An Academic Perspective: Establishing Standards for Therapeutic Genome Editing

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Conflicts of Interest

CRISPR Therapeutics: Equity and SAB

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

Take Home Points

1. **There are thousands of diseases that affect only a small number of patients and establishing a burdensome regulatory path that is not based on scientific risk will inhibit genome editing based therapies being developed.**

2. **Current clinically relevant methods of genome editing using the CRISPR/Cas9 platform *ex vivo* are highly specific (more specific than life).**

3. **Bioinformatic methods identify the relevant off-target sites using clinically relevant delivery methods.**

4. **There are no validated animal models to predict in human genotoxicity for genome editing.**
   - **Standard NSG mouse xenograft models do not support most human blood cancers (Reinisch et al Nature Medicine (2016) PMID 27213817)**

5. **Translocations between the on-target break and spontaneous breaks will occur but at a frequency that is too low to be detected by current methods.**

6. **The best test of the safety of genome editing based therapeutics is phase I trials for serious diseases with unmet medical needs with reasonable follow-up.**

Genotoxicity vs functional toxicity

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.

Nuclease Genotoxicity in HSPCs for an FDA Approved HSPC Editing Clinical Trial

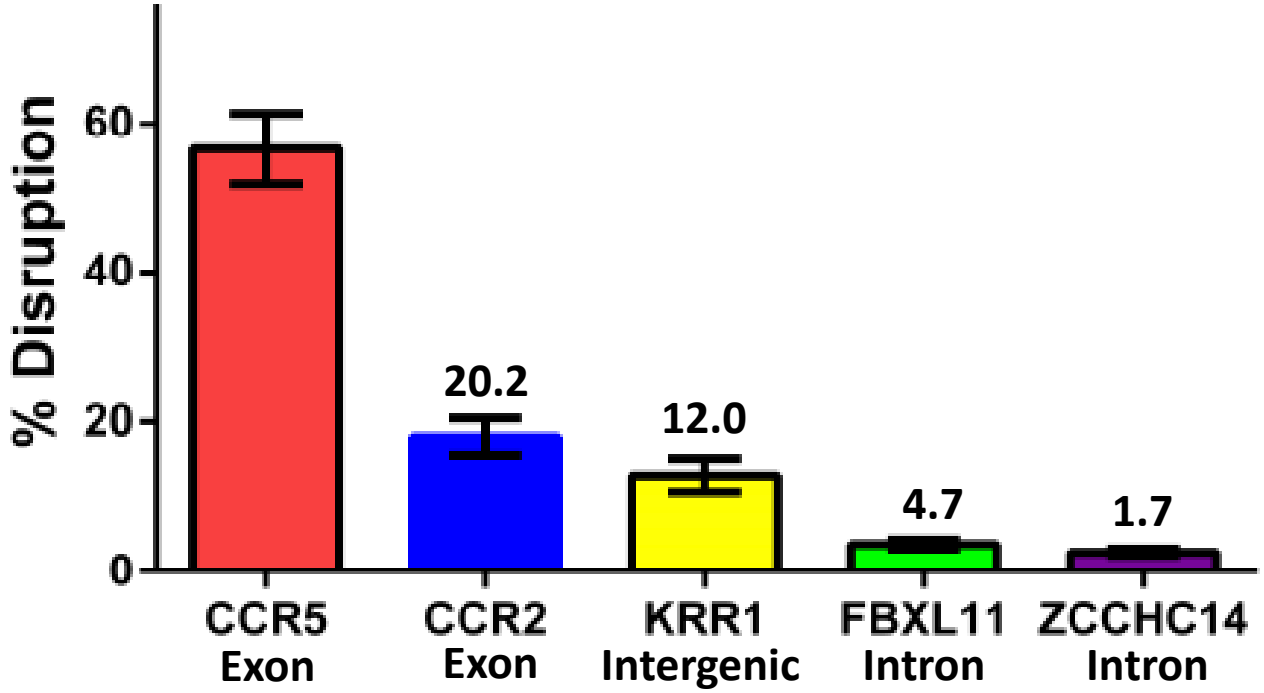
Citation: Molecular Therapy — Methods & Clinical Development (2016) **3,** 16067; doi:10.1038/mtm.2016.67
Official journal of the American Society of Gene & Cell Therapy

www.nature.com/mtm

ARTICLE

Preclinical development and qualification of ZFN-mediated CCR5 disruption in human hematopoietic stem/progenitor cells

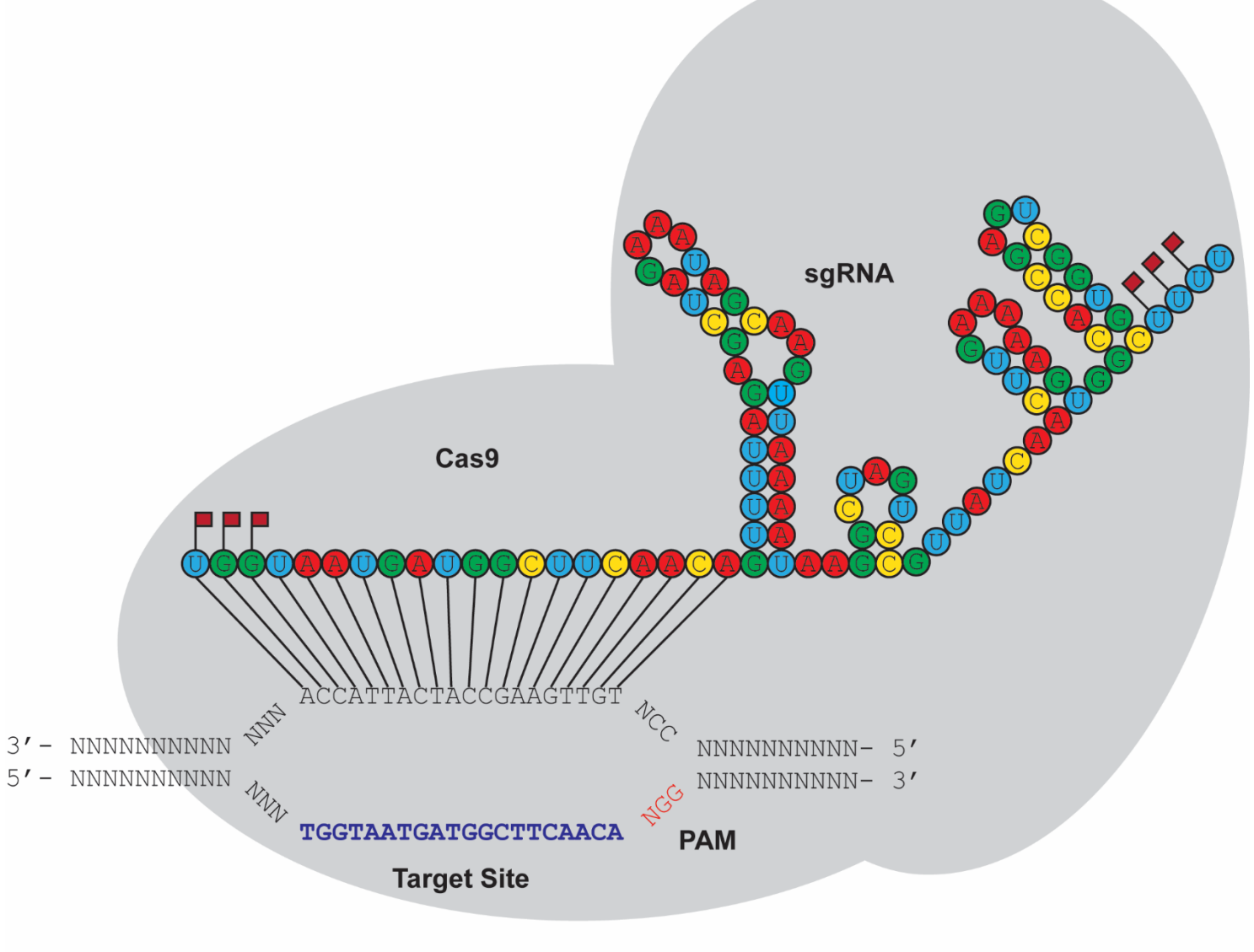
David L DiGiusto[1,2], Paula M Cannon[3], Michael C Holmes[4], Lijing Li[1], Anitha Rao[1], Jianbin Wang[4], Gary Lee[4], Philip D. Gregory[4], Kenneth A Kim[4], Samuel B Hayward[4], Kathleen Meyer[4], Colin Exline[3], Evan Lopez[3], Jill Henley[3], Nancy Gonzalez[1], Victoria Bedell[5], Rodica Stan[6] and John A Zaia[6]



Qualitative Biochemical Understanding of Nuclease Specificity

$$p(\text{Editing}) \approx c_1[\text{conc}] * c_2(\text{Time}) * c_3(\text{Kd}) \text{ (on/off)} * c_4(\text{Kcat}) * c_5(p(\text{repair fidelity}))$$

$$\text{Kd} \approx (\text{guideRNA binding energy})(\text{Cas9-DNA binding energy})$$



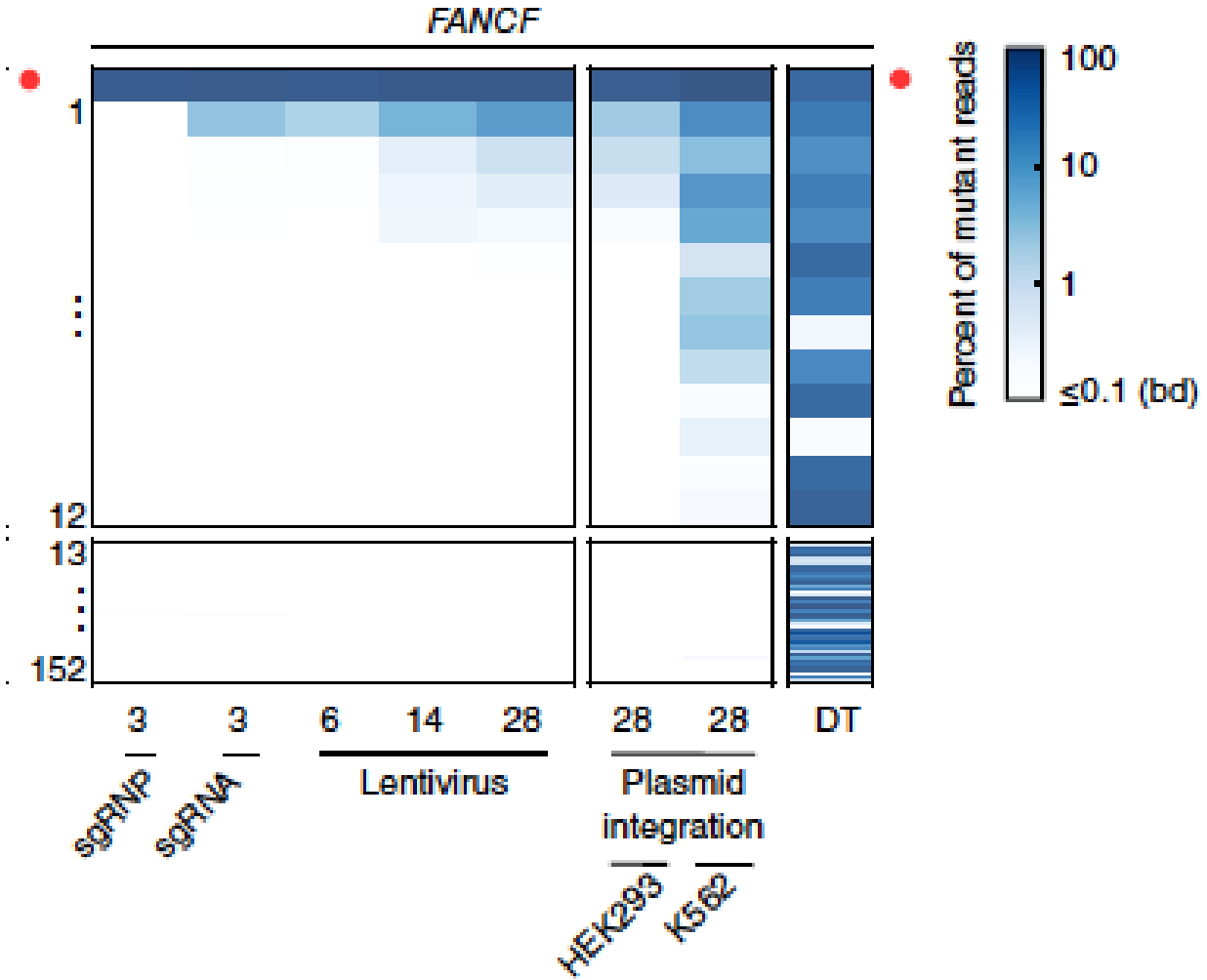
Ribonucleoprotein (RNP): Purified Cas9 protein complexed to synthetic stabilized guide molecule (gRNA) as the best method to engineer cells ex vivo

Importance of Delivery and Cell Type on Specificity

Mapping the genomic landscape of CRISPR–Cas9 cleavage

Peter Cameron[1,4], Chris K Fuller[1,4], Paul D Donohoue[1], Brittnee N Jones[1,3], Matthew S Thompson[1], Matthew M Carter[1], Scott Gradia[1], Bastien Vidal[1], Elizabeth Garner[1], Euan M Slorach[1], Elaine Lau[1], Lynda M Banh[1], Alexandra M Lied[1], Leslie S Edwards[1], Alexander H Settle[1], Daniel Capurso[1], Victor Llaca[2], Stéphane Deschamps[2], Mark Cigan[2,3], Joshua K Young[2] & Andrew P May[1,3]

Nature Methods (2017)



Multiple Methods to Identify Potential Off-Target Sites (ways of creating more lamp posts)

Bioinformatic

COSMID: A Web-based Tool for Identifying and Validating CRISPR/Cas Off-target Sites

Thomas J Cradick[1], Peng Qiu[1], Ciaran M Lee[1], Eli J Fine[1] and Gang Bao[1]

Oligo capture

GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases

Shengdar Q Tsai[1–3,5], Zongli Zheng[1–5], Nhu T Nguyen[1,2], Matthew Liebers[1,2], Ved V Topkar[1,2], Vishal Thapar[1,2], Nicolas Wyvekens[1,2], Cyd Khayter[1,2], A John Iafrate[1–3], Long P Le[1–3], Martin J Aryee[1–3] & J Keith Joung[1–3]

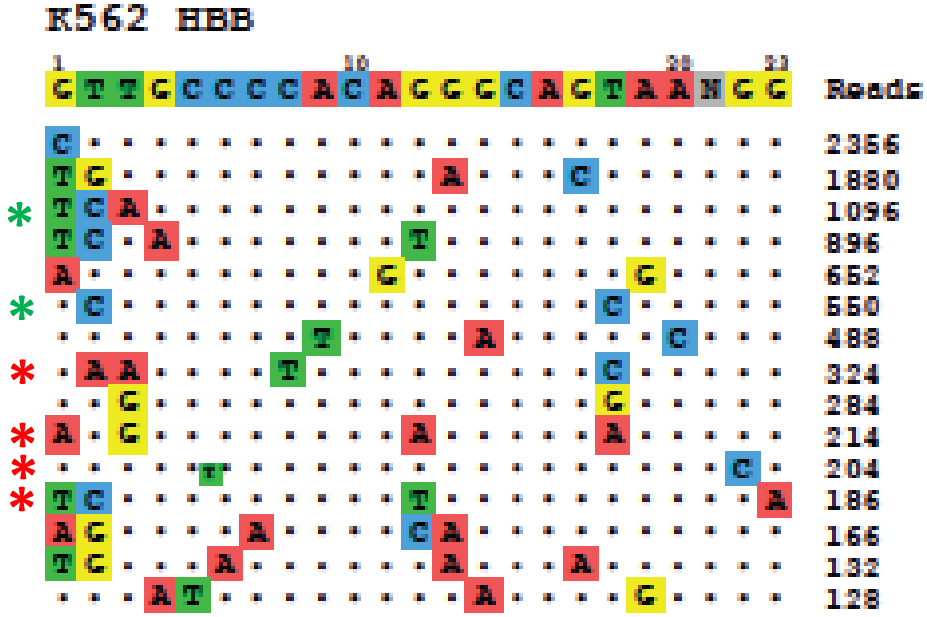
***In vitro* Cas9/gDNA cleavage**

CIRCLE-seq: a highly sensitive *in vitro* screen for genome-wide CRISPR–Cas9 nuclease off-targets

Shengdar Q Tsai[1–4,6], Nhu T Nguyen[1–3], Jose Malagon-Lopez[1–5], Ved V Topkar[1–3], Martin J Aryee[1–5] & J Keith Joung[1–4]

In vitro Methods to Identify R-02 *HBB* Potential Off-Target Sites

Guide	Strand	HBB%	210987654321nGG	HBD%
R-03		55	gACGTTCACCTTGCCCCACAnGG	58
R-08		36	gCTGTGGGGCAAGGTGAACGnGG	48
R-01		54	GTGAACGTGGATGAAGTTGGnGG	27
R-04		53	gCACGTTCACCTTGCCCCACnGG	12
R-07		61	gAGGTGAACGTGGATGAAGTnGG	7
R-05		51	gGTCTGCCGTTACTGCCCTGnGG	-
R-02		66	gTTGCCCCACAGGGCAGTAAnGG	-
R-06		59	gGTTACTGCCCTGTGGGGCAnGG	-



CRISPR/Cas9 systems targeting β-globin and *CCR5* genes have substantial off-target activity

Thomas J. Cradick, Eli J. Fine, Christopher J. Antico and Gang Bao*

NAR (2013)

CIRCLE-seq: a highly sensitive *in vitro* screen for genome-wide CRISPR–Cas9 nuclease off-targets

Shengdar Q Tsai[1–4,6], Nhu T Nguyen[1–3], Jose Malagon-Lopez[1–5], Ved V Topkar[1–3], Martin J Aryee[1–5] & J Keith Joung[1–4]

Nature Methods (2017)

All *bonafide* Off-Target Sites (two) in CD34+ HSPCs using RNP were Identified by COSMID

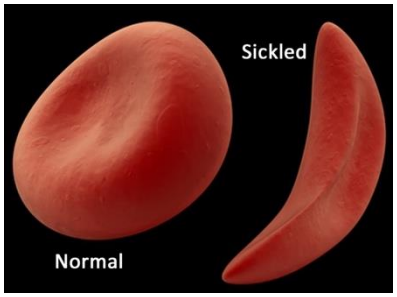
COSMID	GUIDE-Seq	CIRCLE-Seq	Site	Sequence	Closest Gene	Distance (kb)	Feature	hg19 Location	NHEJ	Mock
			R02	CTTGCCCCACAGGGCAGTAANGG	HBB	n/a	Exon	Chr11:5248198-5248220	54.7 (22.0 HR)	0.767
COS1	GS1	CS2	R02_OT1	T**CA**GCCCCACAGGGCAGTAAGGG	GRIN3A	95.004	Intergenic	Chr9:104595866-104595888	16.193	0.076
COS2			R02_OT2	C**C**T**CT**CCCACAGGGCAGTAAAGG	LINC01482	0.034	Intergenic	Chr17:66624239-66624261	0.048	0.041
COS3			R02_OT3	TTT**T**CCCCA**A**AGGGCAGTAAT**A**G	MYO16	n/a	Intron	Chr13:109818336-109818358	0.012	0.007
COS8	GS2	CS7	R02_OT4	**G**T**G**GCCCCACAGGGCAG**G**AANGG	MAGEE2	1.209	Intergenic	ChrX:75006240-75006262	0.003	0.005
COS7	GS3	CS4	R02_OT5	**GC**TGCCCCACAGGGCAG**C**AANGG	FAM101A	3.258	Intergenic	Chr12:124803828-124803850	0.153	0.015
	GS4		R02_OT6	**GA**TGCC**ATT**CA**TA**GCAGT**C**AN**C**G	C22orf34	225.248	Intergenic	Chr22:49582904-49582926	0	0.001
COS23			R02_OT7	CT**C**GCCCC**T**CAGGGCAGTA**G**TGG	GREB1	n/a	Intron	Chr2:11777795-11777817	0.006	0.042
COS9		CS1	R02_OT8	T**G**TGCCCCACAG**A**GCA**C**TAANGG	LOC101929350	1.3kb	Intergenic	Chr22:17230606-17230628	0.028	0.064
COS19		CS3	R02_OT9	ATTGCCCCAC**G**GGGCAGT**G**ANGG	LOC643339	n/a	Intron	Chr12:93549185-93549207	0.054	0.016
COS26		CS5	R02_OT10	GTTGCCCC**T**CAGG**A**CAGTA**C**NGG	LOC105370802	374kb	Intergenic	Chr15:46598112-46598134	n.d.	n.d.
		CS6	R02_OT11	G**AA**GCCC**T**ACAGGGCAG**C**AANGG	NRSN1	416kb	Intergenic	chr6:23709573-23709595	0.024	0.006
COS15		CS8	R02_OT12	AT**G**GCCCCACA**A**GGCAG**A**AANGG	IFI27	2.3kb	Intergenic	Chr14:94585321-94585343	0.013	0.018
		CS9	R02_OT13	A**G**TGCC**A**CACA**CA**GCAGTAANGG	DOCK5(H3K27Ac)	110kb	Intergenic	chr8:24931375-24931397	0.015	0.006
		CS10	R02_OT14	T**G**TGC**A**CCACAG**A**GCA**A**TAANGG	ZNF716	183kb	Intergenic	chr7:57716460-57716482	0.019	0.04
		CS11	R02_OT15	GTT**AT**CCCACAGG**A**CAGT**G**ANGG	SFTA3	53kb	Intergenic	chr14:36889532-36889554	0.055	0.043

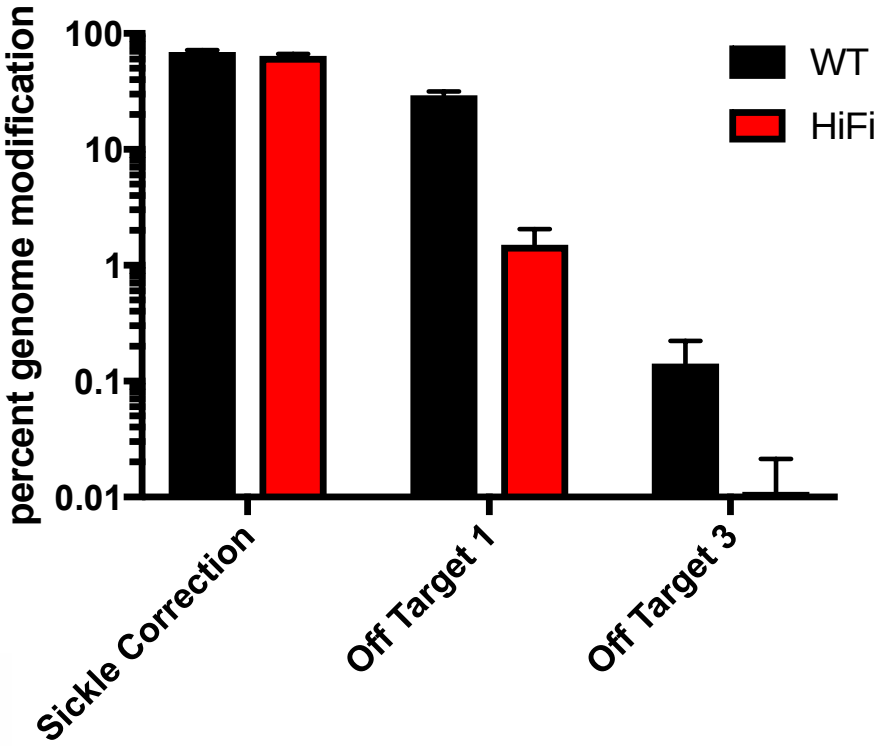
with Ciaran Lee and Gang Bao (Rice University)

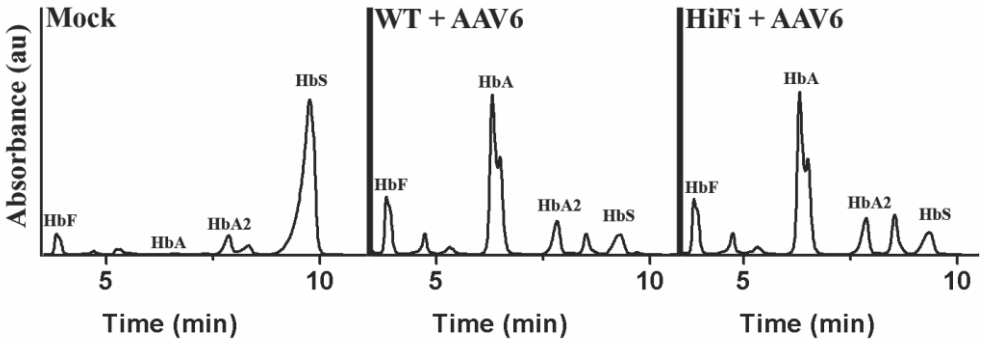
”Log-fold improvements will always be important in making gene therapy safer”
-Paraphrasing Dr. Chris Baum
(*2017 ESGCT Opening Ceremony*)

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

Using SCD-CD34s







Vakulskas, Dever, Camarena, Lee, Bao, Behlke (manuscript under review)

Low Number of Off-Target Sites and Frequency of INDELs in Healthy Donor and Patient Derived CD34+ HSPCs using RNP Delivery

(Both identified by COSMID--Bioinformatics)

Gene Name	Method of Identification		Chromosome Location	Feature	*Expression in Hematopoietic Stem Cells	U2OS	WT CD34+	SCID-X1 CD34+	SCID-X1 CD34+	SCID-X1 CD34+
	Guide Seq	COSMID				Plasmid	20nt RNP (WT *sp*Cas9)	19nt RNP (WT s*p*Cas9)	20nt RNP (HiFi *sp*Cas9)	19nt RNP (HiFi s*p*Cas9)
IL2RG			X	Exon	Yes	81.1%	81.7%	91.7%	94.1%	97.6%
LIN01287	√		7	Intergenic	Data not available	background	background	background	n/s	n/s
MPZL1		√	1	Intron	Yes	1.1%	0.1%	0.1%	background	background
SHQ1	√	√	3	Intron	Yes	1.5%	background	background	n/s	n/s
SMYD3	√		1	Intron	Yes	4.2%	background	background	n/s	n/s
ZFN330		√	4	Intergenic	Data not available	background	0.23%	0.27%	background	background

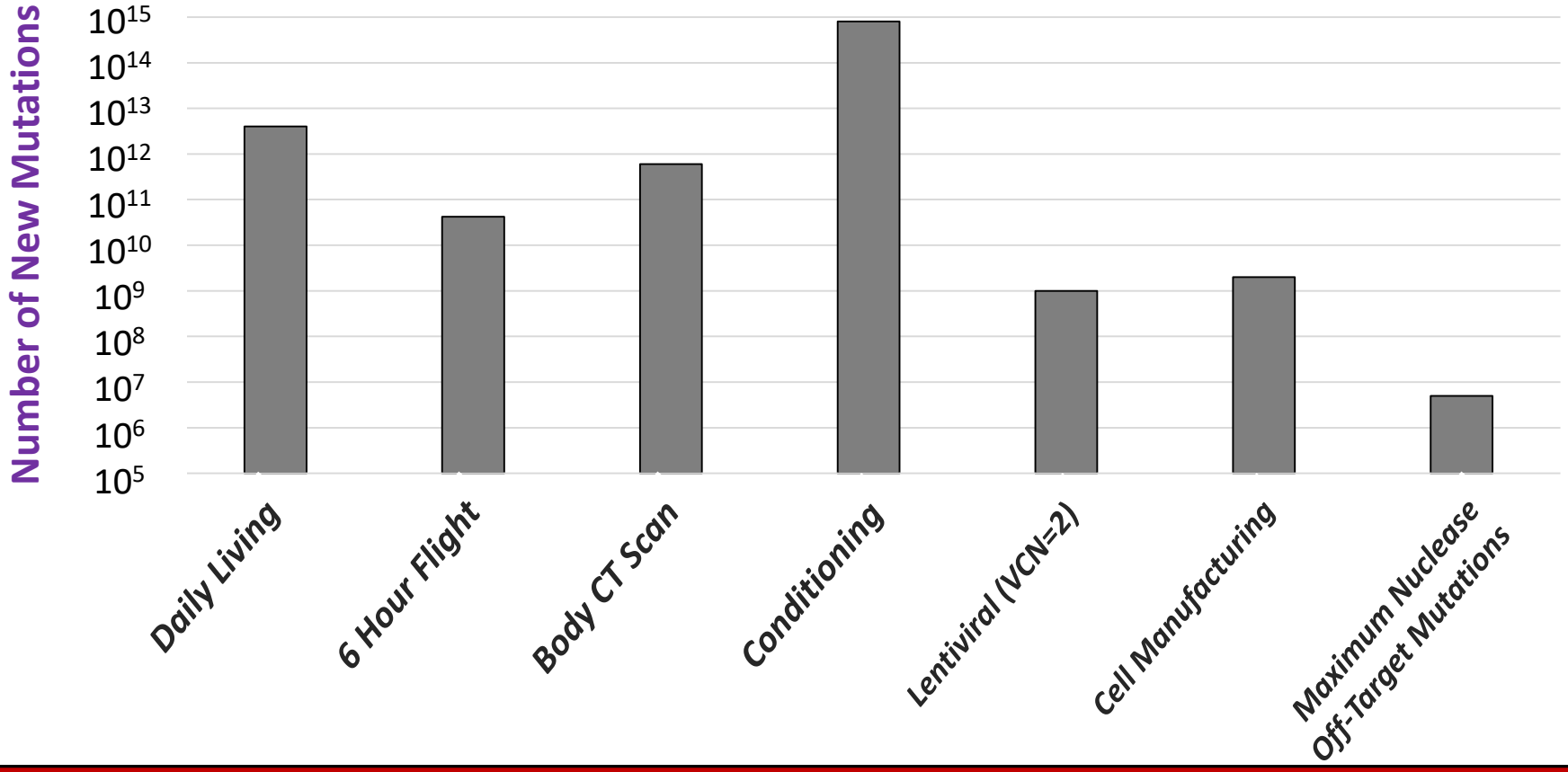
**Expression determined by www.biogps.org and Gene Expression Commons*

48 other potential off-target sites were analyzed with no evidence of INDELs in CD34+ HSPCs modified by RNP

with Ciaran Lee and Gang Bao (Rice University)

Ex vivo Genome Editing is More Precise than Life

- **Tremendous genetic diversity among humans to begin with:**
  Baseline Variation Per Person: 2.4 million SNVs, 500-600K In/Dels (355 Exonic, 91 Frameshift), ~3000 structural variants (i.e. Dewey et al 2014 JAMA)
- **Tremendous ongoing genetic diversity within each person**
  10-20 new mutations per every cell division



Assumes high fidelity of DSB Repair: 90% (9 out of 10 breaks are repaired precisely)

Proposed Standards

1. **Analysis of potential off-target INDELs should be performed at all potential off-target sites in which there are 3 or fewer mismatches (excluding nucleotide 20) and no PAM mismatches identified bio-informatically using the delivery method and cell type that is part of the target product profile (TPP).**
   - **Other techniques can supplement but not replace this analysis of potential off-target sites.**
2. **If on-target site or off-target sites with known INDELs are not associated with cancers (for the tissue type that might be modified), then no further genotoxicity/tumorigenicity studies need to be performed.**
3. **If on-target site or off-target sites with known INDELs are associated with cancers (for the tissue type that might be modified), then thoughtful functional assays for genotoxicity/tumorgenicity should be performed.**
4. **Samples from patients treated with genome edited therapeutics should be archived (15 years?) for analysis if genotoxicity/tumors occur.**

Thank You

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

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