Research* 2010
Lombardo et al *Nature Methods* 2011
Wang et al *Nature Biotechnology* 2015; *NAR* 015

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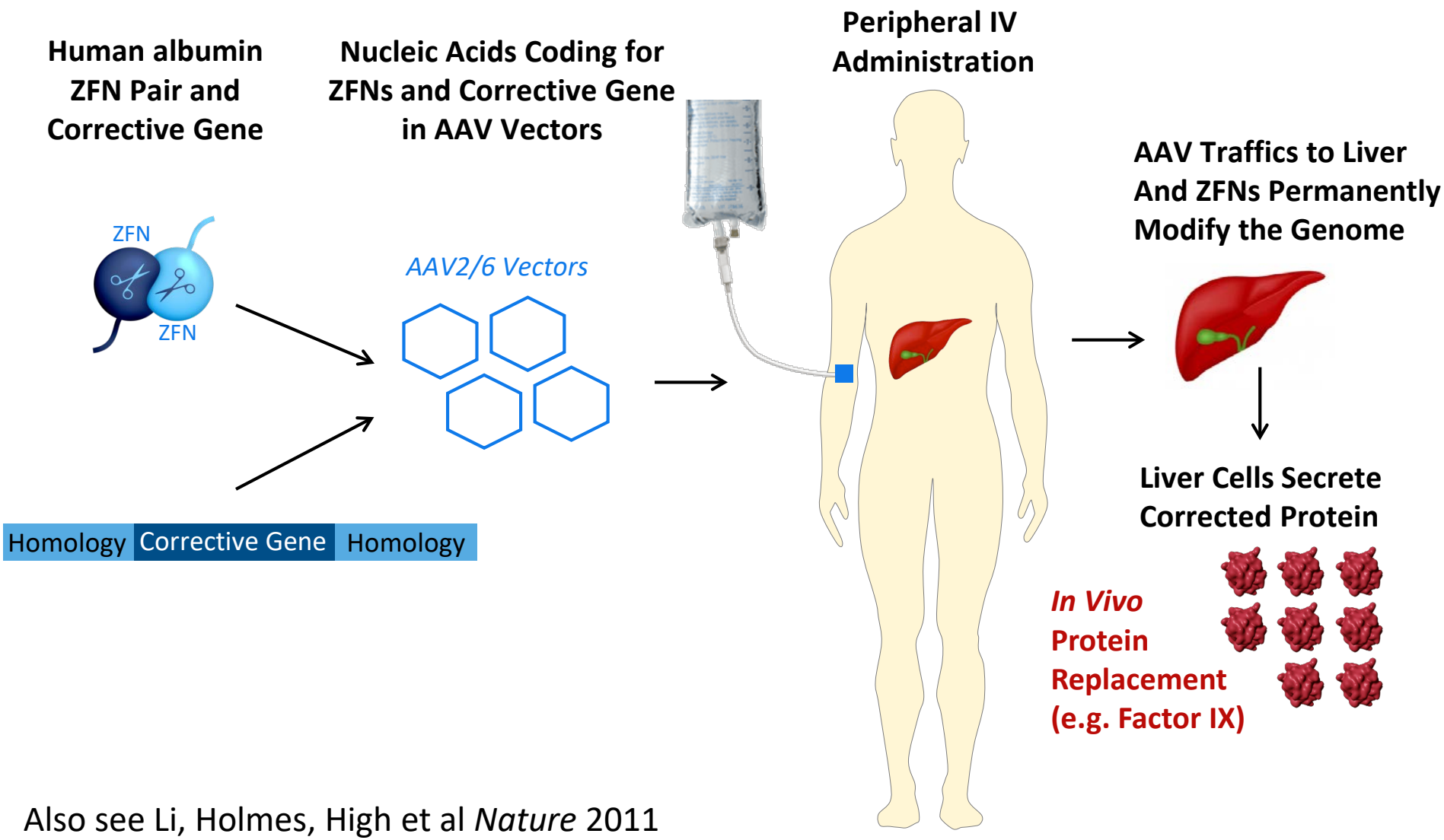
Targeted Gene Integration via Systemic AAV Delivery of ZFP Therapeutics: *In Vivo* Treatment of Monogenic Disease



Also see Li, Holmes, High et al *Nature* 2011

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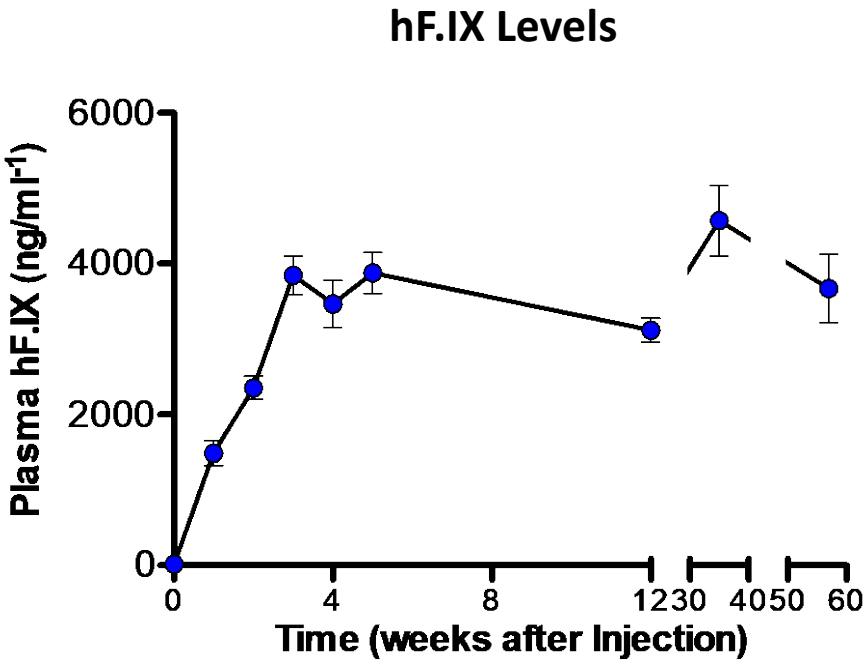
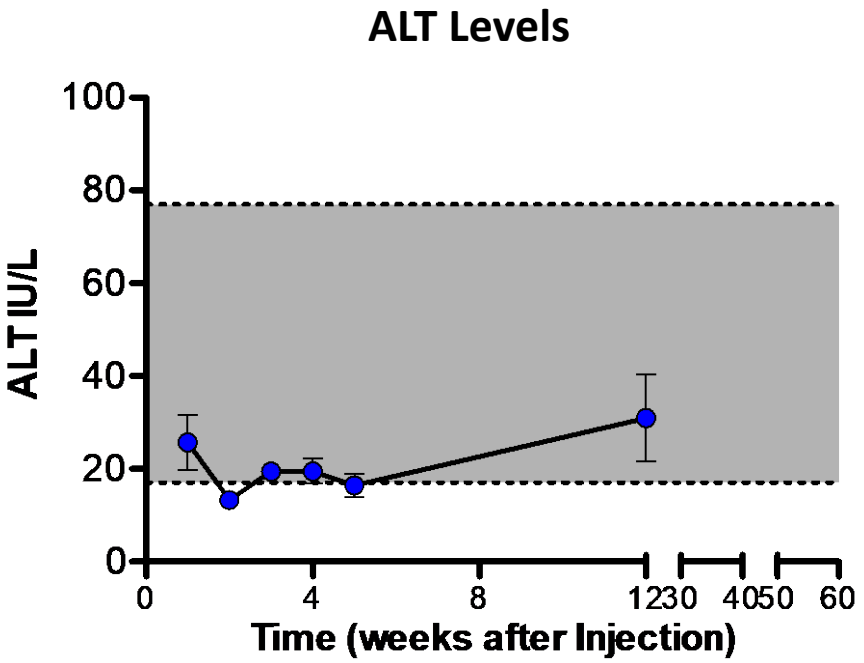
In Vivo Genome Editing: Robust Circulating hF.IX Levels Following Its ZFN-Driven Addition to Albumin – at 60 wks





Therapeutic hF.IX levels observed for 60 weeks (longest time point assayed)
No change in ALT levels

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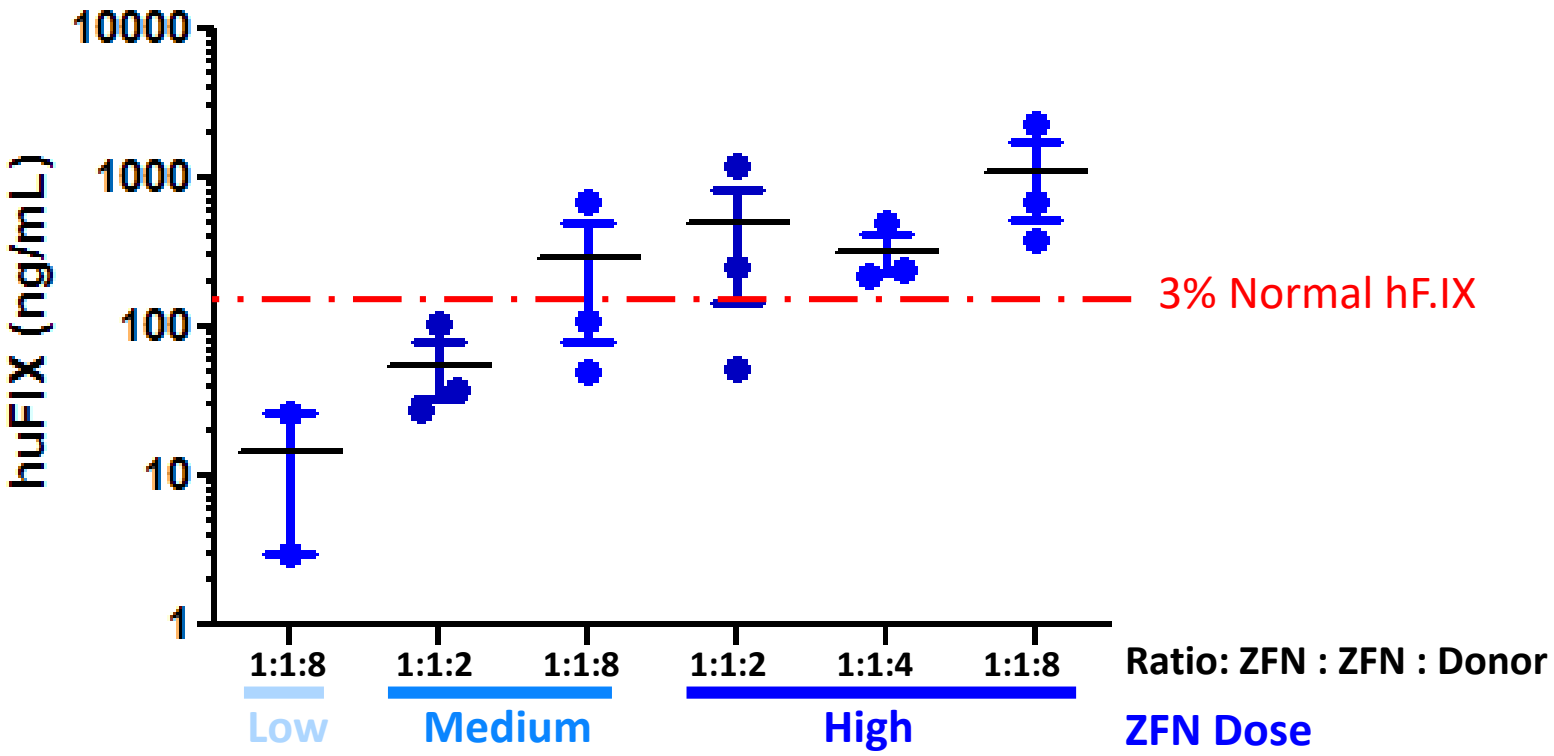
NHP Genome Editing:
Therapeutic hF.IX Levels Following Its Addition to ALB



IND reviewed by FDA and is open – first study of in vivo genome editing

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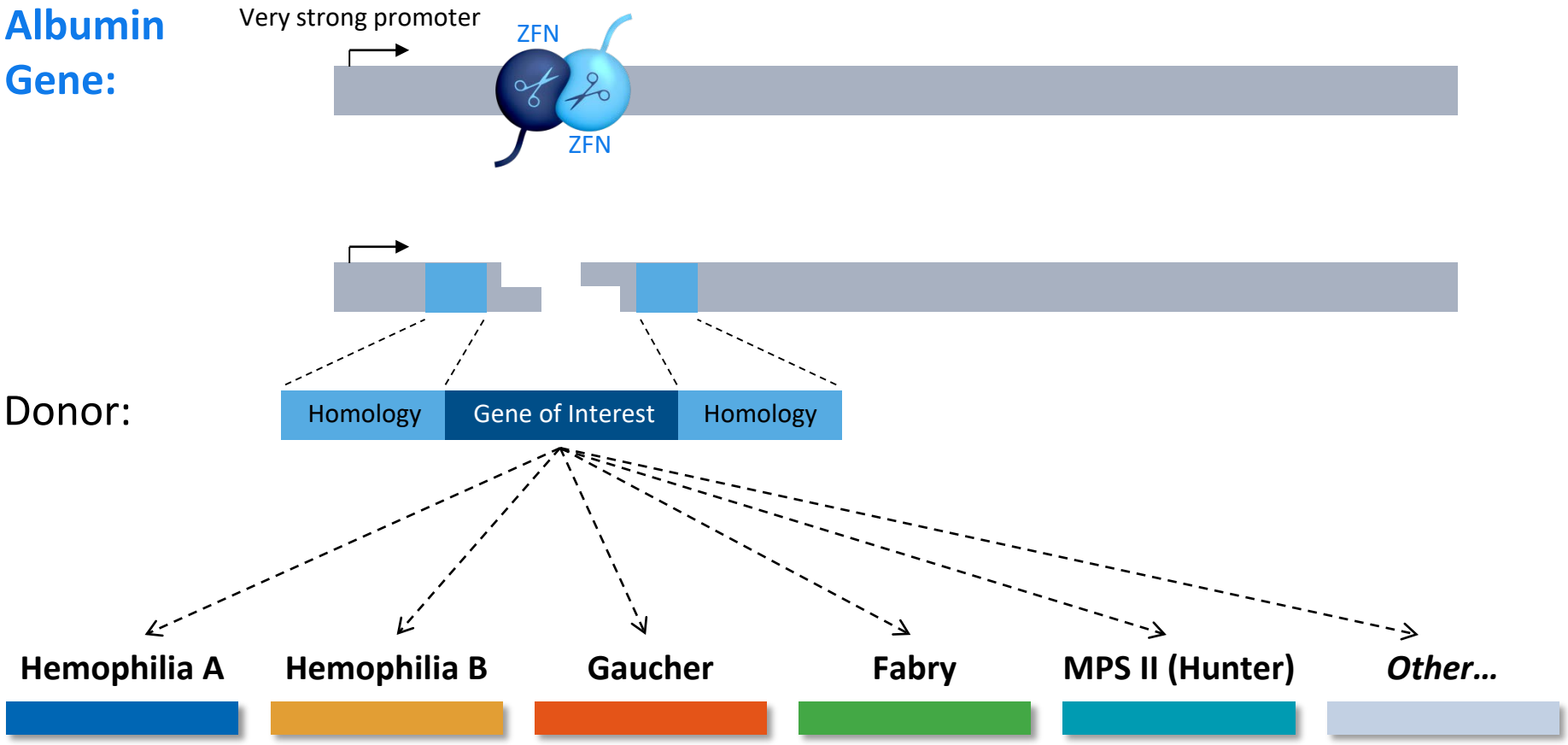
Sangamo's *In Vivo* Protein Replacement Platform

Broadly Leverageable



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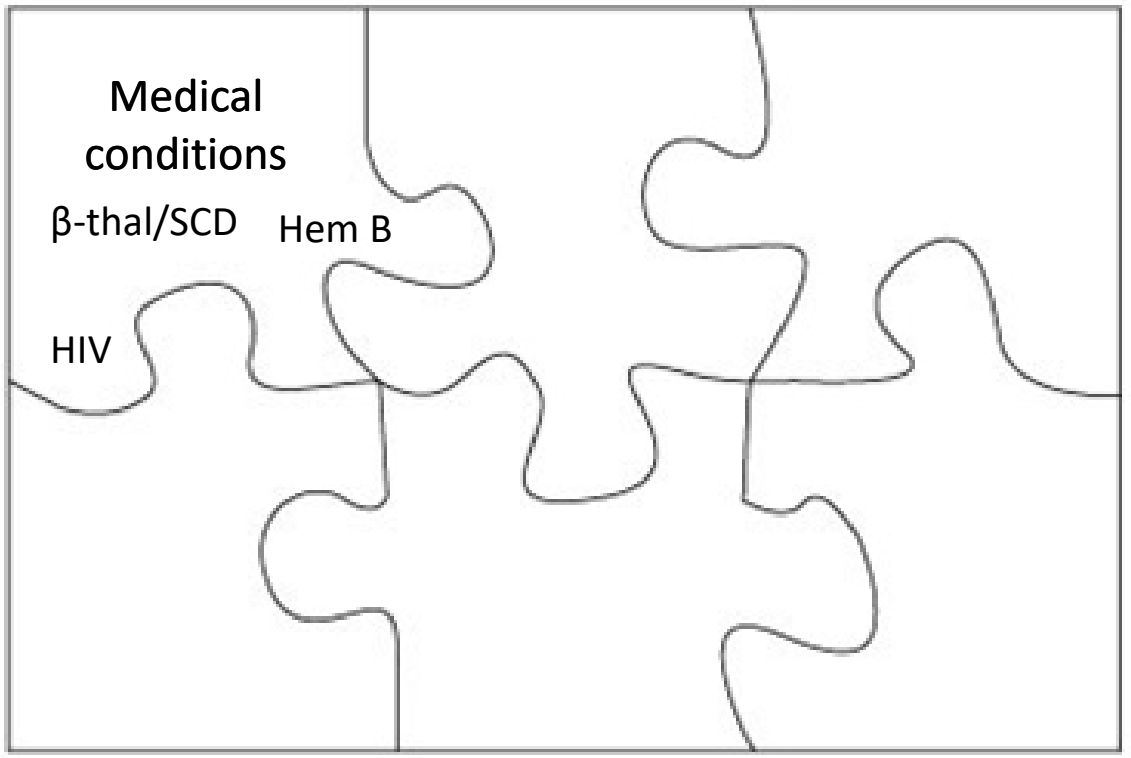
A quote from Sydney Brenner, and a look to the future

"Progress in science depends on new technologies, new ideas, and new discoveries – **in that order**" Sydney Brenner



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A quote from Sydney Brenner, and a look to the future

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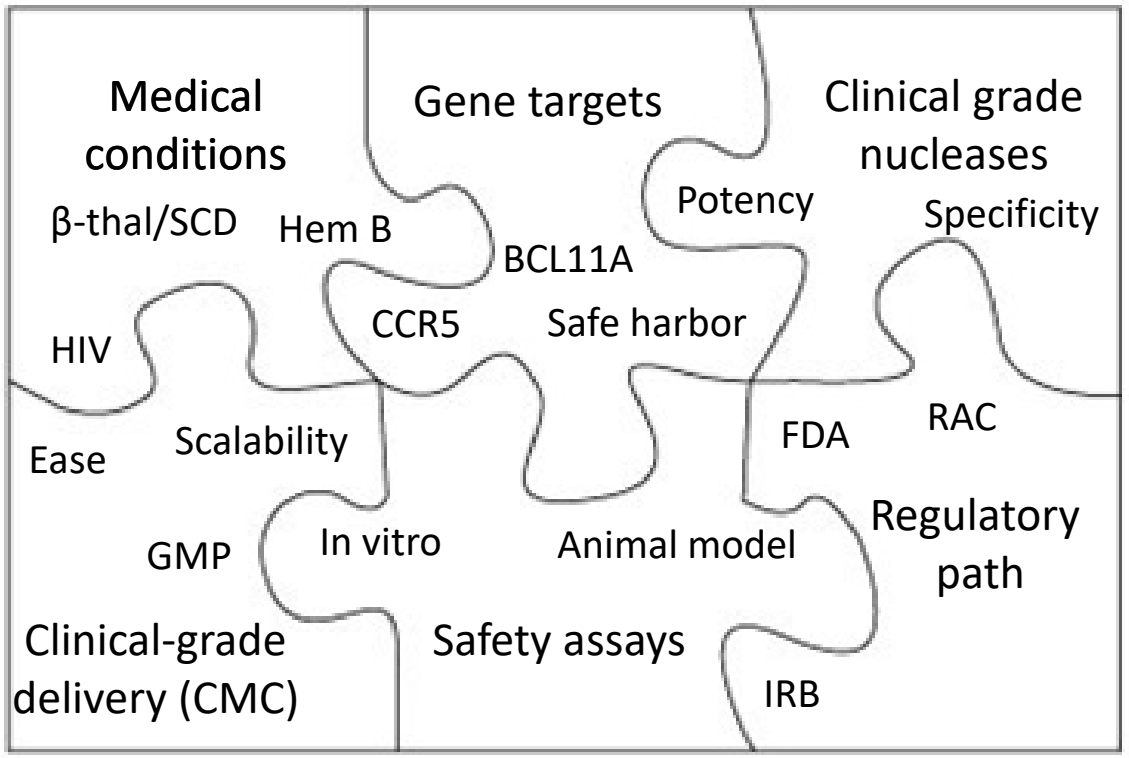
"Pharmacological-grade" genome editing:

- Potency
- Specificity
- Delivery
- Regulatory path



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Debt of gratitude: "academic" research

Zinc finger design

Rebar and Pabo *Science* 1994

Isalan Klug *Nature Biotech* 2001

Editing

Rouet and Jasin MCB, PNAS 1994

Bibikova and Carroll MCB 2001, Genetics 2002, Science 2003

Porteus and Baltimore Science 2003

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Debt of gratitude: collaborators and colleagues

Collaborators

HIV: Carl June, Pablo Tebas, Bruce Levine (Penn); G. Blick, J. Lalezari.

β-thalassemia: George Stamatoyannopoulos, Thalia Papayannopoulou, Kai-Hsin Chang (UW)

Jeff Vierstra, John Stamatoyannopoulos (UW)

Stuart Orkin, Daniel Bauer (Boston Children's/DFCI)

Haiyan Jiang, Siyuan Tan, Kasra Kasraian (Biogen)

Sangamo

Edward Rebar, Jeffrey Miller, Lei Zhang

Andreas Reik

Lisa Fox

Michael Holmes, Thomas Wechsler

Philip Gregory

Dale Ando, Geoff Nichol

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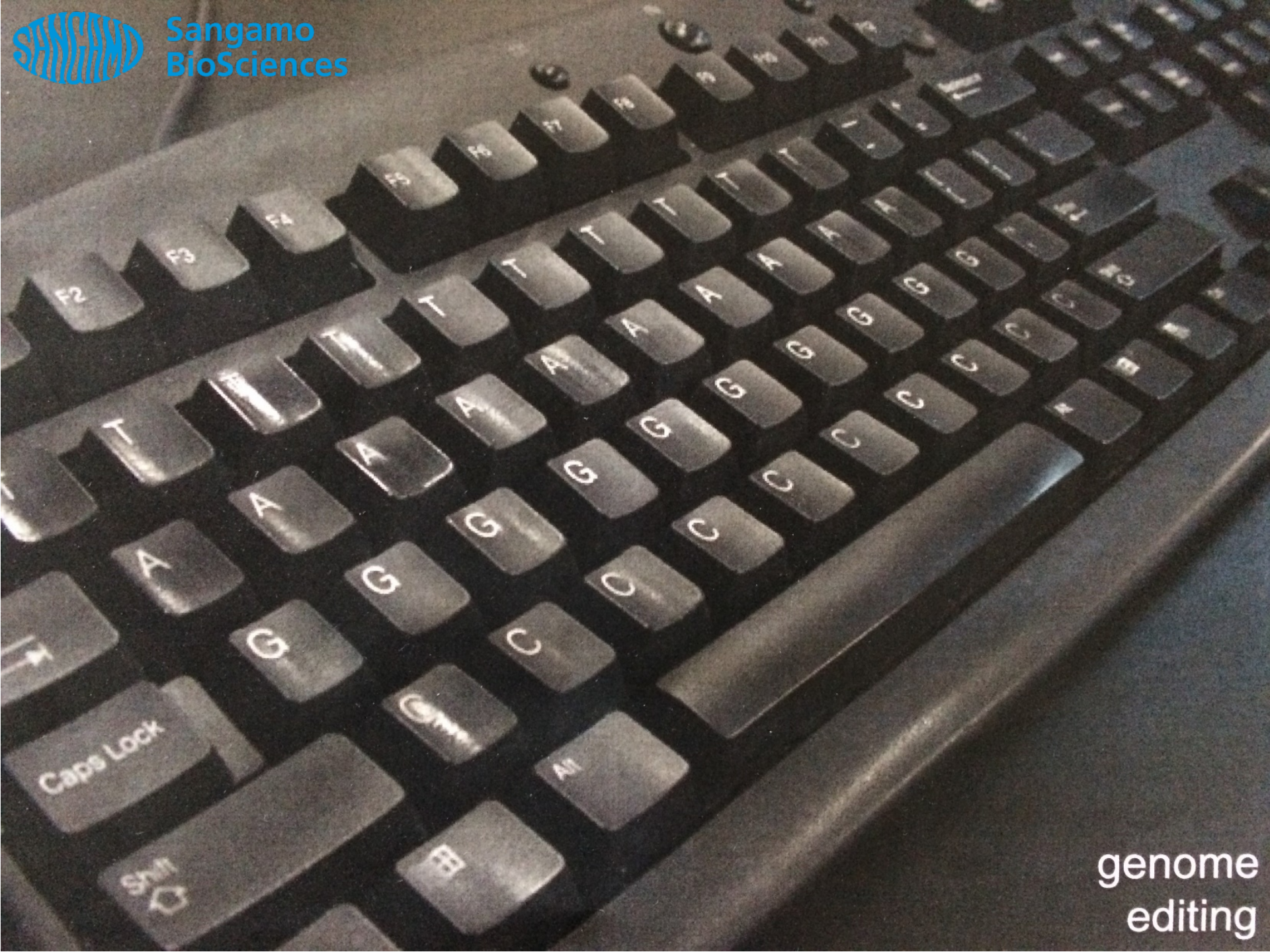
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