Therapeutic Genome Editing of Primary Human Somatic Stem Cells (Personalized Definitive and Curative Cell Based Therapies)

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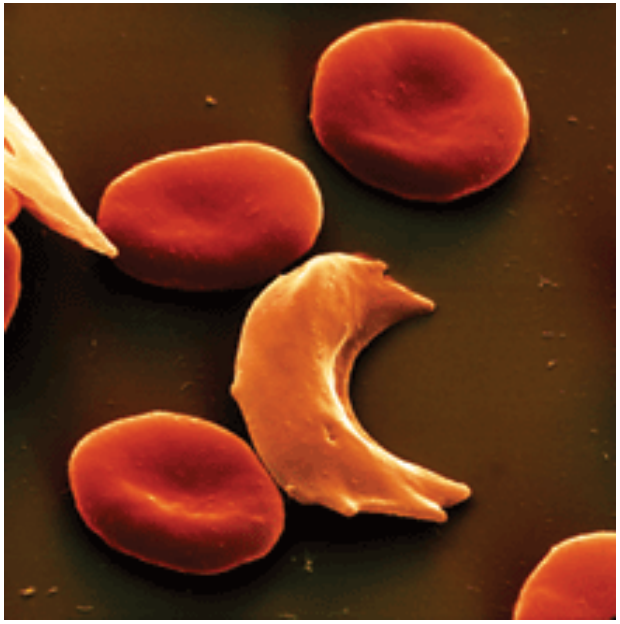
Department of Pediatrics
Stanford University
December 2, 2015

Conflicts of Interest

CRISPR Therapeutics: Equity and Consultancy

Managed through Stanford in accordance with their conflict of interests policy.



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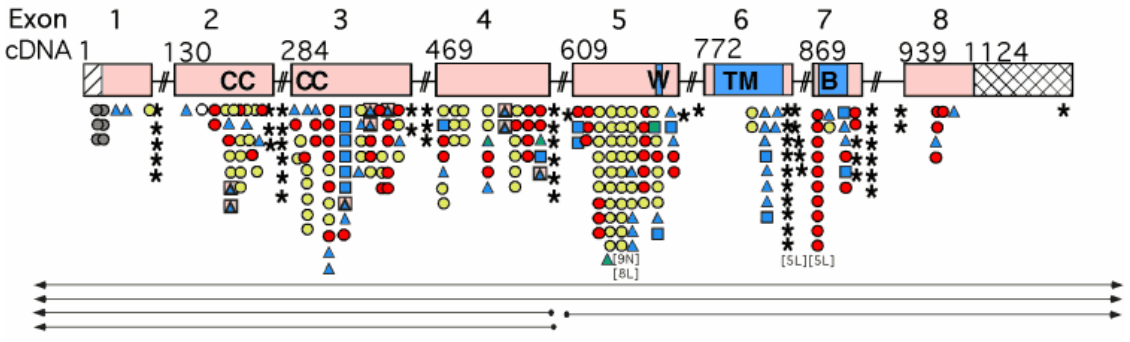
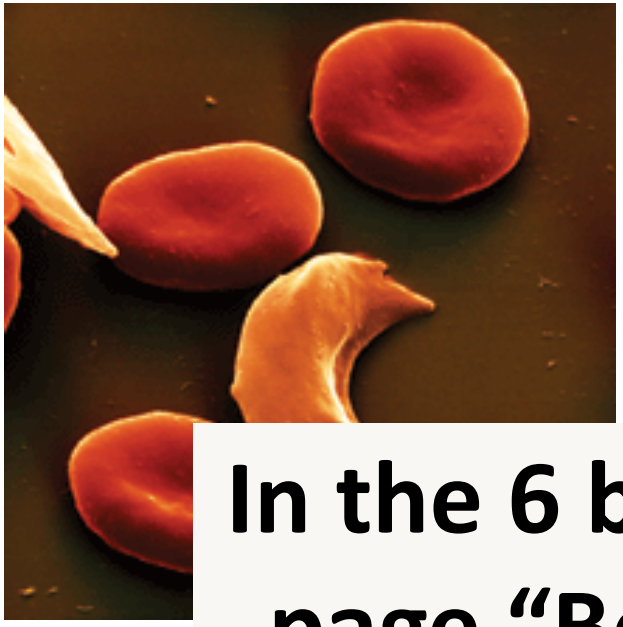
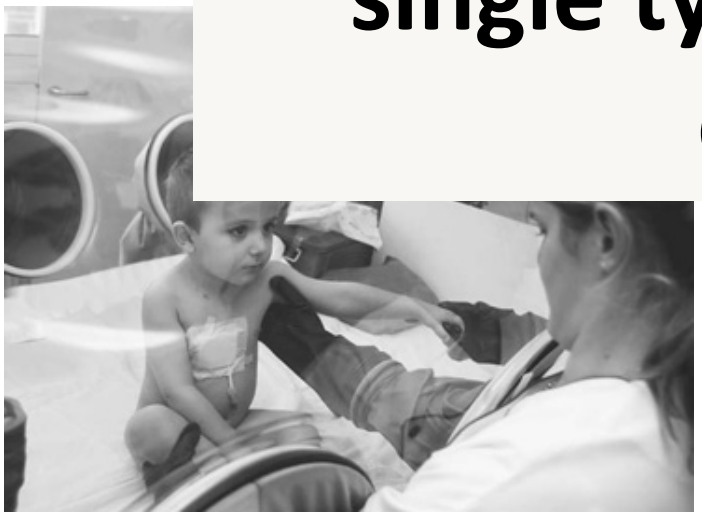
S: …ATG GTG CAC CTG ACT CCT GTG GAG AAG …

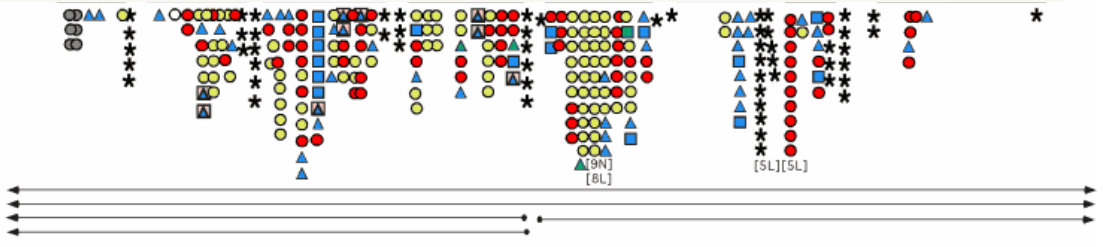




A: …ATG GTG CAC CTG ACT CCT GAG GAG AAG …
S: …ATG GTG CAC CTG ACT CCT GTG GAG AAG …

In the 6 billion character/1.1 million page “Book of Genome” there is a single typographical error that causes disease





Monogenic Diseases Permeate Medicine (>6,000 such diseases)

Hematology: Sickle Cell Disease/Thalassemia
Hematology: Hemophilia
Pulmonary: Cystic Fibrosis
Immunology: Primary Immunodeficiencies (e.g. Severe Combined Immunodeficiency (SCID))
Cardiology: Familial Hypercholesterolemia
Dermatology: Epidermolysis bullosa
Genetics: Muscular Dystrophy, Hurler's Syndrome
Neurology: Huntington's Disease, Myotonic Dystrophy, NGLY1 deficiency

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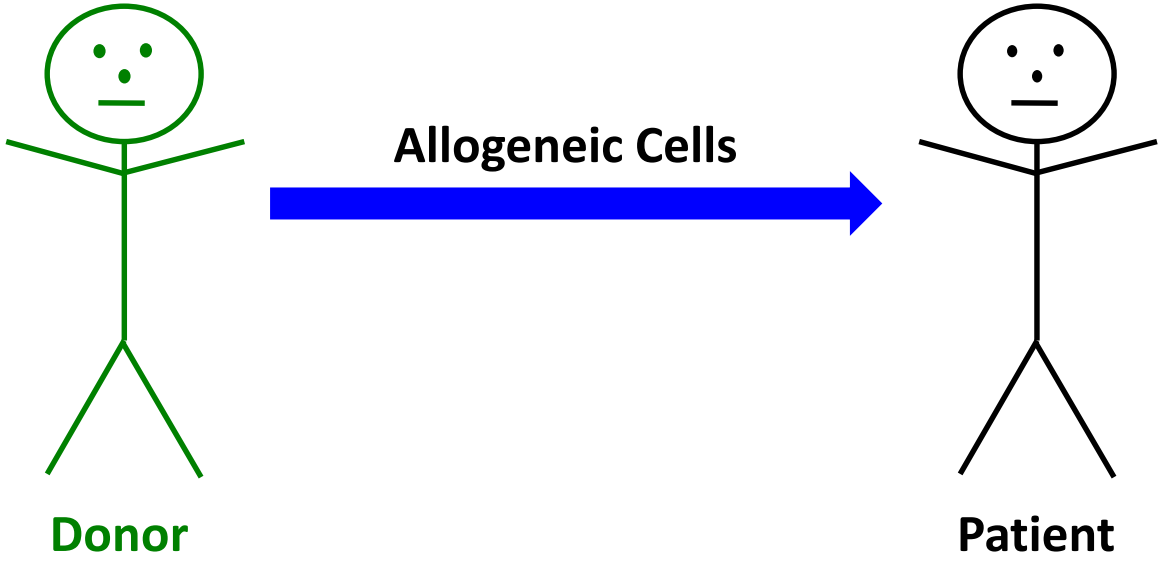
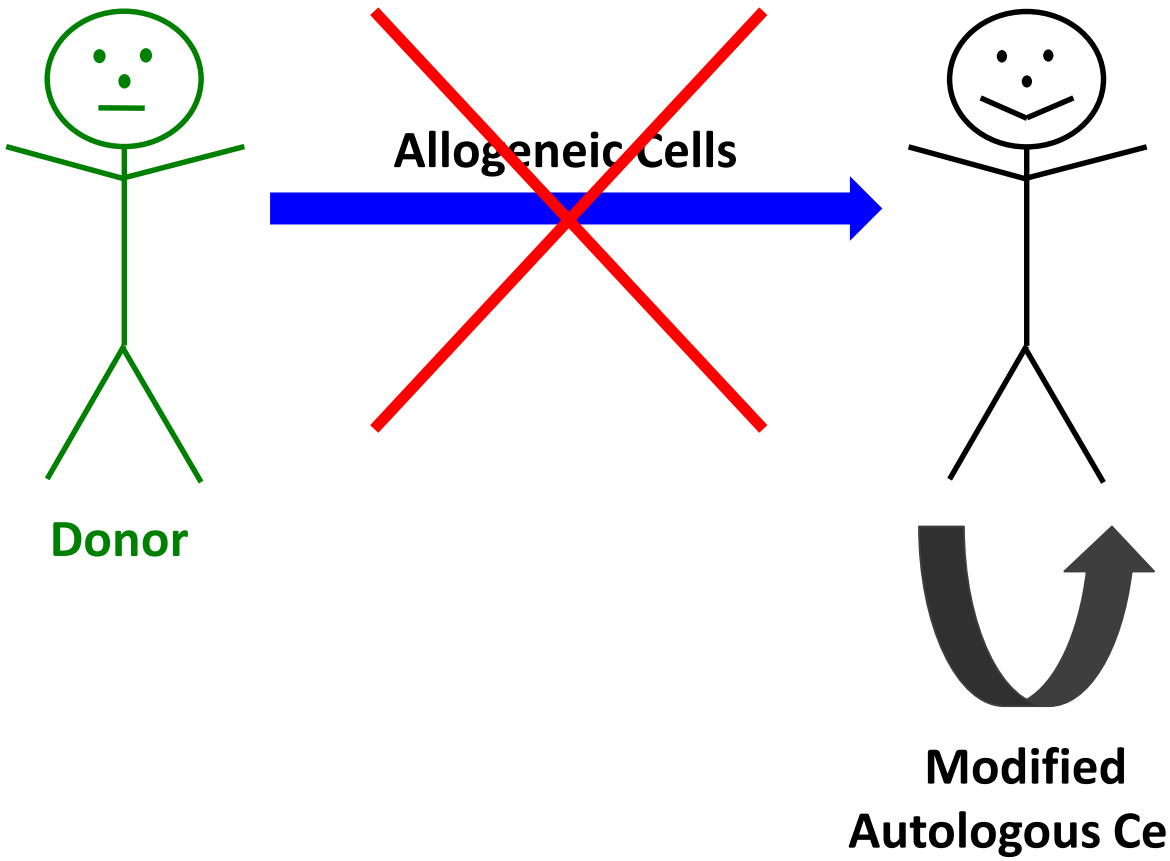
Allo “Gene Therapy” Can Cure Genetic Diseases of the Blood (and then some…)



Replace Allogeneic Cells with Modified Autologous Cells



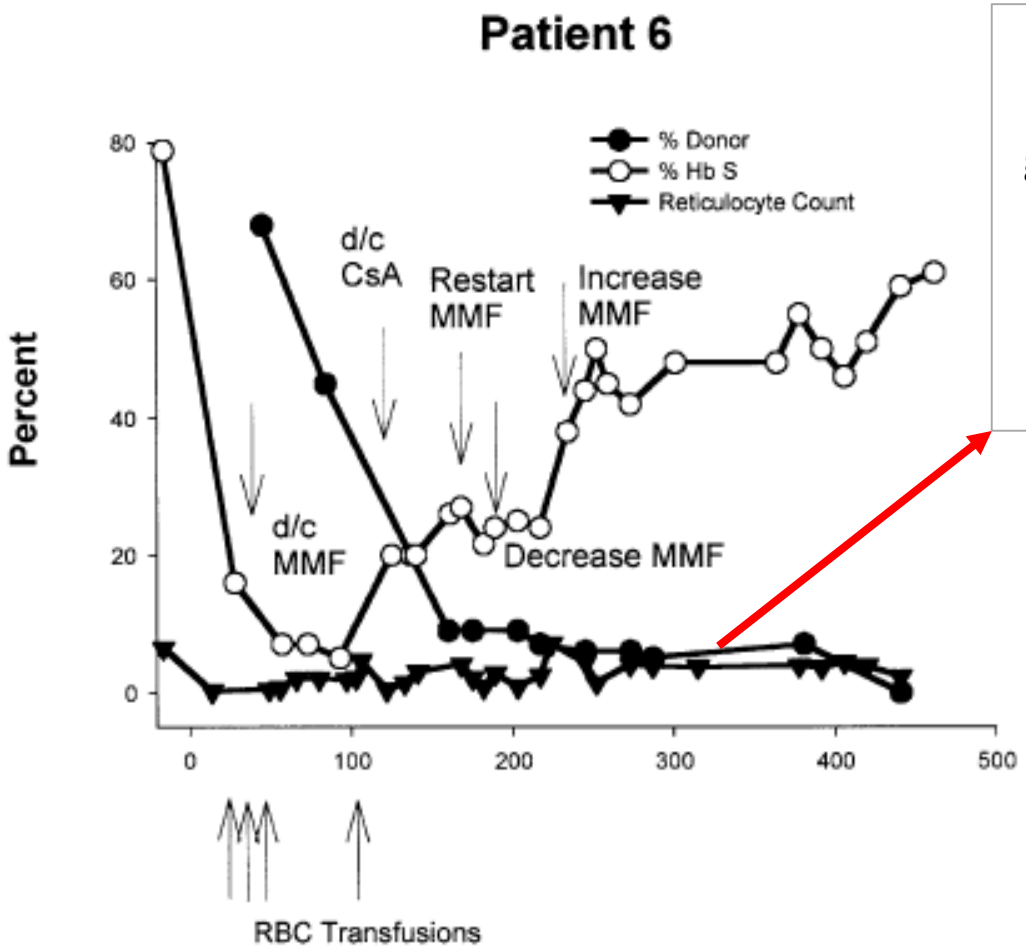
5% Correction of Autologous Cells Would Probably be Sufficient to Cure Sickle Cell Disease

1. **Keeping percentage of sickling red blood cells below 30% cures the symptoms of the disease (shown by chronic transfusions).**

2. **Non-sickling RBCs and RBC precursors have a >30-fold selective advantage (even greater for corrected cells in β-thalassemia)**

Results of Minimally Toxic Nonmyeloablative Transplantation in Patients with Sickle Cell Anemia and β-Thalassemia

Robert Iannone,[1] James F. Casella,[1] Ephraim J. Fuchs,[1] Allen R. Chen,[1] Richard J. Jones,[1] Ann Woolfrey,[2] Michael Amylon,[3] Keith M. Sullivan,[4] Rainer F. Storb,[2] Mark C. Walters[5]



1%-2% WBC chimerism able to give ~50% non-sickle RBC, suppress reticulocyte count and avoid transfusion.

Using Homologous Recombination to Cure Sickle Cell Disease is not a New Idea

Matt ✓

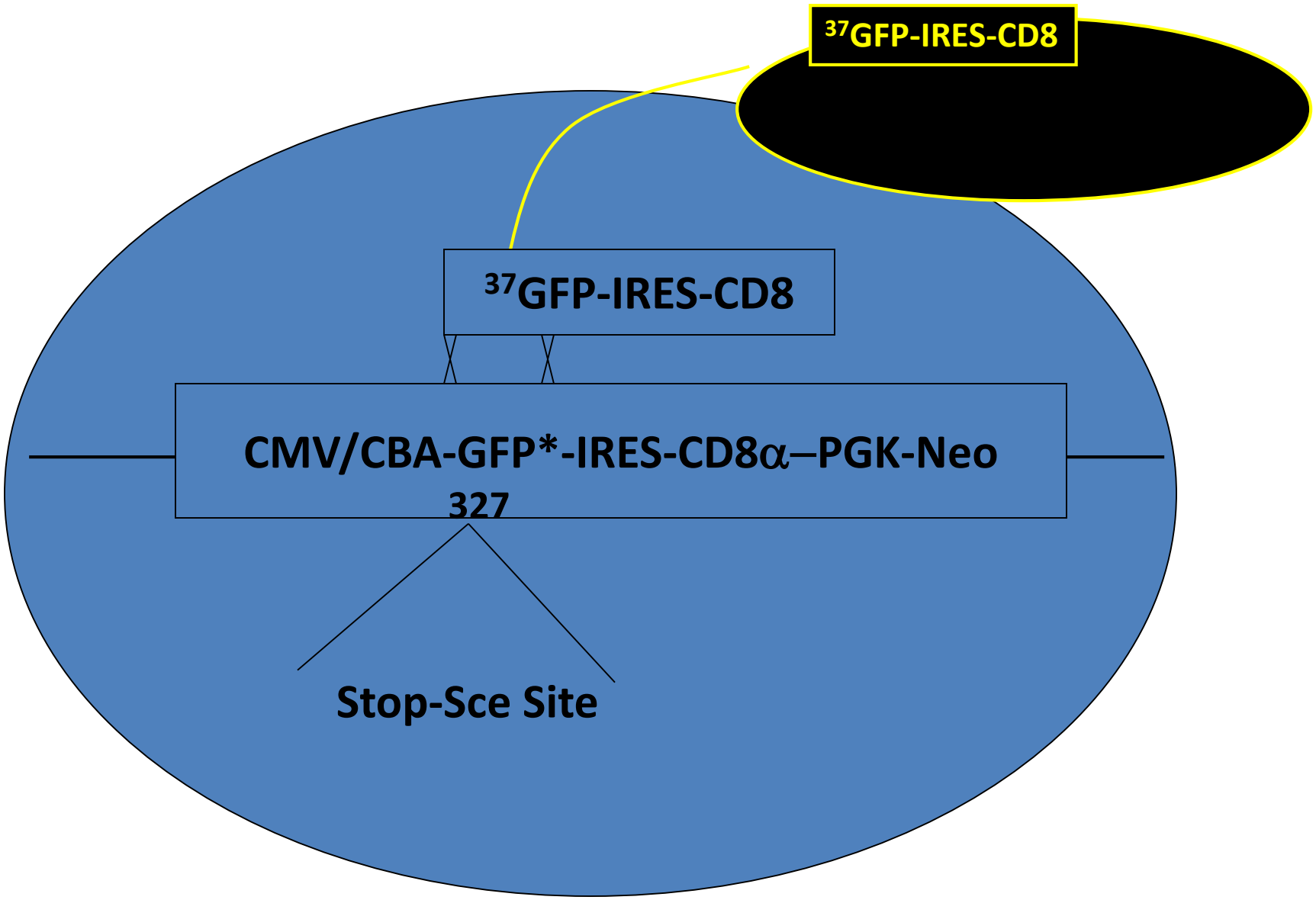
Insertion of DNA sequences into the human chromosomal β-globin locus by homologous recombination

Oliver Smithies*, Ronald G. Gregg*, Sallie S. Boggs†, Michael A. Koralewski* & Raju S. Kucherlapati‡

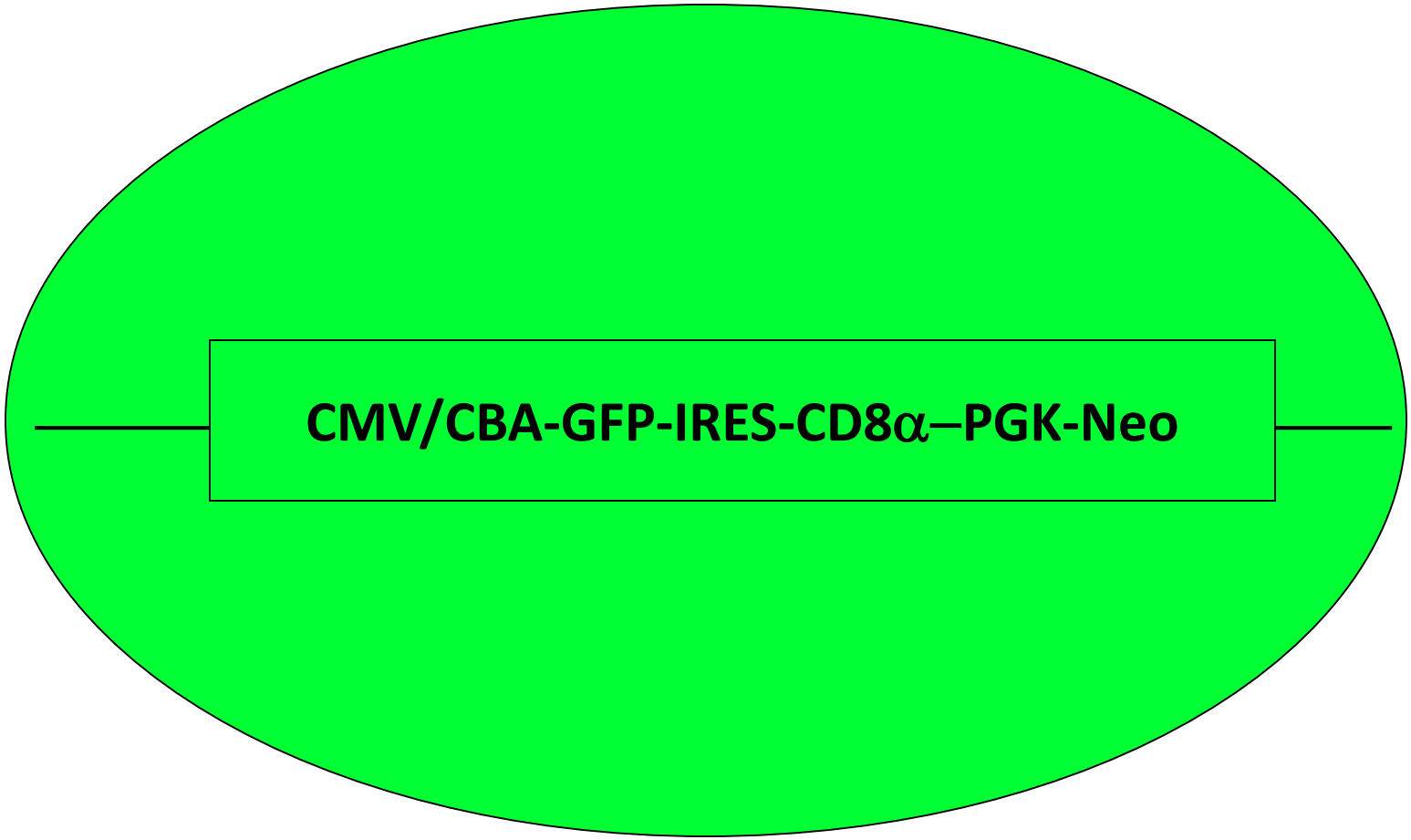
* Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, USA
† School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA
‡ Center for Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612, USA

A 'rescuable' plasmid containing globin gene sequences allowing recombination with homologous chromosomal sequences has enabled us to produce, score and clone mammalian cells with the plasmid integrated into the human β-globin locus. The planned modification was achieved in about one per thousand transformed cells whether or not the target gene was expressed.

GFP Based System to Mimic a Recessive Genetic Disease ("GFP Deficiency")

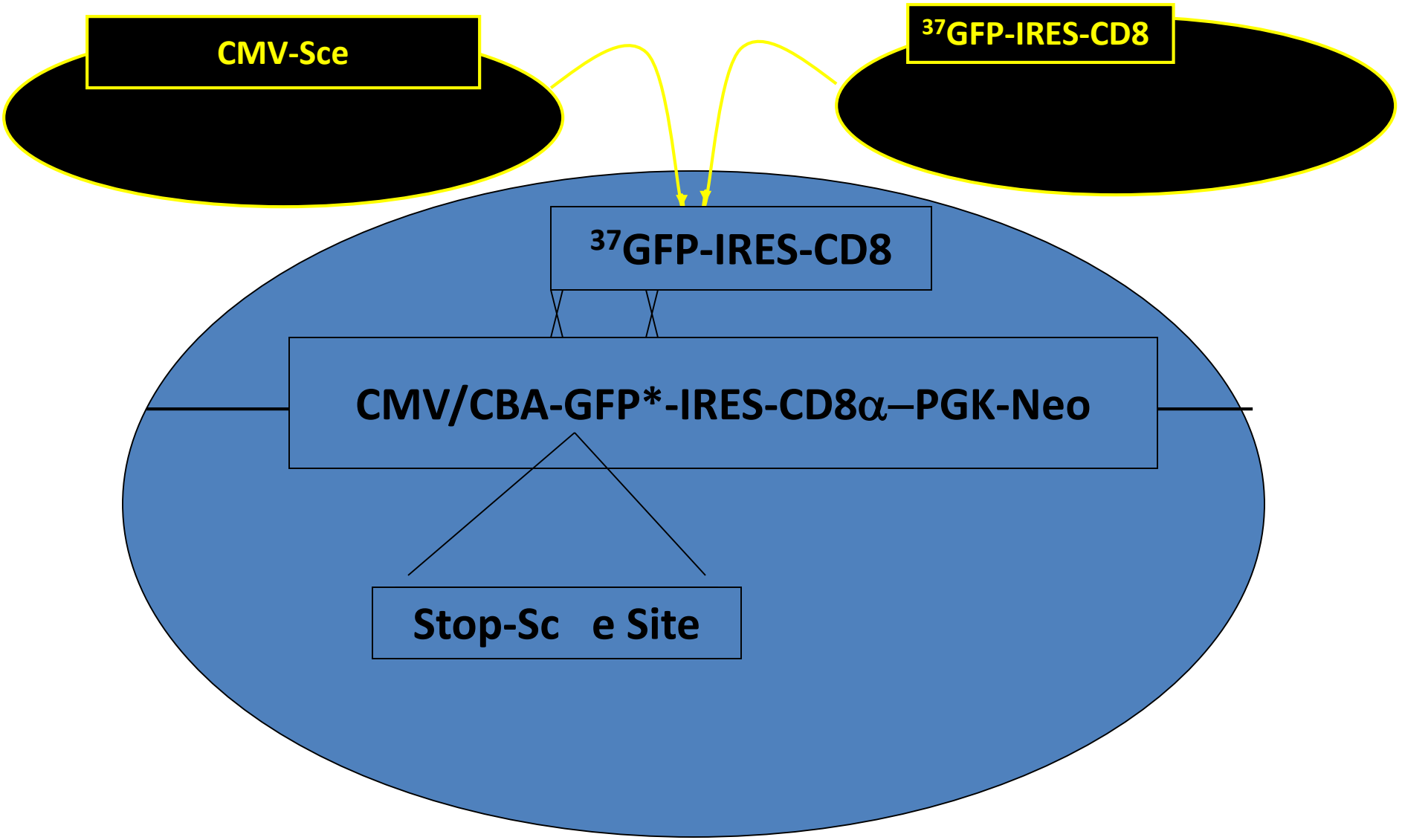


The Problem: The spontaneous rate of gene targeting is too low



Rate= 7.1×10^{-7}
(Approximately 1 per million)

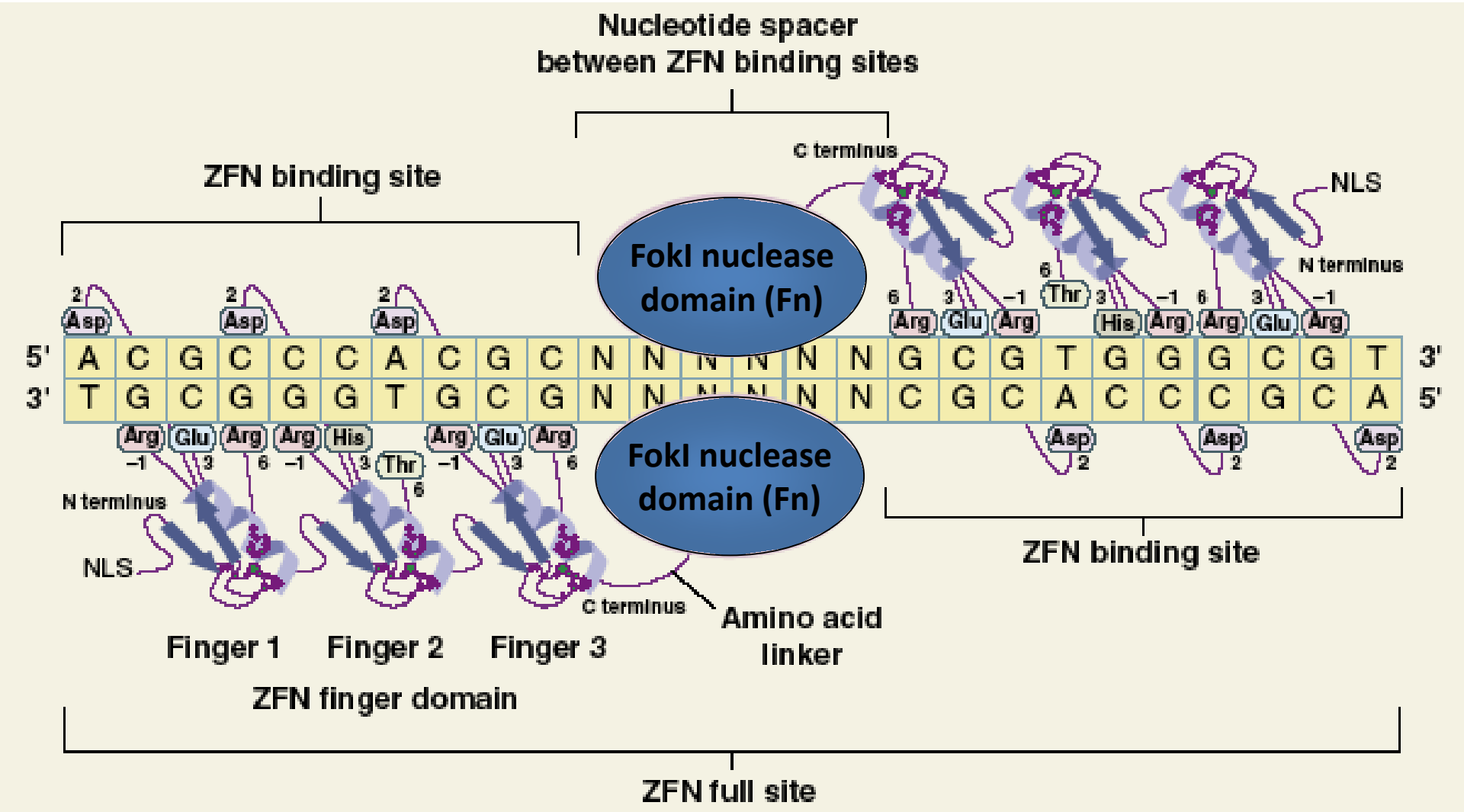
DSB-Mediated Gene Targeting



The Solution: The rate of gene targeting increases to potentially therapeutic levels after the induction of a DSB

Optimized Rate of DSB Mediated Gene Targeting= 3-5 x 10^{-2}
(3-5%)
(builds on pioneering work of Jasin, Nickoloff, Wilson, and Choulika)

Zinc Finger Nucleases as a Method to Create Double-Strand Breaks in Endogenous Genes



Initially developed by labs of Srinivasan Chandrasegaran (Johns Hopkins) and Dana Carroll (Univ. Utah)

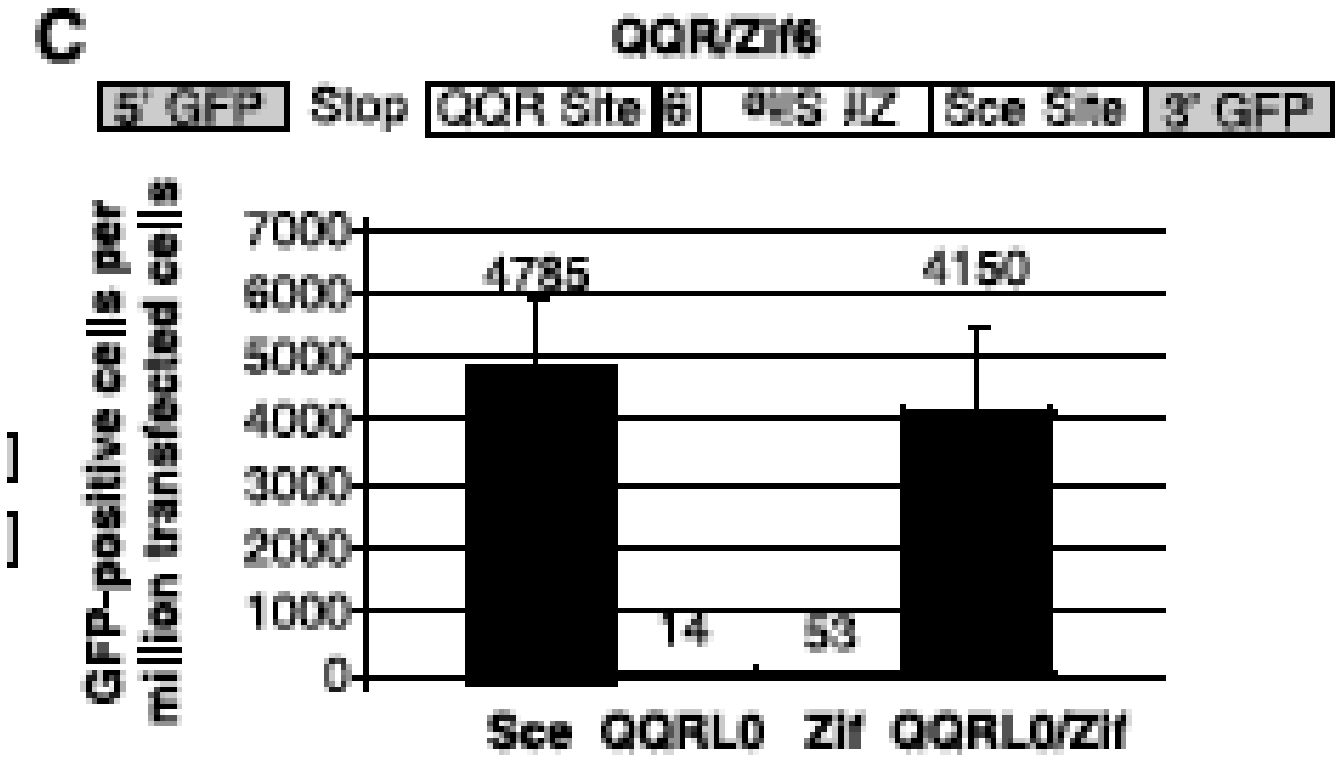
First Use of an Engineered Nuclease to Stimulate Genome Editing in Human Somatic Cells (2003)

"Zinc Finger Nuclease"

"Genome Editing"

Chimeric Nucleases Stimulate Gene Targeting in Human Cells

Matthew H. Porteus* and David Baltimore



Paralleled First Use of Engineered Nucleases to Edit Genome of Model Organisms (2002, 2003)

Targeted Chromosomal Cleavage and Mutagenesis in Drosophila Using Zinc-Finger Nucleases

Marina Bibikova,*,[1] Mary Golic,†,[2] Kent G. Golic†,[2] and Dana Carroll*,[3]

*Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132 and †Department of Biology, University of Utah, Salt Lake City, Utah 84112

Manuscript received January 25, 2002
Accepted for publication March 31, 2002

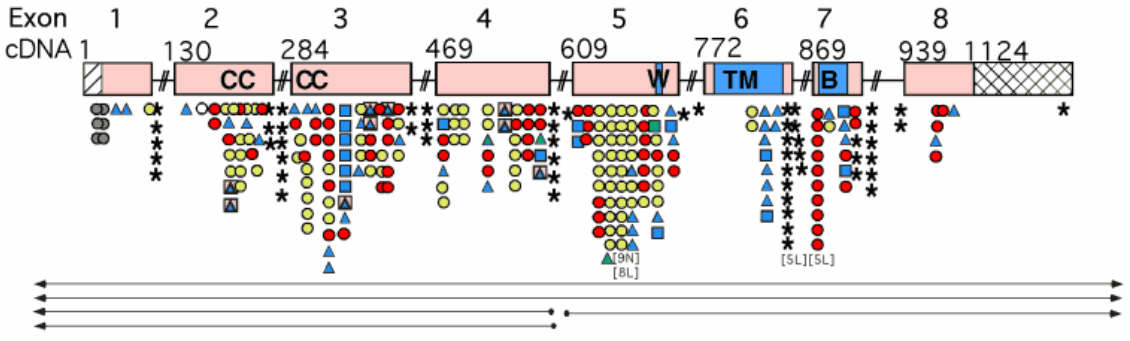
BREVIA

Enhancing Gene Targeting with Designed Zinc Finger Nucleases

Marina Bibikova,* Kelly Beumer, Jonathan K. Trautman, Dana Carroll†

A: …ATG GTG CAC CTG ACT CCT GAG GAG AAG …
S: …ATG GTG CAC CTG ACT CCT GTG GAG AAG …

Genome Editing is the method to correct typographical errors



Detour: Genome Editing of Spermatogonial Stem Cells

Genome Editing in Mouse Spermatogonial Stem/Progenitor Cells Using Engineered Nucleases

Danielle A. Fanslow[1], Stacey E. Wirt[2], Jenny C. Barker[3], Jon P. Connelly[3], Matthew H. Porteus[2*], Christina Tenenhaus Dann[1*]

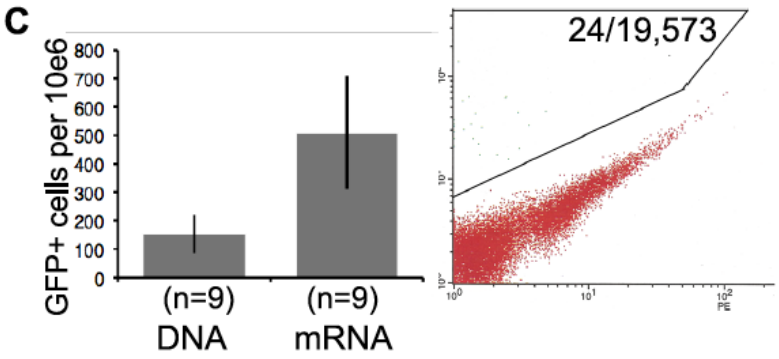
Sato et al (2015) Genome Editing in Mouse Spermatogonial Stem Cell Lines using TALENs and Double-Nicking CRISPR/Cas9. Stem Cell Reports 14: 75-82

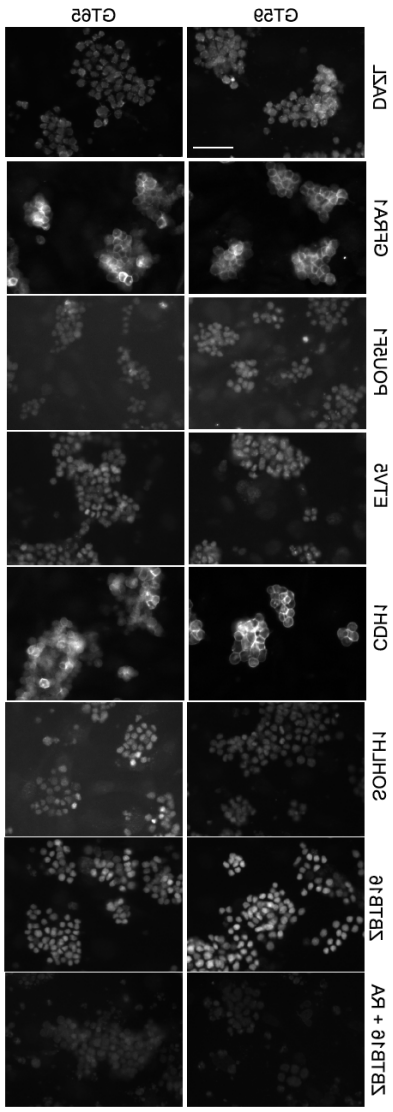
Chapman et al (2015) Targeted Germline Modifications in Rats using CRISPR/Cas9 and Spermatogonial Stem Cells. Cell Reports 10: 1828-35.

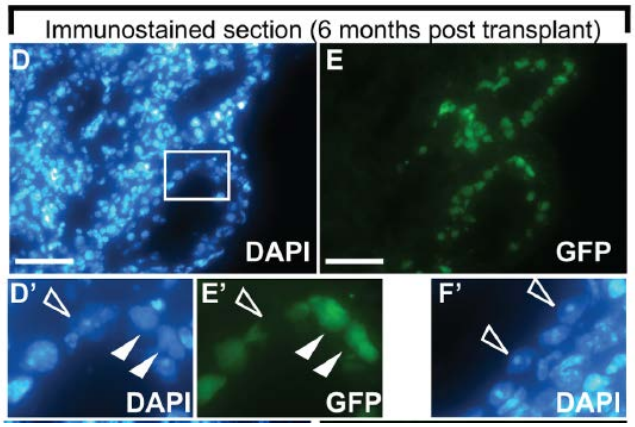
Wu et al (2015) Correction of a genetic disease by CRISPR-Cas9 mediated gene editing in mouse spermatogonial stem cells. Cell Research 25: 67-79.

Genome Editing in Mouse Spermatogonial Stem/Progenitor Cells Using Engineered Nucleases

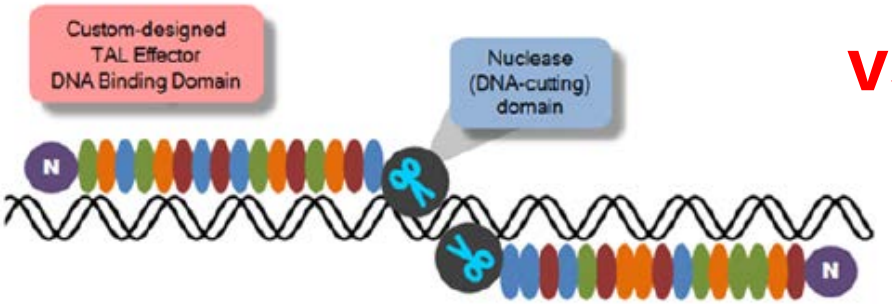
Danielle A. Fanslow[1], Stacey E. Wirt[2], Jenny C. Barker[3], Jon P. Connelly[3], Matthew H. Porteus[2]*, Christina Tenenhaus Dann[1]*





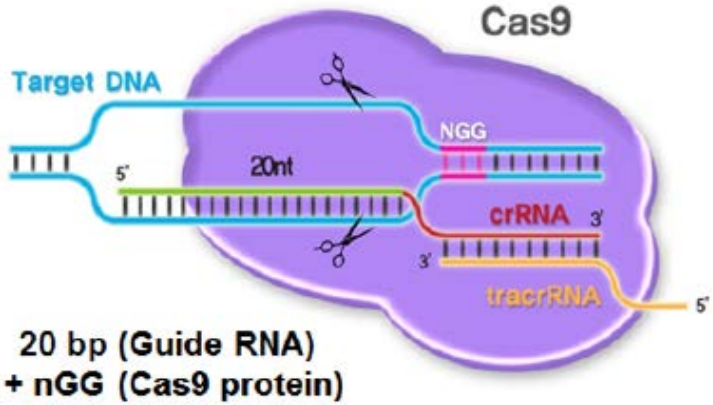


TALEN vs CRISPR/Cas9



TAL Effector Nuclease (TALEN) Dimer

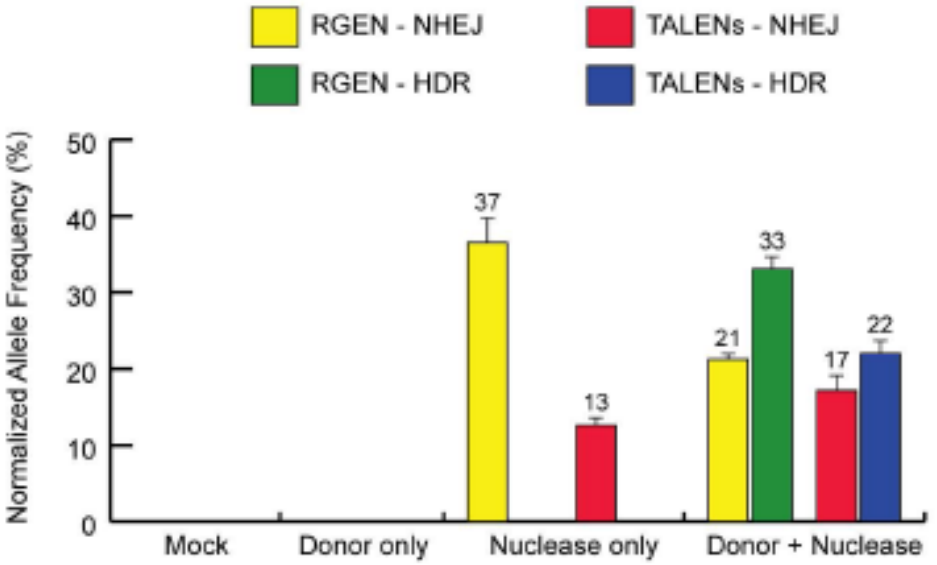
vs.



RNA-Guided Endonucease (RGEN) CRISPR/Cas9

Comparison of Nuclease Platforms in K562 Cells

IL2RG locus target site:



Cell Reports 2014

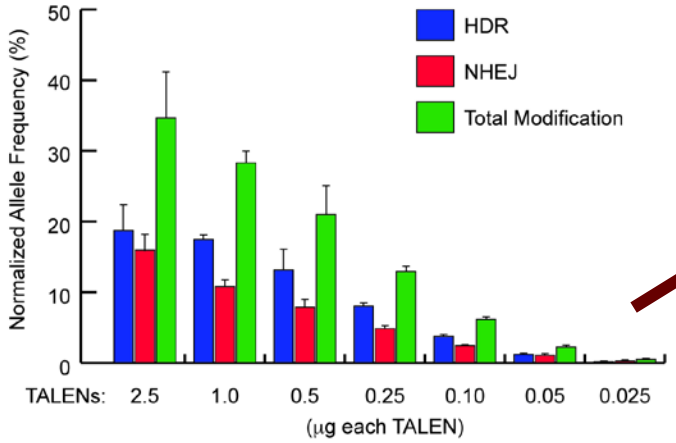
Quantifying Genome-Editing Outcomes at Endogenous Loci with SMRT Sequencing

Ayal Hendel,[1,4] Eric J. Kildebeck,[1,4] Eli J. Fine,[2,4] Joseph T. Clark,[1] Niraj Punjya,[1] Vittorio Sebastiano,[3] Gang Bao,[2] and Matthew H. Porteus[1,*]

[1]Department of Pediatrics, Stanford University, Stanford, CA 94305, USA
[2]Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA
[3]Department of Obstetrics and Gynecology, Stanford University, Stanford, CA 94305, USA
[4]These authors contributed equally to this work
*Correspondence: mporteus@stanford.edu
http://dx.doi.org/10.1016/j.celrep.2014.02.040

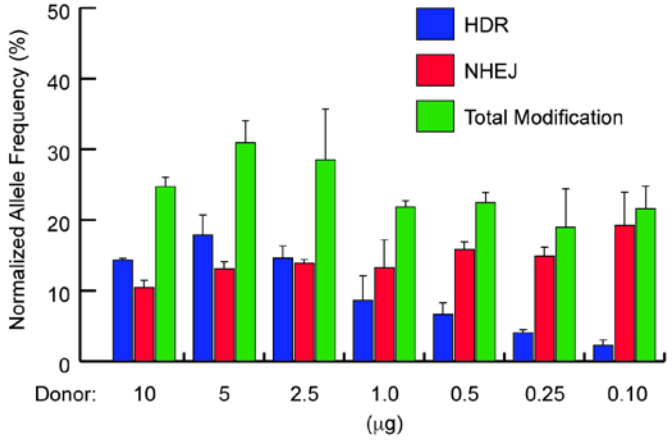
Shifting Genome Editing Outcomes by Changing Different Parameters

A

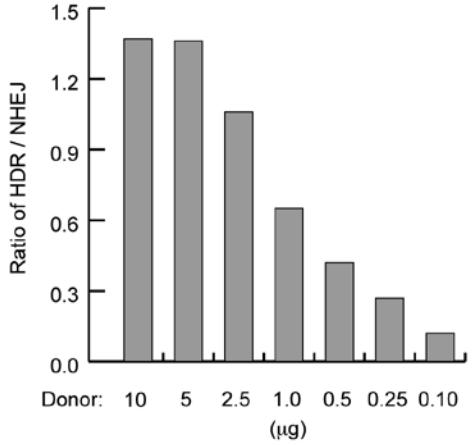


More Nuclease Leads to More Editing (both HR and NHEJ Mediated

B

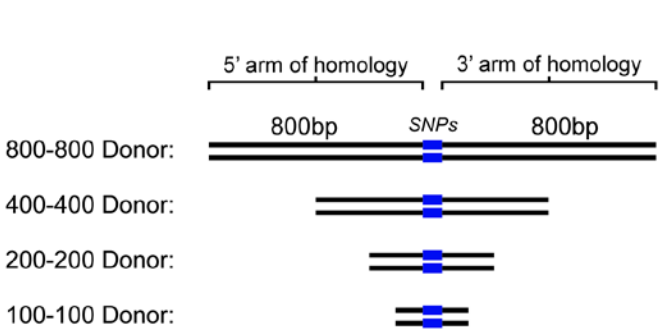


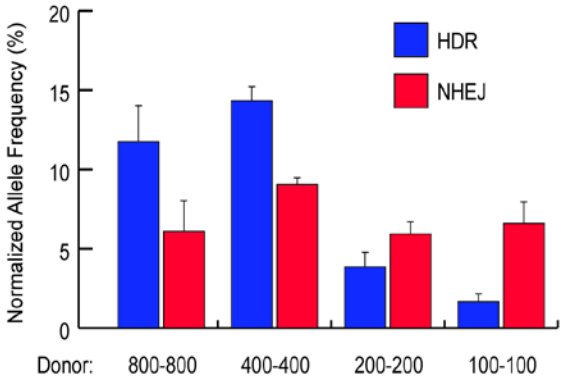
C



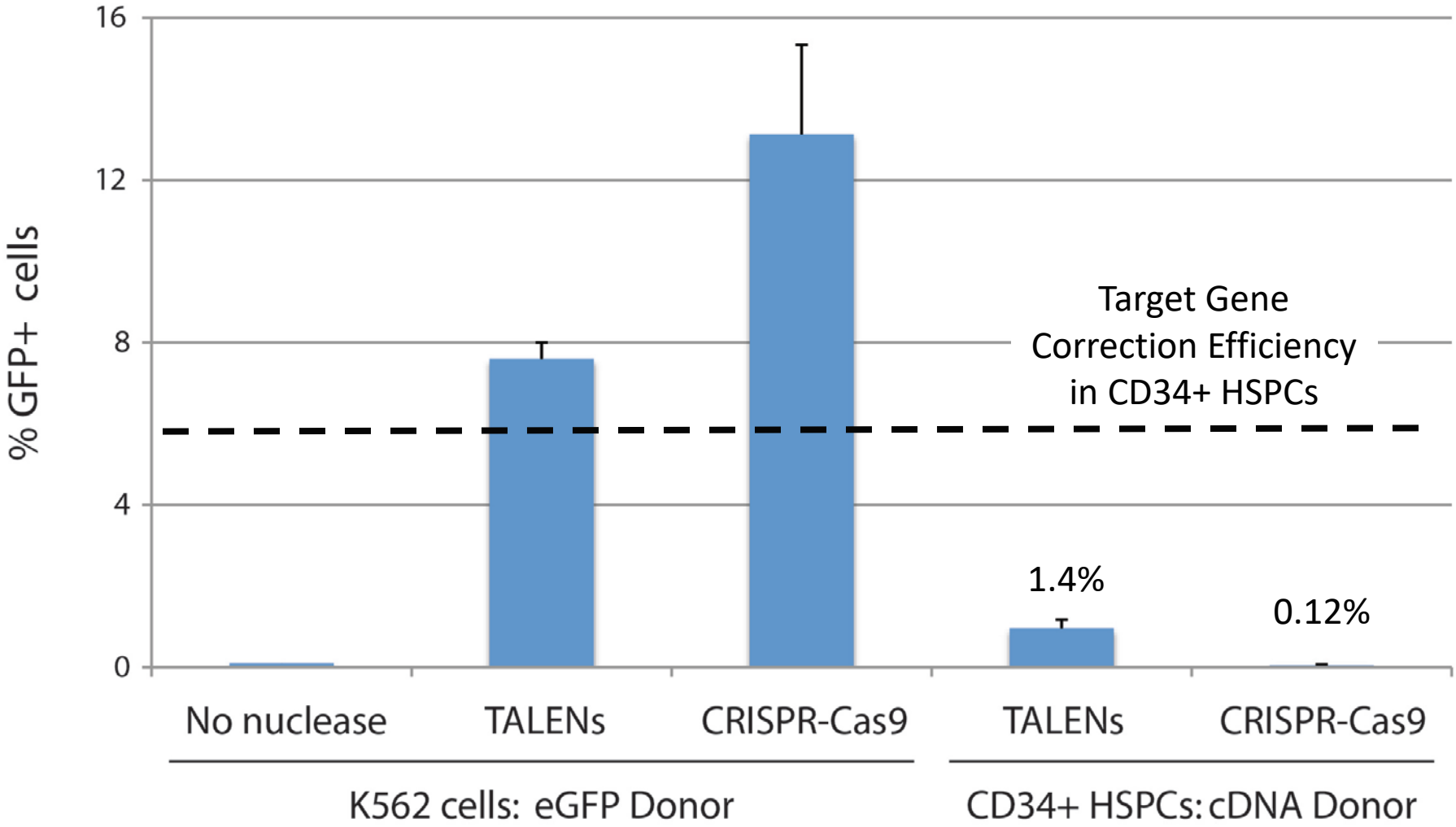
More Donor Shifts to HR Mediated Editing From NHEJ Mediated Editing

D





Genome Editing by Homologous Recombination using CRISPR/Cas9 vs TALENs in different cell types

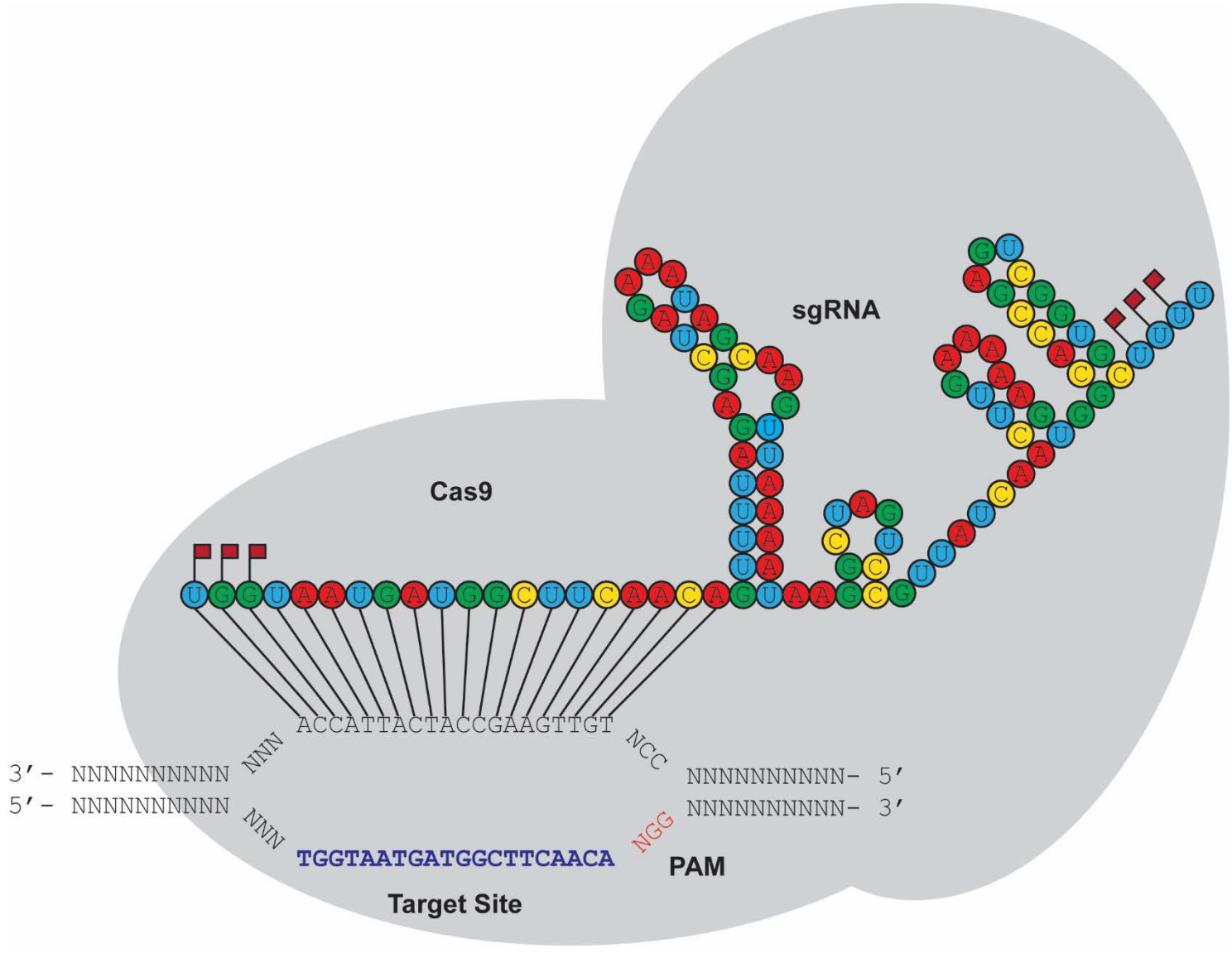


Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells

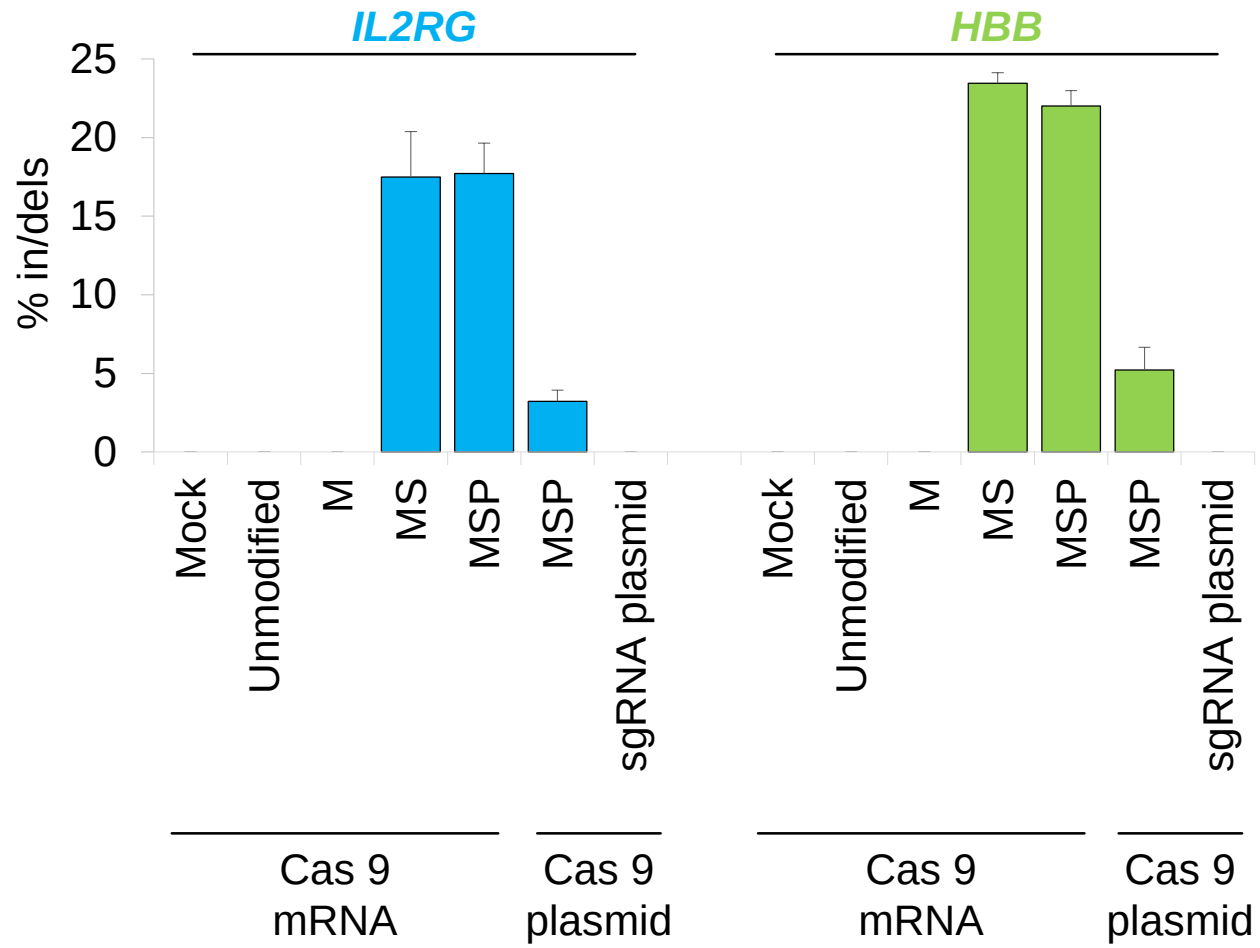
Ayal Hendel[1,5], Rasmus O Bak[1,5], Joseph T Clark[1], Andrew B Kennedy[2], Daniel E Ryan[2], Subhadeep Roy[3], Israel Steinfeld[4], Benjamin D Lunstad[3], Robert J Kaiser[2], Alec B Wilkens[1], Rosa Bacchetta[1], Anya Tsalenko[2], Douglas Dellinger[3], Laurakay Bruhn[2] & Matthew H Porteus[1]

Collaboration with Agilent Research Laboratories

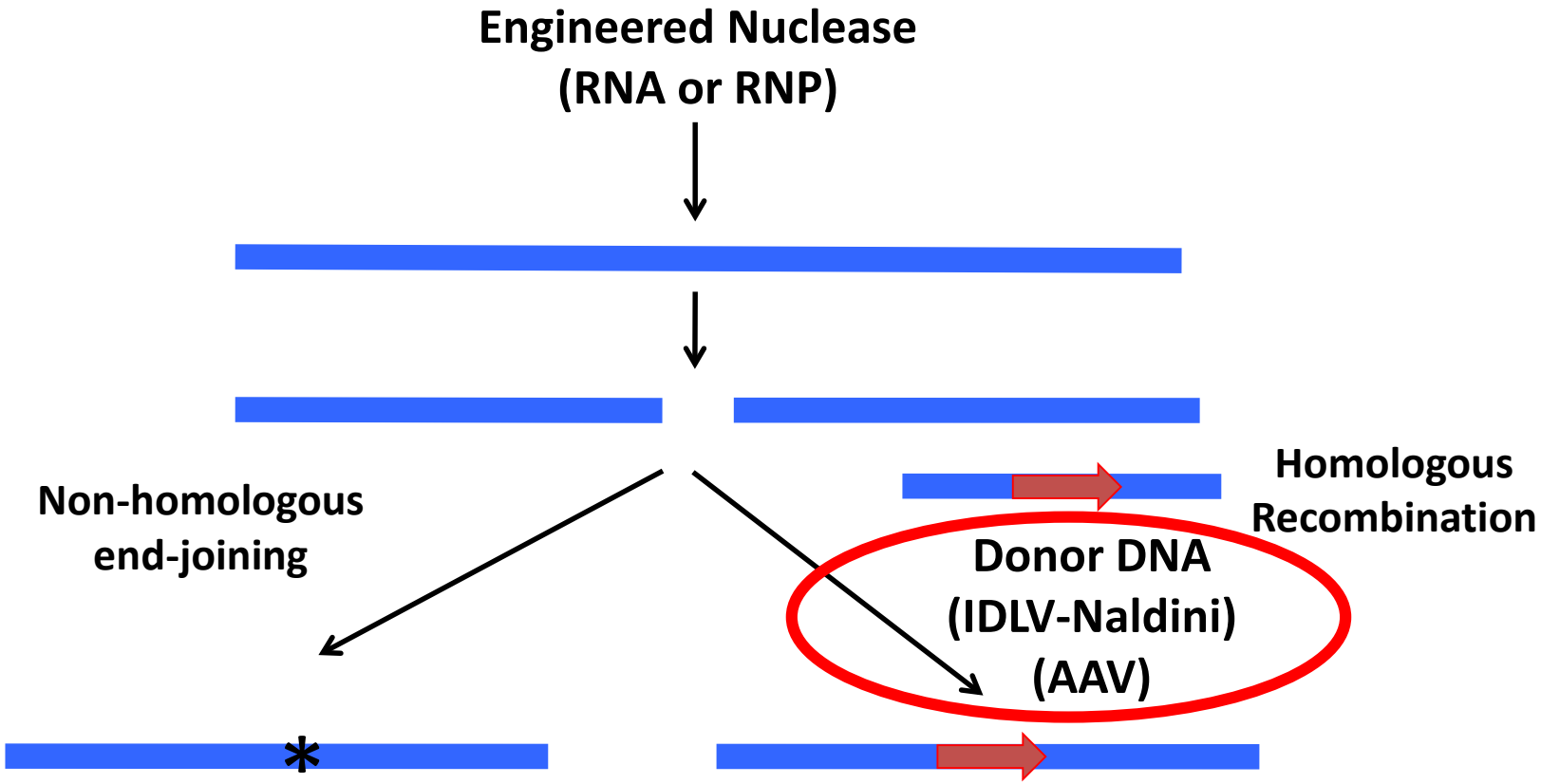
Nature Biotechnology 2015


sgRNA
Cas9
ACCATTACTACCGAAGTTGT
NNN
NCC
3′- NNNNNNNNNN
5′- NNNNNNNNNN
NNN
NNNNNNNNNN- 5′
NNNNNNNNNN- 3′
NGG
TGGTAATGATGGCTTCAACA
PAM
Target Site

Modified sgRNAs Enhance Gene Disruption in CD34+ HSPCs



Components for Genome Editing



AAV is an Efficient Method to Deliver the Donor Molecule

Efficient Gene Targeting Mediated by Adeno-Associated Virus and DNA Double-Strand Breaks

Matthew H. Porteus,[1]* Toni Cathomen,[2] Matthew D. Weitzman,[2] and David Baltimore[1]

California Institute of Technology, Pasadena, California 91125,[1] and Laboratory of Genetics, Salk Institute for Biological Studies, La Jolla, California 92037[2]

Human Gene Targeting by Adeno-Associated Virus Vectors Is Enhanced by DNA Double-Strand Breaks

Daniel G. Miller,[1] Lisa M. Petek,[2] and David W. Russell[2]*

Department of Medicine, Division of Hematology,[2] and Division of Medical Genetics,[1] University of Washington, Seattle, Washington

Gene Therapy (2010) 17, 1175–1180

www.nature.com/gt

ORIGINAL ARTICLE

Self-complementary AAV mediates gene targeting and enhances endonuclease delivery for double-strand break repair

ML Hirsch[1], L Green[1], MH Porteus[2,3] and RJ Samulski[1,4]

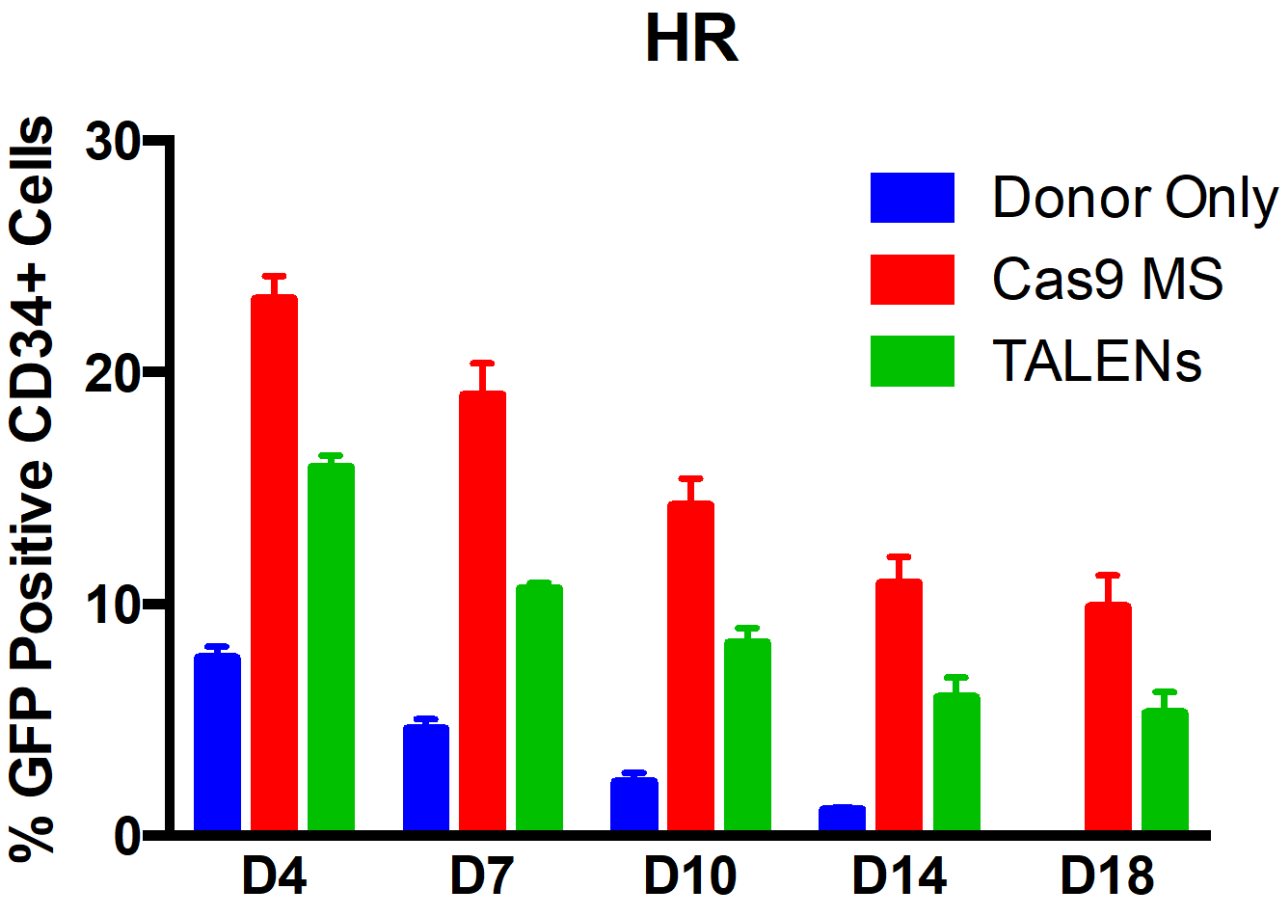
[1]UNC Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [2]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA and [4]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ORIGINAL ARTICLE

Zinc-finger nuclease-mediated gene correction using single AAV vector transduction and enhancement by Food and Drug Administration-approved drugs

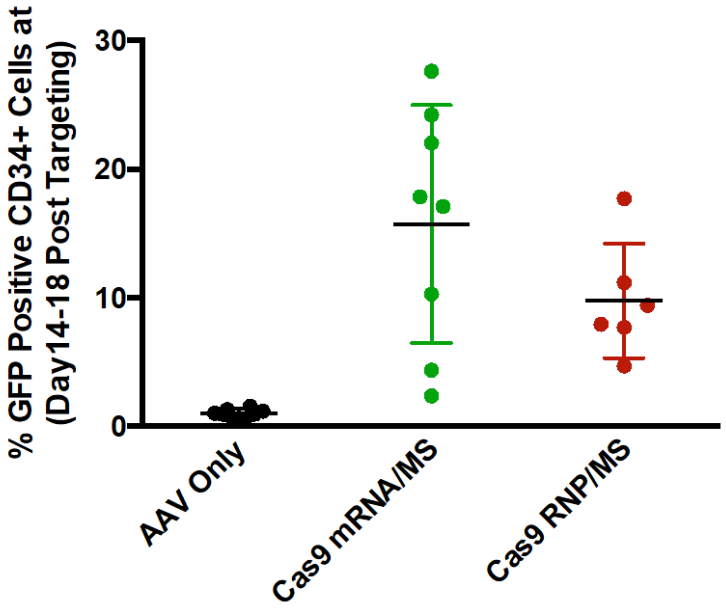
BL Ellis[1,5], ML Hirsch[2,5], SN Porter[3], RJ Samulski[2,4] and MH Porteus[3]

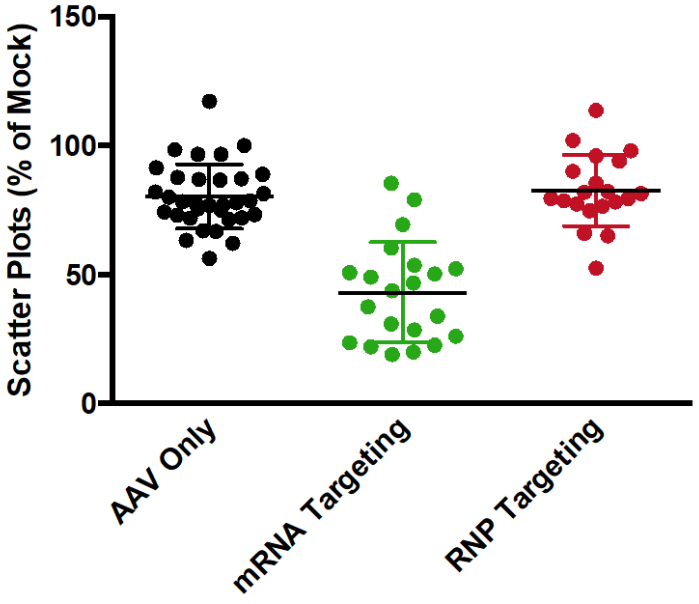
CRISPR/Cas9 AAV6 System Mediates High Frequencies of HR in HSPCs at *HBB* Locus



(patent pending)

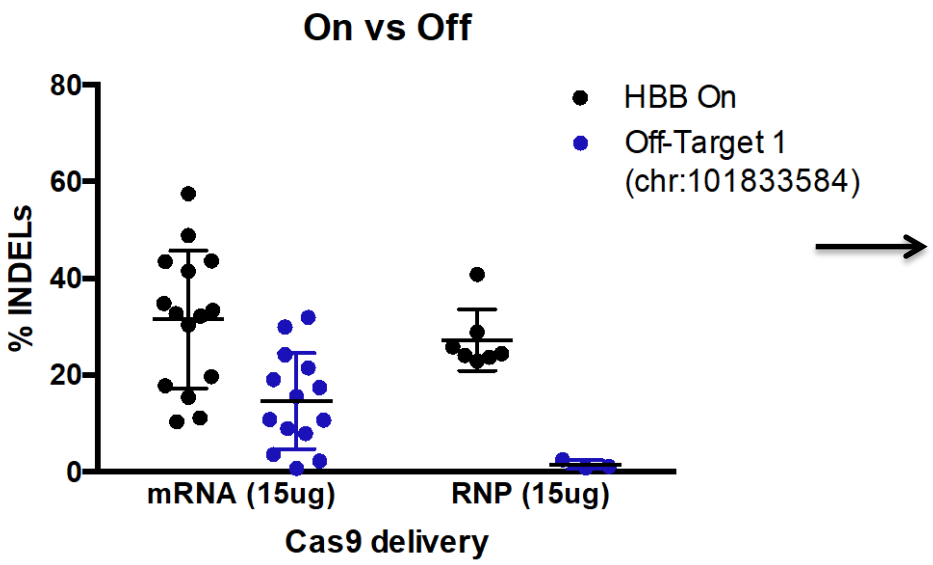
CRISPR/Cas9 Ribonucleoprotein System Superior to "All RNA" System for Cell Viability

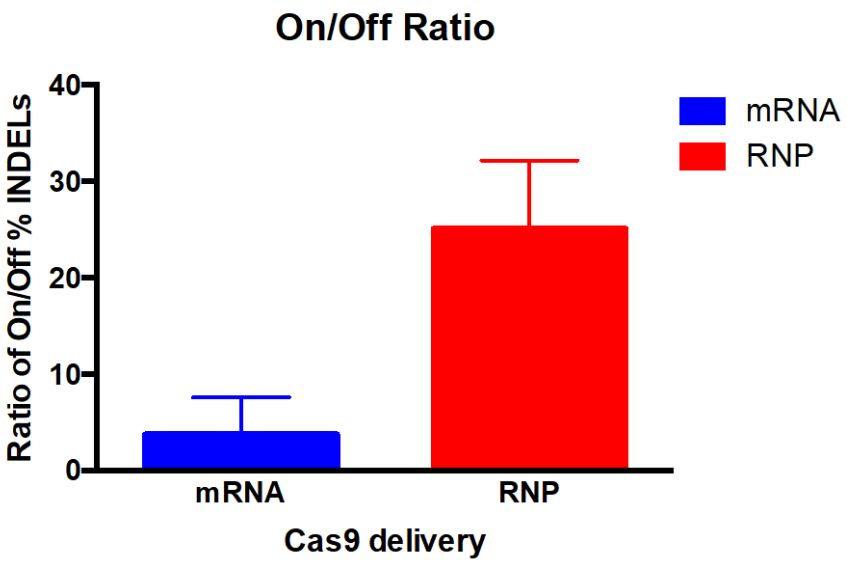




Patent Pending

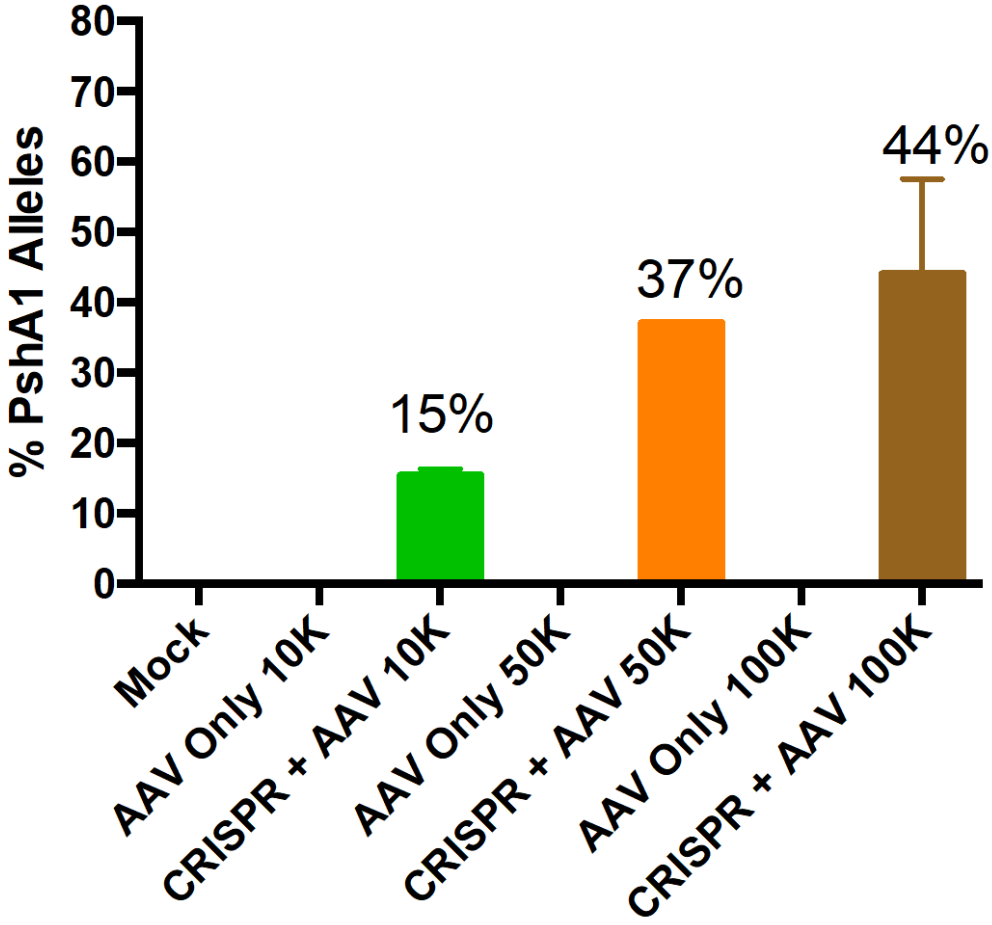
CRISPR/Cas9 Ribonucleoprotein System Superior to "All RNA" System for Off-Target Activity





Patent Pending

Targeting HbS Allele to *HBB* in mPB CD34+ HSPCs using rAAV6 RFLP Donor Vector



Patent Pending

Different Strategies to Assess Safety/Toxicity

(no predictive assays for human safety for gene modified cells)

George Box: "Essentially, all models are wrong, but some are useful"

1. **Bioinformatic Approaches (PROGNOS, COSMID…)**

2. **Unbiased *in vitro* Approaches**
   - SELEX, David Liu's group

3. **Unbiased Approaches in Cells**
   - γH2AX Foci Formation
   - Capture Based Assays (AAV, IDLV, Oligonucleotides…)
   - Translocation Based Assays (HTGTS)
   - BLESS
   - Digenome Seq
   - Whole genome/exome sequencing (useful to evaluate clones)
   - Targeted deep sequencing (not developed)

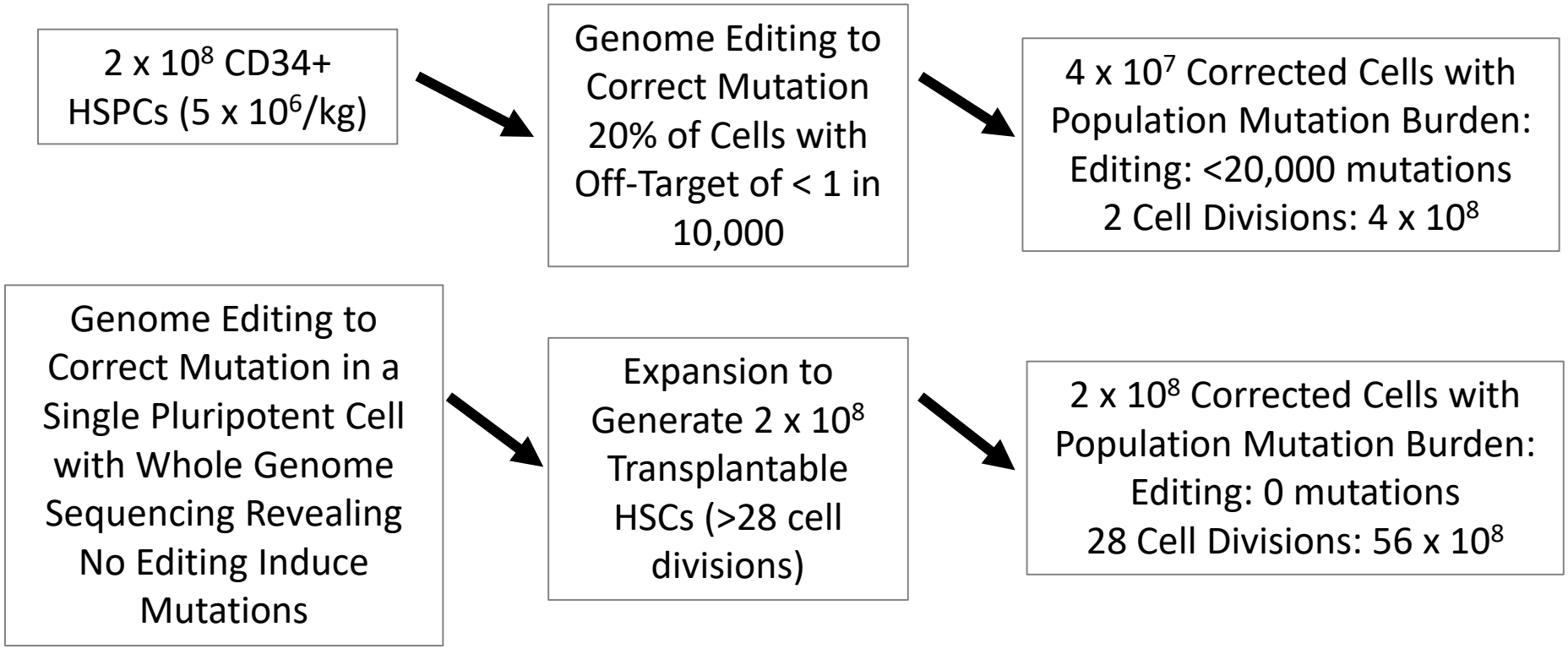
4. **Functional Assays** (a more traditional small molecule pharm-tox approach)
   - Relative Cell Survival
   - Cell Cycle Dynamics
   - Clonal Dynamics
   - Tumorgenicity in mouse models
   - Lineage Reconstitution
   - Mouse Cancer Pre-Disposition (a la Cesana et al (2014))

Genome Engineering in the Context of Natural Genome Instability/Genome Variation

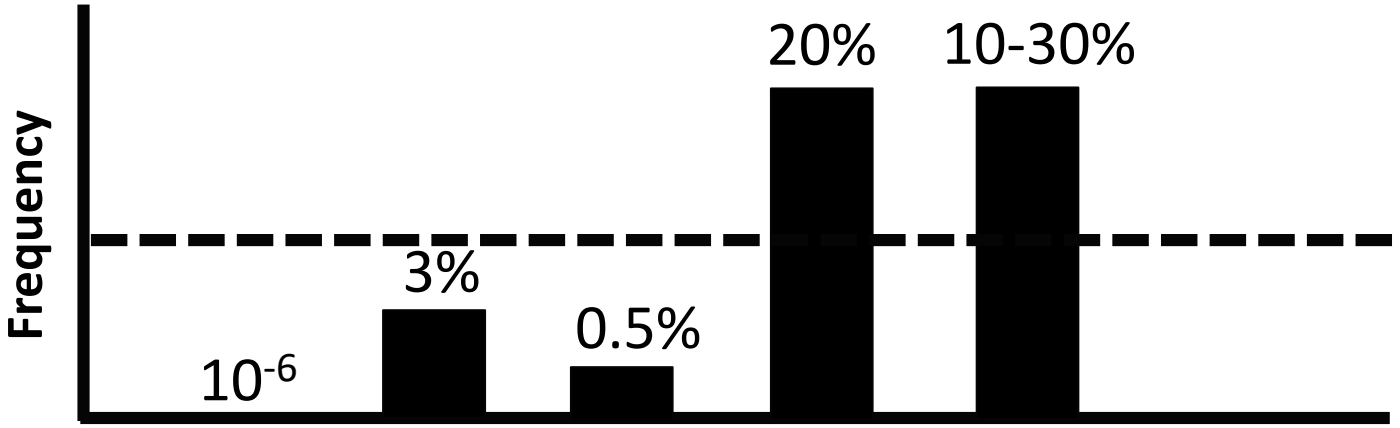
Baseline Per Person: 2.4 million SNVs, 500-600K In/Dels (355 Exonic, 91 Frameshift), ~3000 structural variants (i.e. Dewey et al 2014 JAMA)

Spontaneous: -20 DSBs/day

1-10 mutations/day/cell leading to ~1 million mutations/second/person



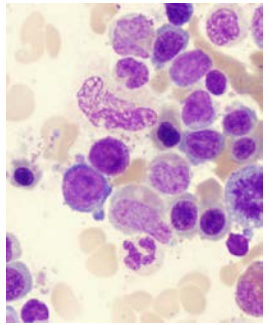
Advances in Genome Editing to Cure Sickle Cell Disease



Year:	1985	1994	2003	2014	2015
Nuclease:	0	I-Sce	ZFN	TALEN	CRISPR/Cas9
Gene:	HBB	Neo	GFP	HBB	HBB
Cell Type:	EJ Carc	3T3	293	K562	CD34+ HSPCs

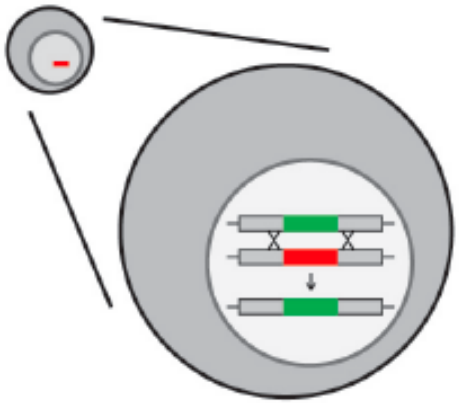
Manufacturing of Autologous Gene Corrected Cell Product

Hematopoietic Stem Cells Harvested from Patient

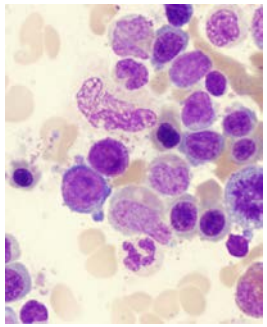


Minimum: 5 x 10^6 CD34/kg
Ideal: >10 x 10^6 CD34/kg

Genome Editing: Gene correction with nuclease mediated homologous recombination



GMP Grade Cas9 Protein
GMP Grade sgRNA (Agilent)
GMP Grade AAV6
GMP Grade Electroporation



Transplant Autologous Gene Corrected Cells Back into Patient

(Busulfan 8 mg/kg)

Minimum: 2 x 10^6 CD34/kg
Ideal: >5 x 10^6 CD34/kg
At
Minimum: 5% Gene Correction
Ideal: >10% Gene Correction
With
No off-target mutations/rearrangements above background and no signs of functional toxicity

Conclusions

1. Advances in genome editing of somatic stem cells may obviate the need to do germline genome editing for many diseases.
   - Also highlights that technology will continue to improve.

2. Genome editing can be used to increase the therapeutic potency of somatic cells (synthetic biology vs “enhancement”).

3. Possible to use engineered nucleases to edit spermatogonial stem cells.

4. There remain challenges in doing *in vivo* genome editing related to the ***immunogenicity*** of the nucleases, delivery vectors, and the risk of ***low-grade on-going DNA damage*** from sustained expression of the nuclease (so need to express nuclease transiently).

5. Need to establish a set of toxicology/safety assays that will satisfy FDA and consenting clinicians.

Thank You

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