Second International Summit on Human Genome Editing

Convened by:

The Academy of Sciences of Hong Kong
The Royal Society
U.S. National Academy of Sciences
U.S. National Academy of Medicine

Lee Shau Kee Lecture Centre
Centennial Campus
The University of Hong Kong

November 27-29, 2018

Gene Correction via Genome Editing of Hematopoietic Stem Cells to Cure Sickle Cell Disease

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28 November 2018

Conflicts of Interest

CRISPR Therapeutics:	Equity and SAB
Allogene Therapeutics:	SAB
Synthego Corporation:	SAB

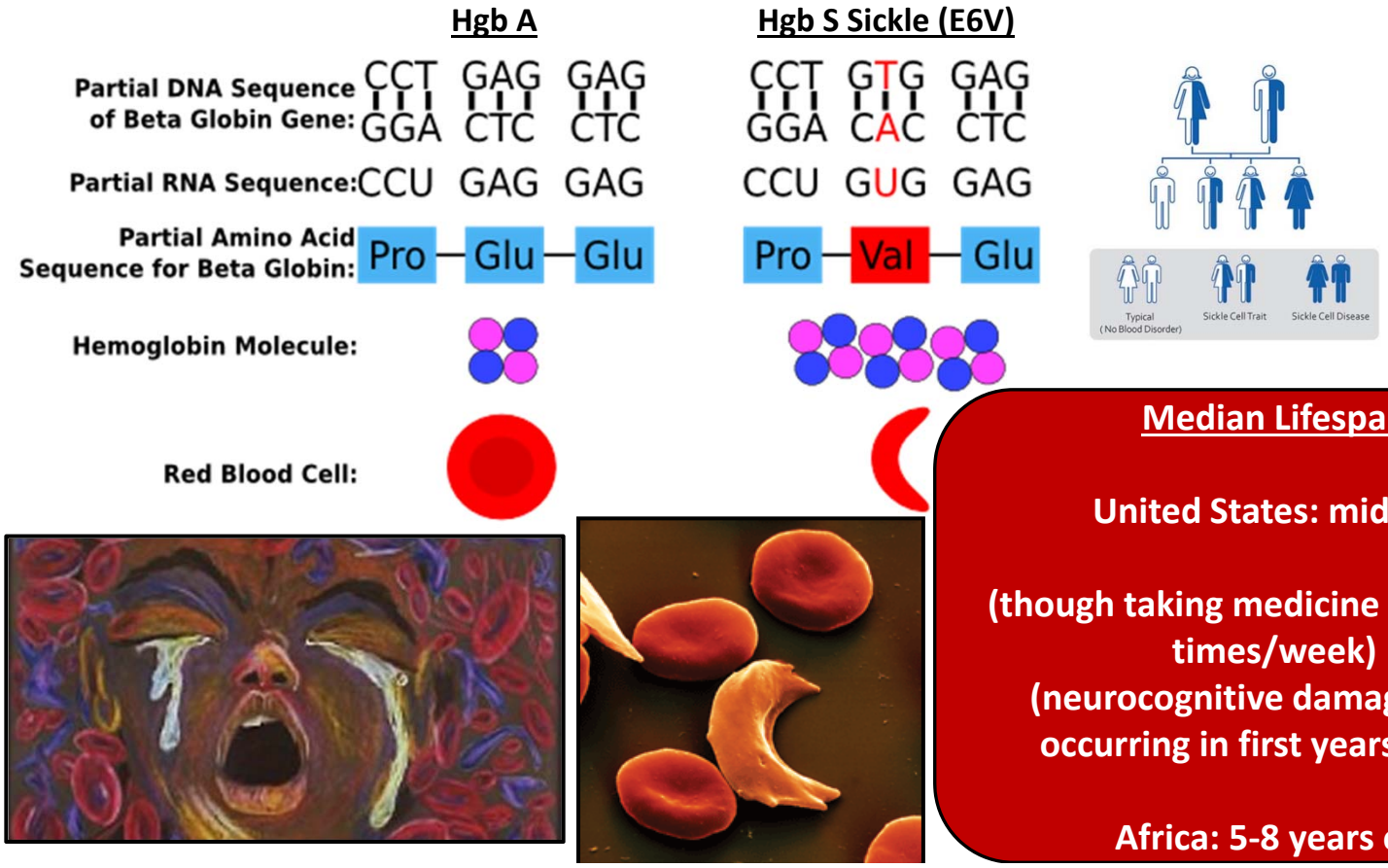
Managed through Stanford in accordance with their conflict of interests policy.

Monogenic Diseases Permeate Medicine
(6,000-10,000 such diseases)
(Patients: 30 million in USA, 350 million worldwide)

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, MPS I
Neurology: Huntington's Disease, Myotonic Dystrophy, NGLY1 deficiency

Each patient affects a larger community of people (echoes of the disease) + life years saved

Sickle Cell Disease is Caused by a Single Nucleotide Variant in the *HBB* Gene



Median Lifespan

United States: mid-40s

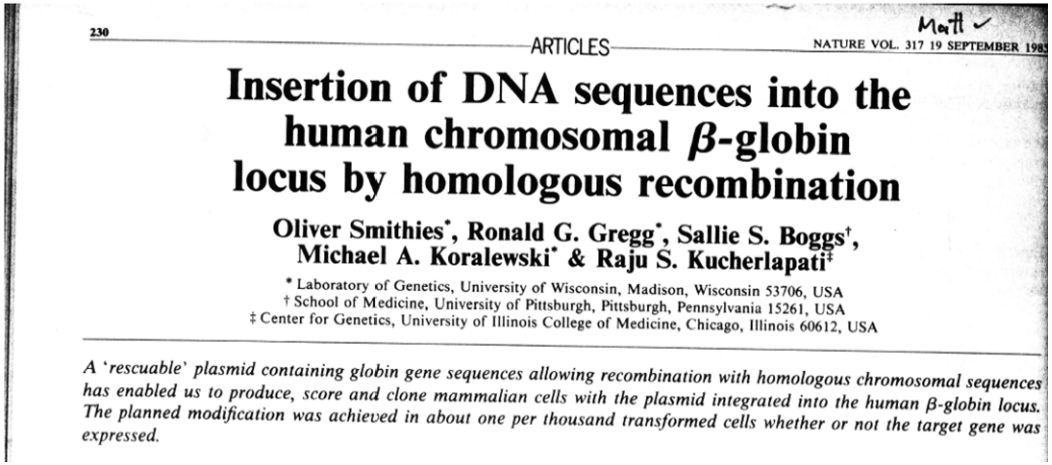
(though taking medicine for pain >3 times/week)
(neurocognitive damage starts occurring in first years of life)

Africa: 5-8 years old

Engineering the DNA of Stem Cells to Cure Genetic Diseases is not a new Idea

". . . many instances of blood disorders, mental problems, and a host of other disabilities are traceable to a malfunctioning gene. It would be a triumph of medicine if the effects of such genes could be countered. . . A patient could, for instance, be treated in this way for a blood disease caused by an abnormal protein made by a mutant gene. A normal gene would be inserted into the precursor cells-immature bone marrow cells that ultimately develop into functioning blood cells. In this way, a normal protein could be made ***in place of****, or along with, the aberrant protein. The genetically altered blood cell precursor could then cure the patient's disease… "*

-David Baltimore (1978)



230 ARTICLES NATURE VOL. 317 19 SEPTEMBER 1985

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

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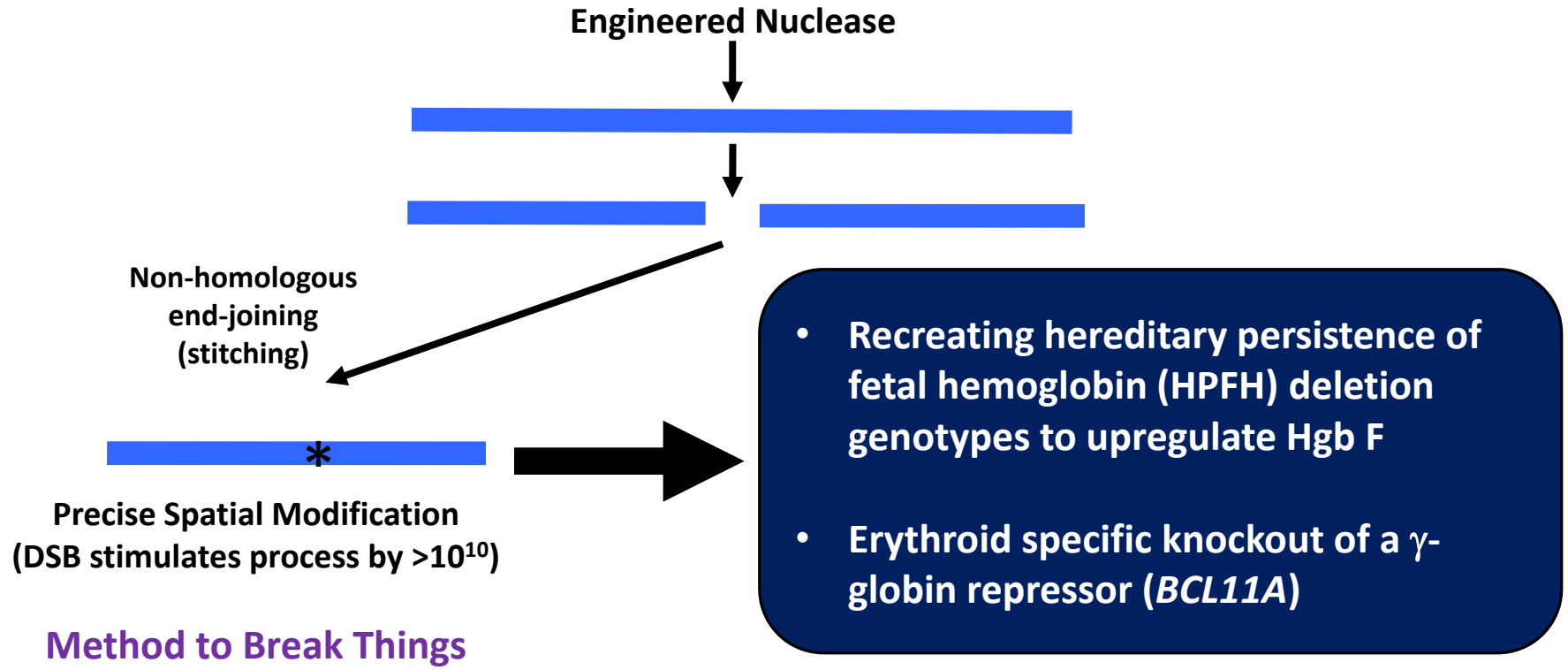
A 'rescuable' plasmid containing globin gene sequences allowing recombination with homologous chromosomal sequences has enabled us to produce, score and clone mammalian cells with the plasmid integrated into the human β-globin locus. The planned modification was achieved in about one per thousand transformed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

Frequency was 10^{-6}

Genome Editing Provides a Precise Method of Genetically Engineering Cells to Treat Sickle Cell Disease (genetic hydroxyurea)



Genome Editing Provides a Precise Method of Genetically Engineering Cells to Cure Sickle Cell Disease

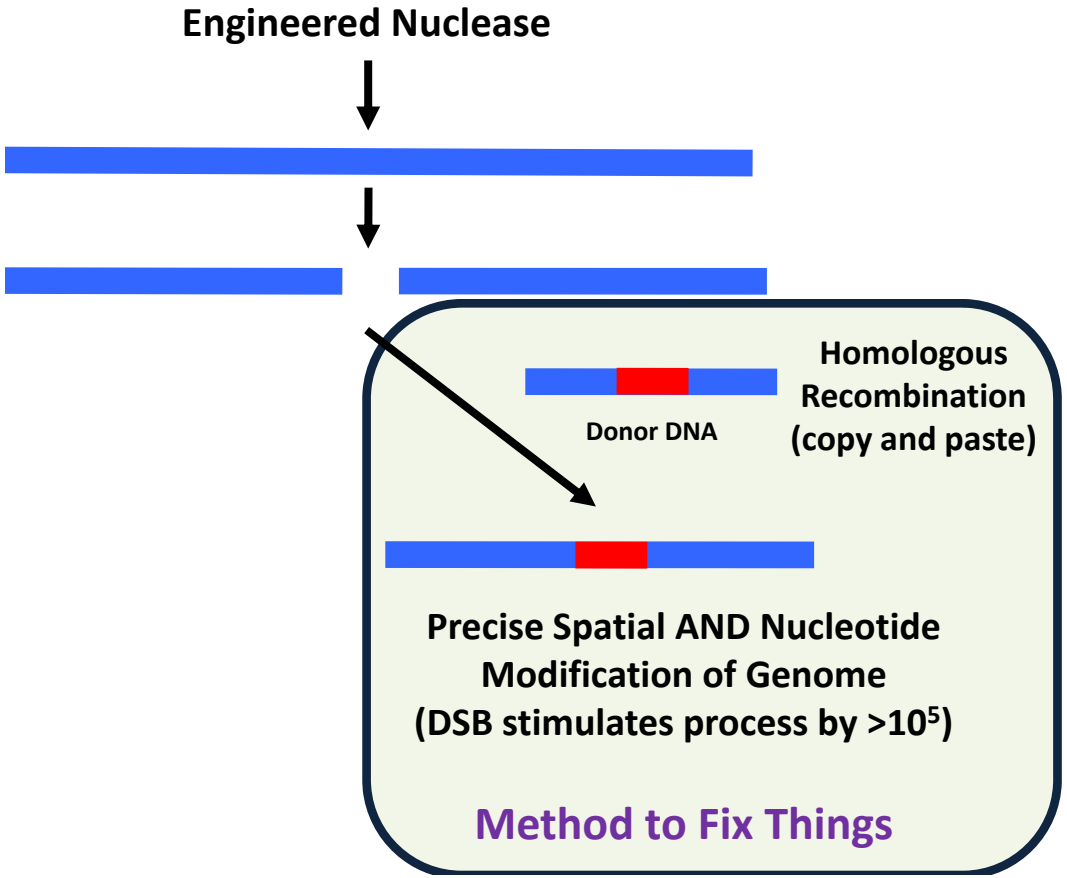
HgbS

CCT GTG GAC

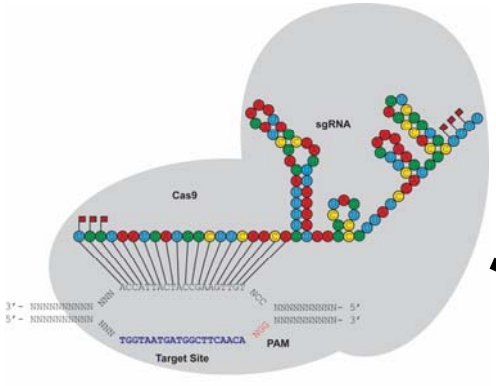
(Genome Editing)

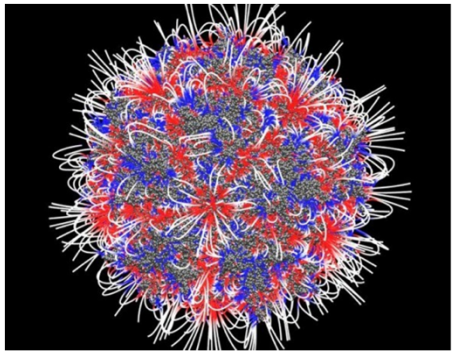
HgbA

CCT GAG GAC

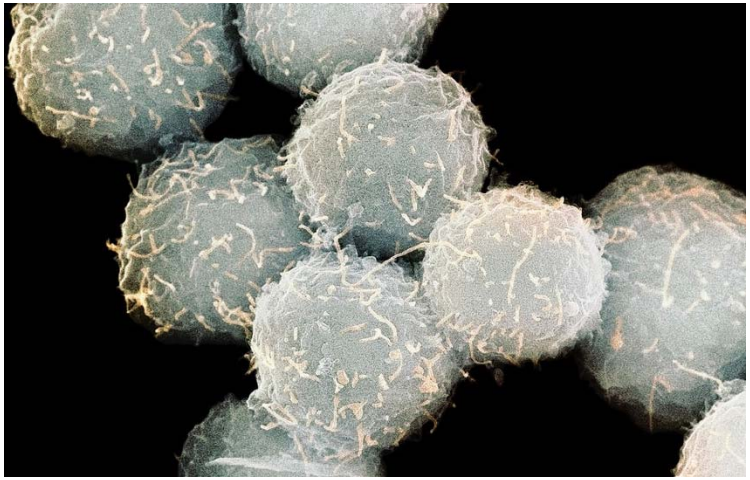


RNP/AAV6 Genome Editing System for CRISPR/Cas9 System



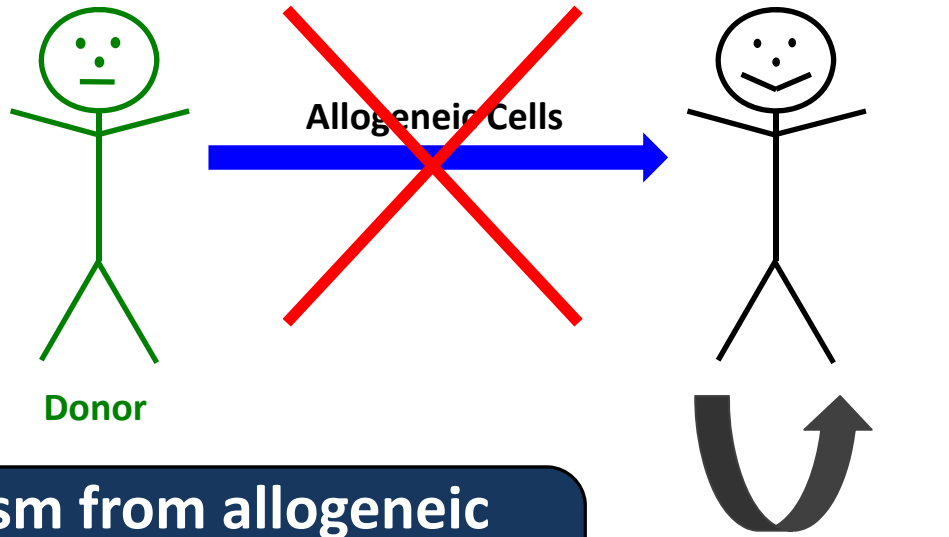


Recombinant Adeno-Associated Virus (AAV6)



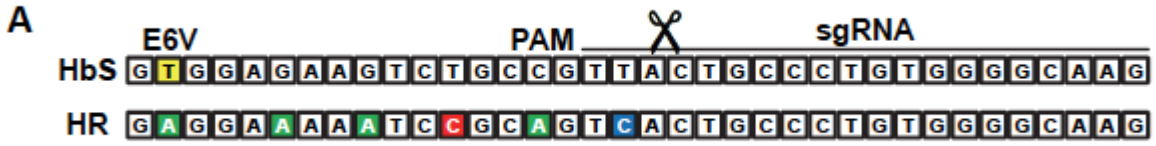
Bak, Dever et al Nature Protocols (2018); Charlesworth, Camarena et al MTNA (2018)

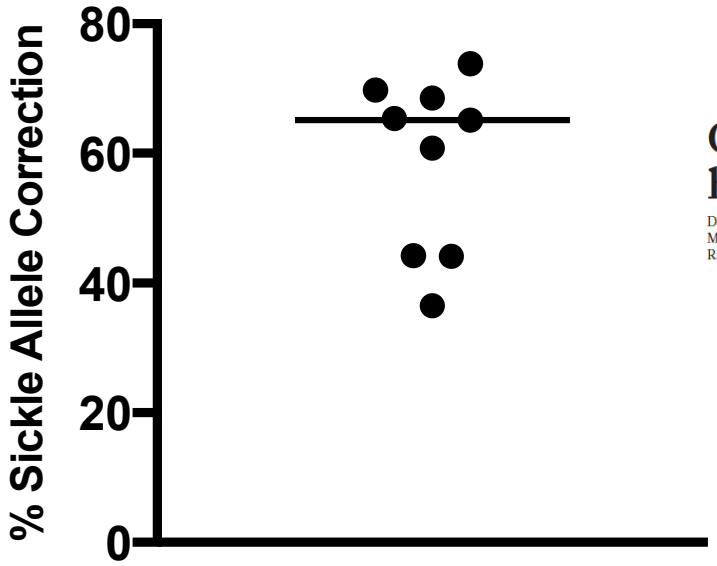
Replace Allogeneic Cells with Corrected Autologous Cells



Mixed chimerism from allogeneic HSCT shows 20% corrected cells can cure the disease

Editing Sickle Variant in CD34+ HSPCs from Sickle Cell Disease Patients





CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells

Daniel P. Dever[1]*, Rasmus O. Bak[1]*, Andreas Reinisch[2], Joab Camarena[1], Gabriel Washington[1], Carmencita E. Nicolas[1], Mara Pavel-Dinu[1], Nivi Saxena[1], Alec B. Wilkens[1], Sruthi Mantri[1], Nobuko Uchida[3]†, Ayal Hendel[1], Anupama Narla[4], Ravindra Majeti[2], Kenneth I. Weinberg[1] & Matthew H. Porteus[1]

Nature 2016

RNP and AAV change gene expression much less significantly than Cas9 mRNA in CD34+ HSPCs

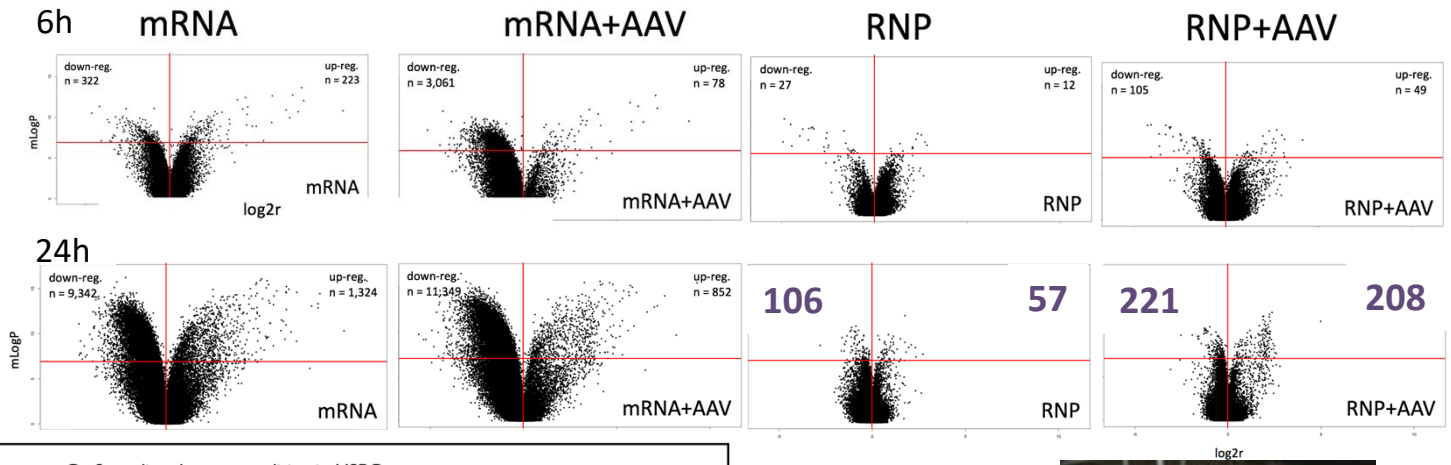
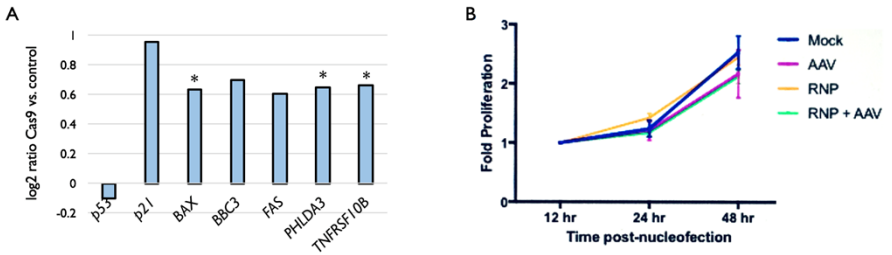


Figure 1: Cellular response to Cas9-mediated genome editing in HSPCs



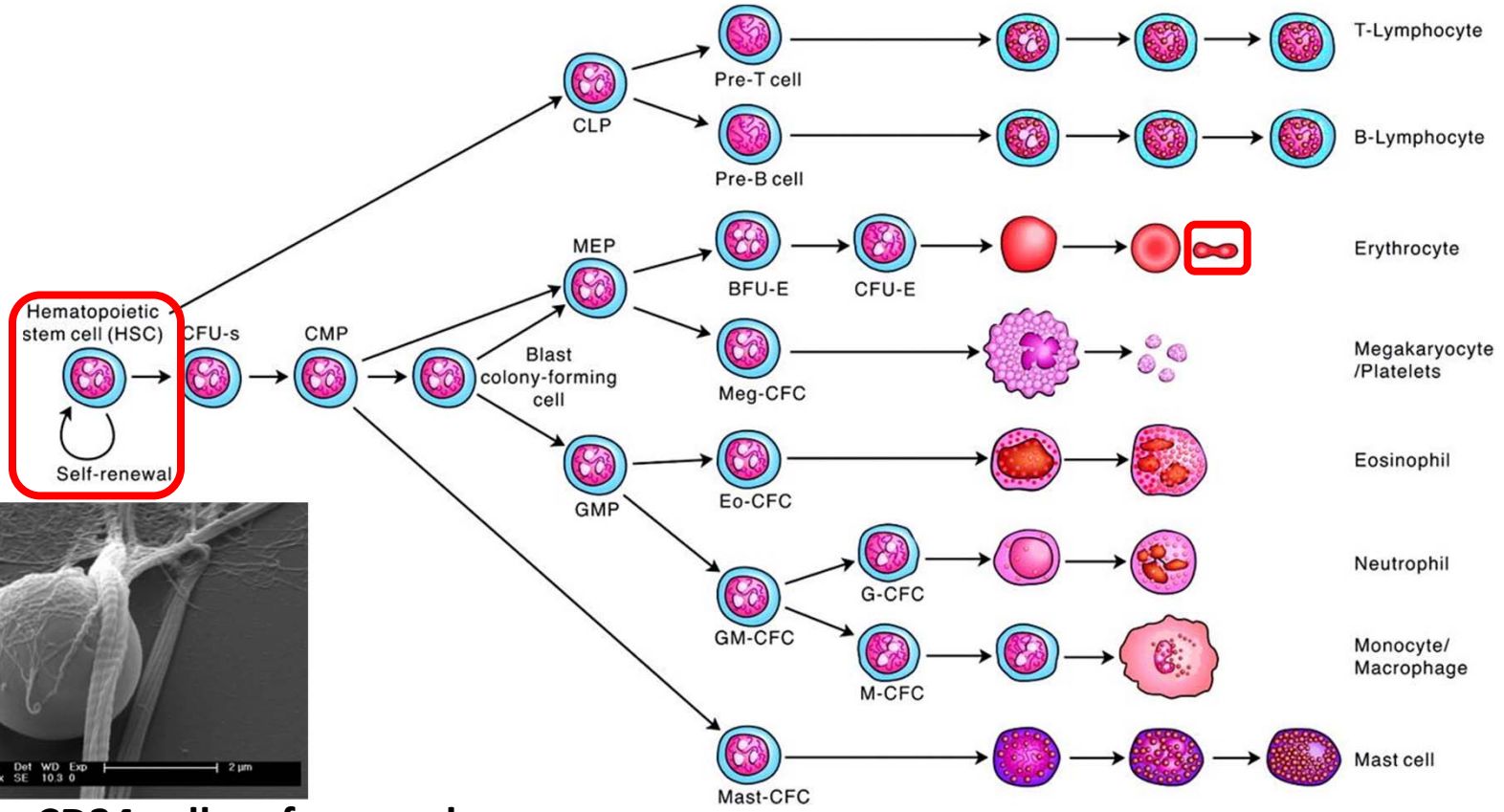
(**A**) At 24h post-treatment, log2 of the ratio of the probe expression difference between Cas9 RNP treatment and mock electroporation control in RNA isolated from four HSPC donors. * indicates significance after Bonferroni correction. (**B**) Post-treatment proliferation rate in plerixafor-mobilized HSPCs. AAV was transduced immediately following electroporation at MOI of 5E4. No differences among treatments were determined to be statistically significant. (N=5)

Cromer et al (2018) Molecular Therapy with Ayal Hendel and Agilent

Do the gene corrected stem cells do what they are supposed to do?

1. Make non-sickling red blood cells

2. Retain their stem cell properties



Marked by CD34 cell surface marker

Gene Corrected Patient Derived CD34+ HSPCs Differentiated *in vitro* into RBCs Generate High Levels of HgbA and Low Levels of HgbS

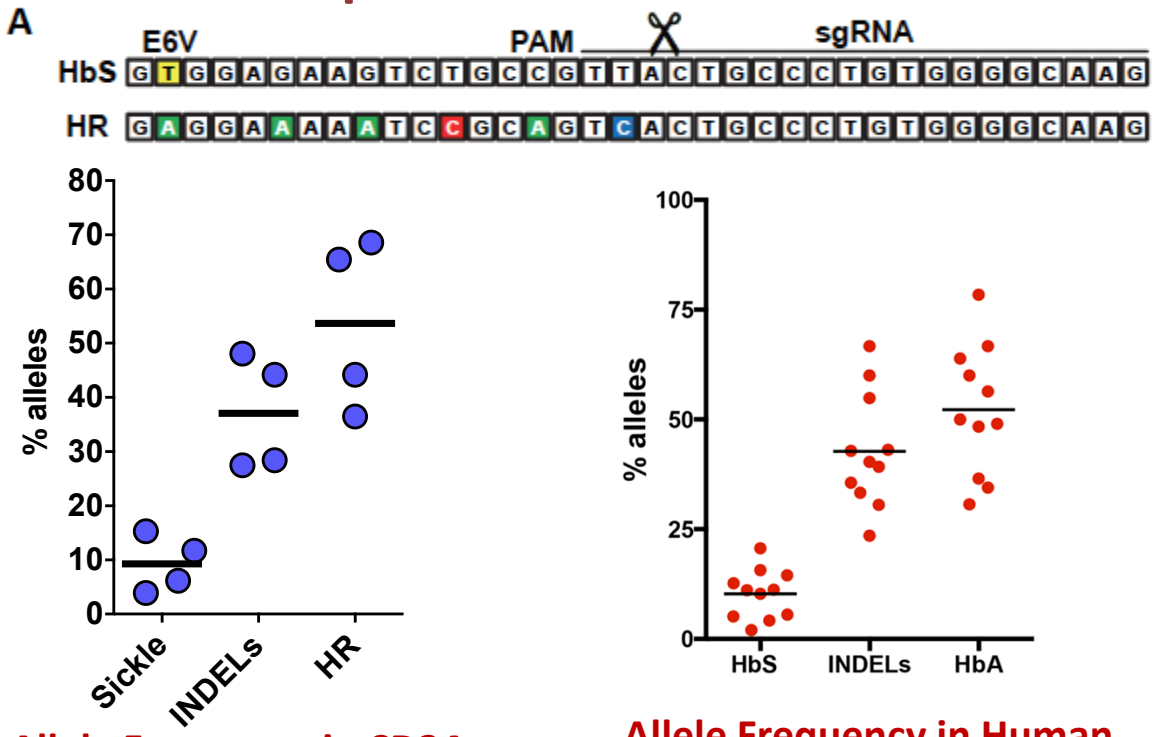
Percent of Beta-Globin Derived Hemoglobin in Total RBC Population (no selection)

Sample	Hgb A	Hgb S
Mock	0.73 +/- 1.0	99.27 +/- 1.0
Gene Corrected (RNP+AAV6)	92.5 +/- 4.3	7.5 +/- 4.3

N=6

Keeping the %S <30% stops disease progression

High Frequencies of Gene Correction at *HBB* in Patient Derived CD34+ HSPCs is maintained after transplantation into NSG mice



Allele Frequency in CD34+ HSPCs Prior to Transplant into NSG Mice

Allele Frequency in Human Cells at 16 Weeks Post-Transplant into NSG Mice

What about potential off-target INDELs?

Peter Marks (head of CBER, FDA): "We don't want off-target events leading to serious adverse events."

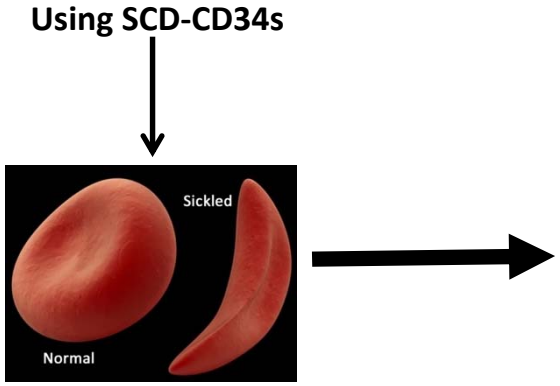
Implication: Off-target changes *per se* are not serious adverse events—only if they lead to functional adverse events.

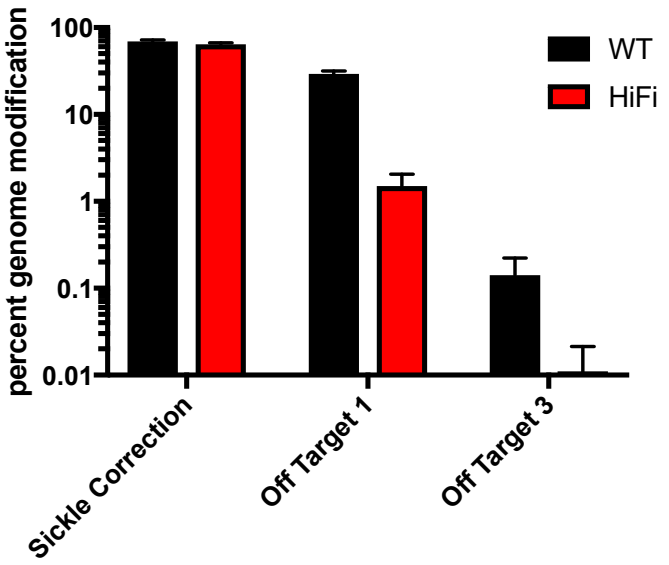
Using Three Different Methods to Identify Potential Off-Target Sites, only Two *bonafide* Sites Identified in CD34+ HSPCs

COSMID	GUIDE-Seq	CIRCLE-Seq	Site	Sequence	Closest Gene	Distance (kb)	Feature	hg19 Location	NHEJ	Mock
			R02	CTTGCCCCACAGGGCAGTAANGG	HBB	n/a	Exon	Chr11:5248198-5248220	54.7	0.767
COS1	GS1	CS2	OT1	TCAGCCCCACAGGGCAGTAAGGG	GRIN3A	95.004	Intergenic	Chr9:104595866-104595888	16.2	0.076
COS2			OT2	CCTCTCCCACAGGGCAGTAAAGG	LINC01482	0.034	Intergenic	Chr17:66624239-66624261	0.048	0.041
COS3			OT3	TTTTCCCCAAAGGGCAGTAATAG	MYO16	n/a	Intron	Chr13:109818336-109818358	0.012	0.007
COS8	GS2	CS7	OT4	GTGGCCCCACAGGGCAGGAANGG	MAGEE2	1.209	Intergenic	ChrX:75006240-75006262	0.003	0.005
COS7	GS3	CS4	OT5	GCTGCCCCACAGGGCAGCAANGG	FAM101A	3.258	Intergenic	Chr12:124803828-124803850	0.15	0.015
	GS4		OT6	GATGCCATTCATAGCAGTCANCG	C22orf34	225.248	Intergenic	Chr22:49582904-49582926	0	0.001
COS23			OT7	CTCGCCCCTCAGGGCAGTAGTGG	GREB1	n/a	Intron	Chr2:11777795-11777817	0.006	0.042
COS9		CS1	OT8	TGTGCCCCACAGAGCACTAANGG	LOC101929350	1.3kb	Intergenic	Chr22:17230606-17230628	0.028	0.064
COS19		CS3	OT9	ATTGCCCCACGGGGCAGTGANGG	LOC643339	n/a	Intron	Chr12:93549185-93549207	0.054	0.016
COS26		CS5	OT10	GTTGCCCCTCAGGACAGTACNGG	LOC105370802	374kb	Intergenic	Chr15:46598112-46598134	n.d.	n.d.
		CS6	OT11	GAAGCCCTACAGGGCAGCAANGG	NRSN1	416kb	Intergenic	chr6:23709573-23709595	0.024	0.006
COS15		CS8	OT12	ATGGCCCCACAAGGCAGAAANGG	IFI27	2.3kb	Intergenic	Chr14:94585321-94585343	0.013	0.018
		CS9	OT13	AGTGCCACACACAGCAGTAANGG	DOCK5(H3K27Ac)	110kb	Intergenic	chr8:24931375-24931397	0.015	0.006
		CS10	OT14	TGTGCACCACAGAGCAATAANGG	ZNF716	183kb	Intergenic	chr7:57716460-57716482	0.019	0.04
		CS11	OT15	GTTATCCCACAGGACAGTGANGG	SFTA3	53kb	Intergenic	chr14:36889532-36889554	0.055	0.043

with Ciaran Lee and Gang Bao (Rice University)

HiFi SpCas9 (R691A) Mediates High Level Sickle Gene Correction while Reducing Off-Target INDELs by > 1-log in Sickle Cell Patient Derived CD34+ HSPCs



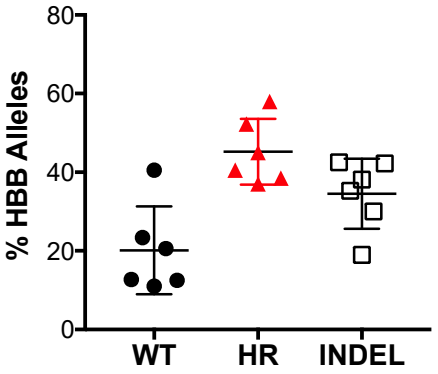


Probably safer than life
(or flying across the Pacific Ocean)

(Vakulskas et al, Nature Medicine, 2018)

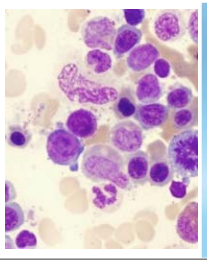
Scaling up the Gene Correction Process

Assay	Specs	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Viability (%)	>70%	90	79	65	75	75	80
Total Viable Cell Count	Report	100 million	24 million	29 million	73 million	111 million	145 million
Phenotypic Analysis	>80% CD34	85.0	99.8	99.7	93.0	92.0	92.0
Percent Allele Correction		56	41	39	37	44.5	52
Percent Cells Corrected	>20%	65	47	53	54	ND	P



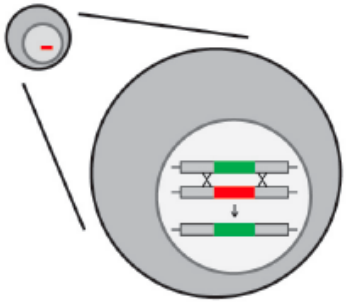
Manufacturing of Autologous HR-Edited Cell Product

Hematopoietic Stem Cells Harvested from Patient



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

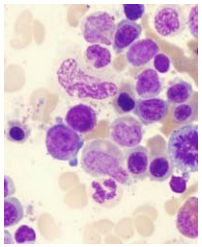
Genome Editing: Gene correction with nuclease mediated homologous recombination



GMP Grade HiFi Cas9 Protein (Aldevron)
GMP Grade sgRNA (Agilent)
GMP Grade AAV6 (CMO)
GMP Grade Electroporation (Lonza)

Transplant Autologous Gene Corrected Cells Back into Patient

HSC Conditioning Needed

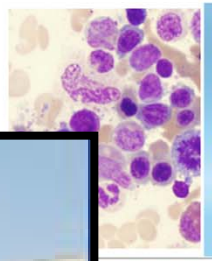


Minimum: 2 x 10^6 CD34/kg
Ideal: 10 x 10^6 CD34/kg

Level of HR modification depends on the disease
Sickle/β-thal (>20%)

Ethics of Genome Editing
(Equity and Distribution (justice))

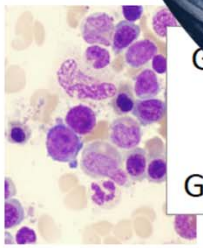
Hematopoietic
Stem Cells
Harvested from



x 10^6 CD34/kg
x 10^6 CD34/kg

nt Autologous
rrected Cells
nto Patient

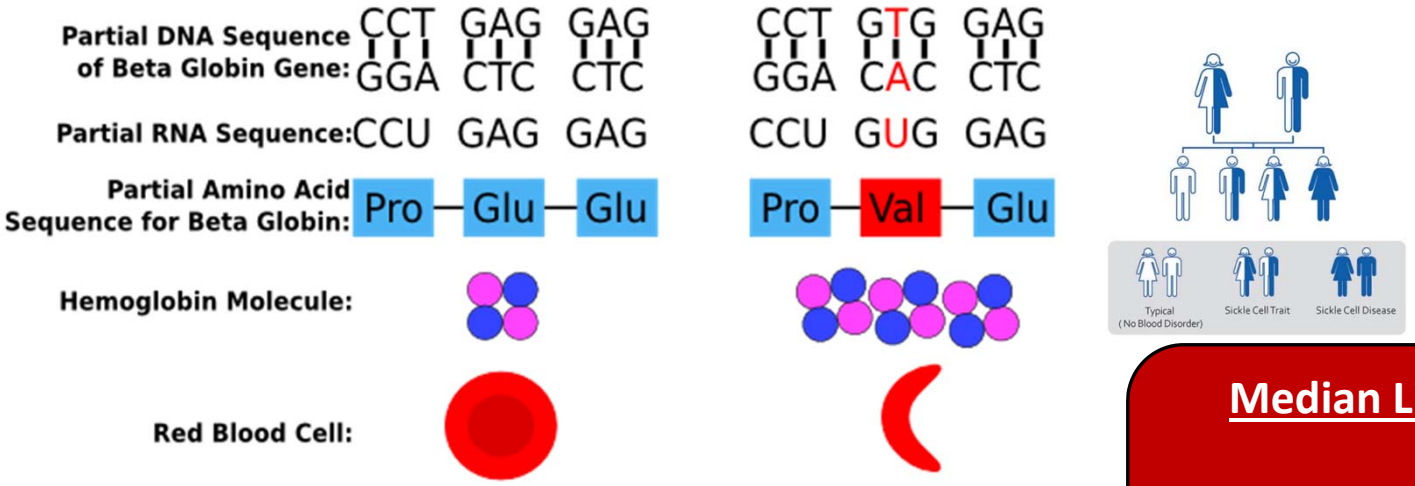
n 8-10 mg/kg)



Ideal: >10% Gene Correction
with
No off-target mutations/rearrangements above
background and no signs of functional toxicity

GMP Grade Electroporation (Lonza)

Genome Editing to Cure Disease using Somatic Stem Cells is an Anti-Eugenics Program



- As somatic cell editing becomes effective, cheap and widespread, the need for germline editing decreases.
- As somatic cell editing becomes effective, cheap, and widespread, the frequency of disease causing alleles in the population will increase. This consequence should be embraced.

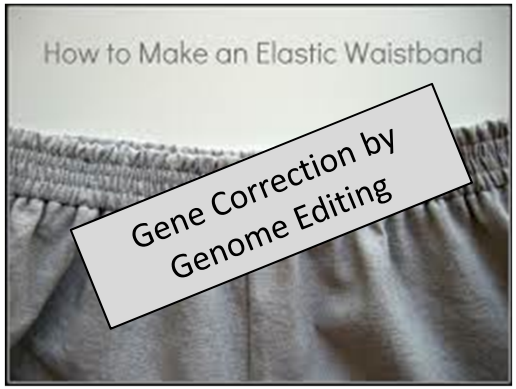
Median Lifespan

United States: mid-40s

Africa: 5-8 years old

There are many different ways to prevent your pants from falling down (including not even wearing pants):

Developing a diverse set of approaches to cure sickle cell disease is a good thing



- **10 years to determine which approach will be best for which patients (patience)**
- **Bakshi et al (2018); Hijmans et al (2010) (impatience)**

Thank You

(for your attention and the opportunity to present our work)

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