



DISCLOSURE: CSO & Commercial interests

INDUCE-seq: Ensuring the safe development of cell and gene therapies by gene editing

Prof. Simon H Reed: reedsh1@cf.ac.uk

simonreed@brokenstringbio.com



3 In vivo vs ex vivo Gene Editing





https://doi.org/10.1038/s41392-019-0089-y

A new paradigm for treating disease

4 Nuclease-dependent genome editing



- Meganucleases
- ZFN
- TALENs
- CRISPR-Cas9
- CRISPR-Cas12a
- Novel/Engineered
 CRISPR Nucleases



5 Nuclease-independent genome editing



GE Delivery Systems can also impinge on genome stability







6

7 Early Gene Editing Successes



in vivo

Intellia, with first results, delivers a 'landmark' for CRISPR gene editing

Study results offer the first clinical evidence that CRISPR gene editing inside the body can be safe and effective.

Jun 21, 2021



Chart 1 - Selected Publicly-Traded Gene Editing Companies

ex vivo

CRISPR/ViaCyte and Genprex Vie for First Diabetes Gene Therapy

Mar 13, 2023



		EV			# of Products								
Company	Ticker	(\$ in M)	On Market	Ph3	Ph1/2	Ph1	Preclinic						
Beam Therapeutics, Inc.	BEAM	\$2,673	0	0	0	1	9	Sickle cell disease (SCD), β-thalassemia					
Caribou Biosciences, Inc.	CRBU	\$368	0	0	0	1	5	Relapsed/refractory B cell non-Hodgkin lymphoma					
Cellectis SA	ALCLS-FR	\$89	0	0	0	6	0	CD19-expressing hematological malignancies					
CRISPR Therapeutics AG	CRSP	\$3,071	0	1	0	3	17	β-thalassemia, SCD					
Editas Medicine, Inc.	EDIT	\$510	0	0	2	0	6	Leber congenital amaurosis-10 (LCA10)					
Graphite Bio, Inc.	GRPH	-\$86	0	0	1	0	3	SCD					
Intellia Therapeutics, Inc.	NTLA	\$3,593	0	0	3	1	11	Transthyretin amyloidosis (ATTR)					
Prime Medicine, Inc.	PRME	\$1,876	0	0 0 0		0	18	Chronic Granulomatous Disease (CDG)					
Sangamo Therapeutics, Inc.	SGMO	\$387	0	1	3	0	14	Hemophilia A					
Verve Therapeutics	VERV	\$1,193	0	0	0	1	1	Heterozygous familial hypercholesterolemia (HeFH)					
Mean		\$1,463											
Median		\$851											

Source: Jefferies, Company Reports, and FactSet

8 Safety concerns for Gene Editing Products





https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-products-incorporating-human-genome-editing https://www.cooley.com/news/insight/2022/2022-04-13-fda-releases-draft-guidance-on-development-of-genome-editing-and-car-t-therapies

9 Current methods for measuring off-target DSBs in cells





Taken from: Bouwman and Crosetto (2018). Genes.

Indirect DSB measurements:

- Using proteins and DSB repair as an indirect measure of breaks in cells
- DISCOVER-seq & GUIDE-seq

Direct DSB measurements:

- Required to determine the mechanism of break formation in the genome
- BLISS | DSBCapture | END-seq

Limitations of existing assays:

- PCR amplification bias
- Not scalable
- Relevant / in vivo context

0 INDUCE-seq solves the problem with a novel use of Illumina Broken Stri flow cells

INDUCE-seq flow cell enrichment was first demonstrated for the measurement of DSBs in cells



INDUCE-seq DSB library fragments are enriched on the flow cell with remarkable efficiency. Their location & frequency measured by sequencing



This makes unbiased, digital mapping of genomic breaks throughout the genome possible



INDUCE-seq flow cell enrichment pulls the needle out of the haystack for direct measurement on the illumina sequencer

INDUCE-seq procedure overview 11





Dobbs, F.M. et al. Precision digital mapping of endogenous and induced genomic DNA breaks by INDUCE-seq. Nat Commun 13, 3989 (2022).

12 INDUCE-seq provides unparalleled signal-to-noise for *in vivo* nuclease-induced break detection





INDUCE-seq sequences an order of magnitude fewer reads than other approaches, while sensitively detecting more AsiSI-induced break sites in the genome

13 INDUCE-seq permits characterization of editinginduced break events at single nucleotide resolution



Broken Strin

14 Comparing CRISPR-induced break structure with mutational (gene editing) outcome



Editing at CRISPR on-target site correlates with break structure

1bp read overlap suggests 1bp 'staggered' break rather than expected blunt DSB

Highest frequency mutation detected by targeted deep sequencing is 1bp insertion (37.24%)



Editing-induced Mutations \rightarrow





INDUCE-seq and Duplex-seq Cross-validation Study



HESI CT-TRACS

A collaborative, multi-sector consortium involving over 30 scientists contributing to all aspects of the study.

16 HESI Project Overview





17 Unbiased discovery of On-Target Editing





18 Phase 1: Off-target nomination process



Unbiased frequency-based break rank list

	N	ICF1UA AAV	51 breakcoul	nts
	chr	Coordir	nates	Breaks
V Target →	19	55627121	55627126	313
	6	36765477	36765483	76
	13	106612926	106612928	27
	12	107486279	107486282	11
	14	107161780	107161789	11
	5	169511418	169511420	7
	11	118717490	118717493	5
	11	62609183	62609185	5
	1	203238430	203238433	5
	1	220445990	220445992	5
	19	8509703	8509707	5
	3	78708691	78708694	5
	10	106321217	106321220	4
	10	132580829	132580833	4
	10	21581841	21581846	4
	10	33482417	33482420	4
	11	15129453	15129460	4
	1	145066102	145066108	4
	1	153072781	153072785	4
	1	160555176	160555178	4
	11	61111252	61111254	4
	1	162106766	162106769	4
	1	168378235	168378237	4
	11	74359766	74359771	4
			Ra	ank n=1



NOTE: Cell type affects 'Off-target' lists

19 Phase 2: Off-target nomination process





20 Curated Off-target Break Classes



				── Off Tar	aets [.] AAV	S1 MC	F10A co	ombined	off-tar	det dataset		
Μ	ICF10A AAVS	51 breakcou	ints		9010.7010			PAM		got_dataoot		
m	mon		Breaks	<mark>G G G G</mark>	CCACT	A <mark>G G G</mark> A	CAGG	A T N G G	Break Num	Mismatches	Coordinates	
19	55627121	55627126	313	• • • • •		• • • • •	• • • •	./././.	337	Target	chr19:55627116-55627139	
6	36765477	36765483	76	• • • A	• • • T C	• • • • •		./.,/.,/.,	80	3	chr6:36765463-36765486	-
13	106612926	106612928	27	• • • •	• • • <mark>A</mark> • i	ΤΑ···	· · · /	.,,,,,,,	33	3	chr13:106612911-106612934	ŧΕ
12	107486279	107486282	11	ΑΤ • •	• • • • •	• A • • •	· · · ///	· A · · ·	11	4	chr12:107486264-10748628	7
4	107161780	107161789	11	• A • •	•••• C	• • • • •	· · ////	Crry	9	3	chr5:169511412-169511435	E
5	169511418	169511420	7	CA··	• • • • C	· · · · T	••//////	7////	5	4	chr11:61111236-61111259	
1	118717490	118717493	5	• • • •	• <u>T T</u> • • •	• <mark>A</mark> • • •	• //////	/./././	4	3	chr19:16174986-16175009	
11	62609183	62609185	5	• • • •	• A • • •	• • <mark>A</mark> • •	•////////	$\cdot \mathbf{A} \cdot \cdot \cdot$	4	3	chr8:22635580-22635603	
1	203238430	203238433	5	• • • •	$\cdot \cdot \mathbf{C} \cdot \cdot \mathbf{c}$	G · · · ·	/,//,/, A		3	3	chr21:42892941-42892964	
1	220445990	220445992	5	• <mark>A</mark> • •	• • • T A	/	///////////////////////////////////////	G / / · /·	3	4	chr10:132580815-132580838	3
	2204433330	0500707	5	• A • •	••• <mark>G</mark> ••• <mark>(</mark>	$G \cdot \cdot \cdot // \cdot$	///////////////////////////////////////	· C · · ·	3	4	chr16:765567-765590	
2	70700001	79709001		• • T •	• • • • C	••••/•//•//•	G - / - / -	· · · · ·	2	3	chr22:44699097-44699120	
3	106201017	1000094	5	• • T •	· · · · ·	· · · C ·	//////	• <mark>G</mark> • • •	2	3	chr8:144885119-144885142	
0	106321217	106321220	4	ACT・	· · T · ·	· · · · · ·	/ · /•/•/•		2	4	chr15:37287856-37287879	
10	132580829	132580833	4	• • T • •	· · · · C	· · · C ·		Τ · · · ·	2	4	chr15:89175079-89175102	
10	21581841	21581846	4	• C A •		0		C • • • •	2	4	chr16:31484902-31484925	
10	33482417	33482420	4	• T • •	G····		••• C	• • • <mark>A</mark> •	2	4	chr5:103932913-103932936	
11	15129453	15129460	4	• T • •	· · · · · /		· CA·	$GG \cdot \cdot \cdot$	2	6	chr14:80822956-80822979	
1	145066102	145066108	4									
1	153072781	153072785	4	Sequ	ence-bas	ed (Gu	ide-like	homol	oav)			
1	160555176	160555178	4						- 377			

Lower down the hierarchy

key: On-target | Common | Unbiaseduniq

168378235 168378237

n=10,000

21 Break Class Hierarchy





22 Phase 2 – Mutational (editing) Outcome



Broken String

The Problem: Signal-to-Noise

Duplex-seq improves average sequencing error rate from 1 in 1,000 to 1 in 10,000,000

DNA sequencing has transformed medicine and biology with the detection of clonal genetic variants, but remains poor at detecting rare, sub-clonal variants.



Easy Genetic difference between two people





Potential off-target sites as discovered by INDUCE-seq



BIOSCIENCES

Off-target likelihood rank

Target site category

COMMON: sequency homology and BS empiric data

SEQUNIQ: sequency homology only

SINGLETONS: singlets supported by sequency homology and BS empiric data

UNBIASEDUNIQ: BS empiric data only

UNBIASEDUNIQ_GM24385: unique to GM24385

UNBIASEDUNIQ_MCF10A: unique to MCF10A

CONTRL: control target sites

- Each guide has >50 target sites:
 - <u>The number of sites in each</u> category varies per guide.
 - The length of target sites varies across categories.
- There are 10 probes (target sites) serving as controls across all guides.
- Internal controls (TP53 and one random mutagenesis region) are not shown here.

For Research Use Only. Not for use in diagnostic procedures. 11

25 **Duplex-seq overall mutation data summary**





Summary

- Treated samples have generated many indels.
- Treated samples have more **indels** than control samples.

26 Characterise Novel off-targets discovered by INDUCE-seq



HBD



BCL11A

key: Common | Sequniq | Singletons | Unbiaseduniq | GM24385 | MCF10A | Control | TP53 | RNG |

27 AAVS1 Mutation Report



Note: Nuclease-induced break recurrency correlates with On and Off-target mutation frequencies

Off-target name chr start off Score treate Control Indel mv sive sive </th <th></th> <th></th> <th></th> <th>INDUCE-</th> <th>seq Brea</th> <th>k Da</th> <th>ata</th> <th></th> <th colspan="9">GM24385</th> <th colspan="11">MCF10A</th>				INDUCE-	seq Brea	k Da	ata		GM24385									MCF10A										
Off-larget name ohr start end Score these Design indel mnv snv sv indel mnv snv snv <th<< th=""><th></th><th></th><th colspan="4">Break MismachrstartendScoretchesn1955,627,12155,627,1277530</th><th>Panel</th><th></th><th>Tre</th><th>ated</th><th></th><th></th><th>Con</th><th>trol</th><th></th><th></th><th>Trea</th><th>ated</th><th></th><th></th><th>Con</th><th>trol</th><th></th></th<<>			Break MismachrstartendScoretchesn1955,627,12155,627,1277530				Panel		Tre	ated			Con	trol			Trea	ated			Con	trol						
01.AdVS1 common 19 55.627.127 753 0 Pass 2244 126 505 143 5 0 14 1 4295 223 739 205 5 0 13 02.AVX51 common 13 06.2223 106.1223 106.1223 106.1223 106.1223 106.1223 106.1223 106.123 10 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1		Off-target name	chr	start	end	Score	tches	Design	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	mnv	snv	sv				
O2 AVS1 common 6 36,765,477 36,765,473 3783 Pass 7452 50 1 0 1 1 3776 65 1 0 0 03 AAVS1 common 13 106,612,923 106,612,923 107,486,224 40 4 Pass 992 36 73 0 2 0 1 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 <th< td=""><td></td><td>01_AAVS1_common</td><td>19</td><td>55,627,121</td><td>55,627,127</td><td>753</td><td>0</td><td>Pass</td><td>294<mark>4</mark></td><td>136</td><td>595</td><td>143</td><td>5</td><td>0</td><td>14</td><td>1</td><td>4395</td><td>223</td><td>739</td><td>205</td><td>5</td><td>0</td><td>13</td><td>0</td></th<>		01_AAVS1_common	19	55,627,121	55,627,127	753	0	Pass	294 <mark>4</mark>	136	595	143	5	0	14	1	4395	223	739	205	5	0	13	0				
03 AAVS1_common 13 106,612,923 106,612,923 106,612,923 107,462,822 40 4 Pass 1002 27 140 8 0 0 1 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 4 0 0 0 4 0 0 0 4 0 0 0 1 0 10 0 0 10 0 0 10 10 10 10 0 0 11 0 10 0 0 11 0 10 10 10 <th< td=""><td></td><td>02_AAVS1_common</td><td>6</td><td>36,765,477</td><td>36,765,483</td><td>196</td><td>3</td><td>Pass</td><td>2342</td><td>105</td><td>592</td><td>50</td><td>1</td><td>0</td><td>1</td><td>1</td><td>3925</td><td>159</td><td>777</td><td>65</td><td>1</td><td>0</td><td>0</td><td>0</td></th<>		02_AAVS1_common	6	36,765,477	36,765,483	196	3	Pass	2342	105	592	50	1	0	1	1	3925	159	777	65	1	0	0	0				
04_AAVS1_common 12 07/466_278 0 <		03_AAVS1_common	13	106,612,923	106,612,929	73	3	Pass	1052	27	140	8	0	0	1	0	3795	162	477	40	0	0	4	0				
O5 AVX51_common 5 199,511,417 199,511,421 40 3 Pass 560 21 85 1 1 0 13 0 1621 67 172 5 2 0 9 06 AAVS1_common 10 61,111,254 24 4 Pass 57 1 2 2 0 0 0 144 4 15 0 0 2 0 0 144 4 15 0 0 0 0 0 144 4 15 0 0 0 0 0 144 4 15 0 0 0 0 144 4 4 10 0 0 0 10 228 7 21 1 0 <th< td=""><td></td><td>04_AAVS1_common</td><td>12</td><td>107,486,278</td><td>107,486,282</td><td>40</td><td>4</td><td>Pass</td><td>992</td><td>36</td><td>73</td><td>0</td><td>2</td><td>0</td><td>2</td><td>0</td><td>1298</td><td>55</td><td>64</td><td>1</td><td>1</td><td>0</td><td>2</td><td>0</td></th<>		04_AAVS1_common	12	107,486,278	107,486,282	40	4	Pass	992	36	73	0	2	0	2	0	1298	55	64	1	1	0	2	0				
O6_AAVS1_common 11 61,111,251 61,111,251 61,111,254 24 4 Pass 57 1 2 1 0 0 0 0 0 2 0 0 0 144 44 15 0 0 3 0 2 0 0 0 144 44 15 0 0 0 0 144 44 15 0 0 3 0 2 0 0 0 144 44 15 0 0 0 0 144 44 15 0 0 0 0 144 44 15 0 0 0 0 144 44 15 0 0 0 0 0 144 44 15 0 0 0 0 0 0 144 44 15 0		05_AAVS1_common	5	169,511,417	169,511,421	40	3	Pass	560	21	85	1	1	0	13	0	1621	67	172	5	2	0	9	0				
O7_AAVS1_common 10 132_580,828 132_580,833 15 4 Pass 57 1 2 1 0 0 3 0 144 4 15 0 0 0 3 0 0 3 0 <th< td=""><td></td><td>06_AAVS1_common</td><td>11</td><td>61,111,251</td><td>61,111,254</td><td>24</td><td>4</td><td>Pass</td><td>218</td><td>6</td><td>25</td><td>2</td><td>2</td><td>0</td><td>0</td><td>0</td><td>615</td><td>23</td><td>66</td><td>7</td><td>0</td><td>0</td><td>2</td><td>0</td></th<>		06_AAVS1_common	11	61,111,251	61,111,254	24	4	Pass	218	6	25	2	2	0	0	0	615	23	66	7	0	0	2	0				
08 AAVS1_common 8 144,885,132 144,812,114,913 144,812,114,913 144,812,114,913 144,812,114,914,914 144,812,114,914,914 144,91		07_AAVS1_common	10	132,580,828	132,580,833	15	4	Pass	57	1	2	1	0	0	3	0	144	4	15	0	0	0	3	0				
09_AAVS1_common 19 16,174,992 16,174,995 12 3 Intermediate 10_AAVS1_common 8 22,635,586 22,635,586 11 3 Pass 177 4 24 0 1 0 3 0 7 43 2 0 0 7 1 11_AAVS1_common 15 67,137,238 9 4 Pass 5 0 4 1 0 0 5 0 12 14 1 0 0 12 14 1 0 0 12 14 1 0 0 12 14 1 0 0 12 12 14 14 1 0 0 12 12 14 14 1 0 0 12 12 14 14 1 0 0 1 12 13 12 13 12 1 10 10 12 12 14 14 10 0 0 0 1 12 14 14 10 0 0	L	08_AAVS1_common	8	144,885,132	144,885,136	14	3	Pass	61	0	1	0	0	0	1	0	282	7	21	1	1	0	10	0				
I0_AAVS1_common 8 22,635,586 24,636,586 24,636,586 24,636,586 24,636,586 24,636,586 25,573 76,575 5 4 10,10 9 0 10,	ğ	09_AAVS1_common	19	16,174,992	16,174,995	12	3	Intermediate																				
PG 11_AAVS1_common 11 118,717,490 118,717,493 9 5 Fail 12_AAVS1_common 15 67,137,278 67,137,283 9 4 Pass 5 0 4 1 0 0 5 0 14 1 0 0 12 0 13_AAVS1_common 7 73,521,158 9 4 Pass 13 0 2 0<	Ľ	10_AAVS1_common	8	22,635,586	22,635,589	11	3	Pass	177	4	24	0	1	0	3	0	579	7	43	2	0	0	7	0				
O 12_AAVS1_common 15 67,137,278 67,137,283 9 4 Pass 5 0 4 1 0 0 5 0 12 1 14 1 0 0 12 1 13_AAVS1_common 7 73,521,155 73,521,158 9 4 Pass 5 0 4 1 0	Ę	11_AAVS1_common	11	118,717,490	118,717,493	9	5	Fail																				
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	\mathbf{O}	12_AAVS1_common	15	67,137,278	67,137,283	9	4	Pass	5	0	4	1	0	0	5	0	22	1	14	1	0	0	12	0				
14_AAVS1_common 11 65,297,767 65,297,771 8 4 Pass 13 0 2 0	U	13_AAVS1_common	7	73,521,155	73,521,158	9	4	Fail																				
15_AAVS1_common 10 121,198,694 121,198,697 7 4 Pass 51 0 13 1 0 6 1 124 1 14 0 0 0 8 0 16_AAVS1_common 5 175,794,470 175,794,474 6 5 Fail -		14_AAVS1_common	11	65,297,767	65,297,771	8	4	Pass	13	0	2	0	0	0	0	0	26	1	10	0	0	0	1	0				
16_AAVS1_common 5 175,794,470 175,794,474 6 5 Fail 17_AAVS1_common 6 53,200,090 53,200,095 6 6 Pass 55 0 20 0 5 0 6 0 28 0 13 0 0 0 3 0 18_AAVS1_common 15 90,476,691 90,476,696 5 4 Pass 23 0 22 0 1 0 9 0 49 1 24 0 1 0 14 14 0		15_AAVS1_common	10	121,198,694	121,198,697	7	4	Pass	51	0	13	1	1	0	6	1	124	1	14	0	0	0	8	0				
17_AAVS1_common 6 53,200,090 53,200,090 53,200,095 6 6 Pass 55 0 20 0 5 0 6 0 28 0 13 0 0 0 3 0 10 0 13 0 0 0 1 0 10 <td< td=""><td></td><td>16_AAVS1_common</td><td>5</td><td>175,794,470</td><td>175,794,474</td><td>6</td><td>5</td><td>Fail</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>		16_AAVS1_common	5	175,794,470	175,794,474	6	5	Fail																				
18_AAVS1_common 15 90,476,691		17_AAVS1_common	6	53,200,090	53,200,095	6	6	Pass	55	0	20	0	5	0	6	0	28	0	13	0	0	0	3	0				
19_AAVS1_common 16 765,573 765,575 5 4 Intermediate 20_AAVS1_common 19 13,906,202 13,906,206 1 4 Intermediate No breaks		18_AAVS1_common	15	90,476,691	90,476,696	5	4	Pass	23	0	22	0	1	0	9	0	49	1	24	0	1	0	14	0				
20_AAVS1_commo 19 13,906,202 13,906,206 1 4 Intermediate No breaks detected region 5020 - - Pass 3 0 54 1 4 0 26 1 0 6 0 0 1 0 3 1 60 0 3 <th< td=""><td></td><td>19_AAVS1_common</td><td>16</td><td>765,573</td><td>765,575</td><td>5</td><td>4</td><td>Intermediate</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></th<>		19_AAVS1_common	16	765,573	765,575	5	4	Intermediate																				
No breaks betected → regin 5020 - Pass 3 0 54 1 4 0 26 1 60 0 3 3 3 3 3 3 3 3 3 3 3 1 60 0 3 1 60 0 3 3 3 3 3 3 3 3 3 1 60 0 3 1 60 0 3 1 3 1 60 0 3 3 3 3 3 3 3 3 1 1 0 0 0 0 1 0 <td></td> <td>20_AAVS1_common</td> <td>19</td> <td>13,906,202</td> <td>13,906,206</td> <td>1</td> <td>4</td> <td>Intermediate</td> <td></td>		20_AAVS1_common	19	13,906,202	13,906,206	1	4	Intermediate																				
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No breaks tps3 exon05 - Pass 0 0 1 0 0 tps3 exon06 - - Pass tps3 exon07 - Pass tps3 exon08 - Pass tps3 exon09 - Pass tps3 exon09 - Pass tps3 exon09. - Pass		Nie breeke	ar	tp53_exo	on04		-	- Pass	1		0	6 0	0		0	3 0	0		0	7 0	2		0	3 1				
total total <thtotal< th=""> <thtotal< th=""> <thto< td=""><td></td><td>ino preaks</td><td></td><td>tp53_exo tp53_exo</td><td>on05 on06</td><td></td><td>-</td><td>- Pass - Pass</td><td>· · · · · · · · · · · · · · · · · · ·</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0</td><td></td><td>0</td><td>1 0 3 0</td><td>0</td><td></td><td>0</td><td>0 0 1 0</td></thto<></thtotal<></thtotal<>		ino preaks		tp53_exo tp53_exo	on05 on06		-	- Pass - Pass	· · · · · · · · · · · · · · · · · · ·								0		0	1 0 3 0	0		0	0 0 1 0				
OCICULOU 5 tps3 exon08 - Pass 0 0 0 0 0 9 1 0 0 6 1 <td></td> <td>detected ></td> <td>- Trc</td> <td>tp53_exo</td> <td>on07</td> <td></td> <td>-</td> <td>- Pass</td> <td>0</td> <td></td> <td>0</td> <td>9 0</td> <td>0</td> <td></td> <td>0</td> <td>6 0</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		detected >	- Trc	tp53_exo	on07		-	- Pass	0		0	9 0	0		0	6 0												
tp53_exon09a_9b - Pass 0 0 1 0 0 0			00 -	tp53_exo tp53_exo	nus n09			- Pass - Pass			0	2 0	0		0	0 0	0		0	<u>9 1</u> 0 1	0		0	<u>6 0</u> 0 0				
			S –	tp53_exon09a	1_9b		-	- Pass									0		0	0 1	0		0	0 1				
upp3_exoniu - Pass tp53 exon11 - - Pass 0<			⊢ –	tp53_exo tp53_exo	on10 on11			- Pass - Pass	0		0	2 0	0		0	1 0	0		0	1 0	0		0	0 0				

28 BCL11A Mutation Report





			INDUCE-	seq Brea	ak Da	ta	GM24385								MCF10A										
					Break	Misma	Panel		Trea	ated			Cor	ntrol			Tre	ated				Cor	ntrol		
	Off-target name	chr	start	end	Score	tches	Design	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	Freq	D2B*	mnv	snv	sv
	01_BCL11A_common	2	60,722,398	60,722,406	702	0	Pass	308 <mark>8</mark>	122	575	<mark>1</mark> 04	0	0	10	0	4804	225	904	249	0			0	6	0
	01_BCL11A_sequniq	2	211,395,382	211,395,405	8	4	Pass	0	0	2	0	1	0	0	0	3	1	1	0	0			0	0	0
	02_BCL11A_sequniq	8	141,153,116	141,153,139	5	3	Pass	15	0	12	0	4	0	7	0	18	0	16	0	4			0	8	0
	03_BCL11A_sequniq	6	29,936,060	29,936,083	3	4	Fail																		
	04_BCL11A_sequniq	10	34,042,251	34,042,274	2	4	Pass	2	0	9	0	1	0	3	0	5	0	6	0	3			0	2	0
	05_BCL11A_sequniq	11	18,336,599	18,336,622	2	6	Fail																		
	06_BCL11A_sequniq	1	18,342,060	18,342,083	2	6	Pass	3	0	6	0	1	0	3	0	4	0	17	0	1			0	8	0
	07_BCL11A_sequniq	1	203,687,903	203,687,926	2	6	Fail					<u></u>													
<u> </u>	08_BCL11A_sequniq	12	105,662,939	105,662,962	2	6	Pass	0	0	6	0	0	0	3	0	1	0	3	0	0			0	0	0
Ĭ _	09_BCL11A_sequniq	1	236,099,848	236,099,871	2	6	Fail					<u></u>													
5	10_BCL11A_sequniq	14	58,659,277	58,659,300	2	6	Pass	1	0	4	0	1	0	0	1										
<u>5</u> _	11_BCL11A_sequniq	16	7,469,218	7,469,241	2	6	Fail											•							
D	12_BCL11A_sequniq	18	77,689,301	77,689,324	2	6	Pass	1	0	7	1	0	0	4	0	0	0	12	0	0			0	8	0
,	13_BCL11A_sequniq	2	99,730,584	99,730,607	2	6	Pass	1	0	7	0	0	0	3	0	0	0	10	1	0			0	5	0
	14_BCL11A_sequniq	4	100,540,897	100,540,920	2	6	Pass	3	0	7	0	0	0	4	0	1	0	15	0	0			0	11	0
	15_BCL11A_sequniq	4	10,747,452	10,747,475	2	6	Pass	3	0	11	0	1	0	6	0	0	0	11	0	0			0	5	0
	16_BCL11A_sequniq	4	119,091,045	119,091,068	2	6	Fail																		
	17_BCL11A_sequniq	4	18,109,458	18,109,481	2	6	Fail																		
	18_BCL11A_sequniq	5	114,678,383	114,678,406	2	6	Pass	0	0	7	0	0	0	3	0	0	0	9	0	0			0	5	0
	19_BCL11A_sequniq	5	55,996,084	55,996,107	2	6	Pass	4	0	2	0	3	0	0	0	2	0	13	0	1			0	7	0
	20_BCL11A_sequniq	7	116,548,904	116,548,927	2	6	Fail																		

This guide RNA is being used by Vertex and CRISPR Therapeutics and is in BLA for treatment of Sickle Cell Disease

29 Closing Remarks



- INDUCE-seq: An unbiased, genome-wide measurement of On and Off-target DNA breaks
 - Generating a ranked list of induced breaks easily identifies On Target editing.
 - Off-target editing based on induced break recurrency and structure can also be observed.
- **Duplex-seq**: A sensitive, targeted mutation assay revealed the mutational outcome of editing.
- What is the relationship between DSB induction at On and Off-targets and editing outcome?
 - -1. Nuclease-induced break recurrency is highly correlated with mutation frequency.
 - -2. Induced break features can be used to predict editing outcomes.

On and Off-target break and mutation data publicly available in due course.

30 Acknowledgments



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HESI is a global non-profit, scientific organization for PUBLIC BENEFIT. It facilitates public-private partnerships to address contemporary issues in human and environmental health and safety, collaboratively, through the engagement of scientists from academia, government, industry, NGOs, and other strategic partners.

Cell **T**herapy – **TR**acking, **C**irculation and Safety (CT-TRACS)

SECURING THE SAFETY OF CELL THERAPIES TO REALIZE THEIR POTENTIAL

MISSION: To facilitate the translation of cell-based therapies to the clinic by driving: the development of tools, methods and knowledge required to evaluate safety and fate of therapeutic cells.



- >100 participants
- >35 organizations

Co-Chairs:

- Mick Fellows (AstraZeneca)
- Tineke van den Hoorn (CBG-MEB)

Points of Administration/ **Biodistribution WG**

- Localization, persistencee, survival, proliferation
- Types of effects, ability to detect, ability to assess fate/distribution

Tumorigenicity WG

 Need for prediction, assays, consensus

Scientific Program Managers:

Lucilia Mouriès, Imouries@hesiglobal.org

Connie Chen, cchen@hesiglobal.org

WG Leaders:

- Vladimir Ponomarev (MSKCC)
- Ridhirama Bhuwania (Bayer)

WG Leaders:

- Charlotte de Wolf (CBG-MEB)
- Silvana Libertini (Novartis)

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Appendix Supplementary

33 HBD Mutation Report



		l	INDUCE-	seq Brea	ta					GM24385					N				ICF10A				
	-				Break	Misma	Panel		Trea	ated			Cor	ntrol			Trea	ated			Cor	trol	
	Off-target name	chr	start	end	Score	tches	Design	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	mnv	snv	sv	indel	mnv	snv	sv
	01_HBD_unbiaseduniq	2	91,816,830	91,816,841	12		Fail																
_	02_HBD_unbiaseduniq	2	214,013,745	214,013,753	12		Fail																
	03_HBD_unbiaseduniq	5	74,632,729	74,632,742	12		Pass	355	18	55	2	1	0	4	0	13	0	7	0	7	0	3	0
	04_HBD_unbiaseduniq	20	35,402,159	35,402,169	12		Fail																
-	05_HBD_unbiaseduniq	1	174,968,472	174,968,476	11		Fail																
e –	06_HBD_unbiaseduniq	1	249,120,945	249,120,952	11		Fail	-	-	-													
δĹ	07_HBD_unbiaseduniq	12	40,501,557	40,501,562	11		Pass	191	7	22	0	1	0	7	0	0	0	10	0	0	0	7	0
Ξ_	08_HBD_unbiaseduniq	13	28,558,530	28,558,541	11		Fail					<u></u>								·			
D _	09_HBD_unbiaseduniq	19	42,724,469	42,724,472	11		Fail																
D _	10_HBD_unbiaseduniq	1	214,758,727	214,758,735	10		Intermediate																
ě	11_HBD_unbiaseduniq	10	59,417,158	59,417,166	10		Fail																
as —	12_HBD_unbiaseduniq	12	4,964,742	4,964,748	10		Fail																
<u> </u>	13_HBD_unbiaseduniq	14	23,058,076	23,058,080	10		Fail																
Ž _	14_HBD_unbiaseduniq	14	50,429,588	50,429,596	10		Fail													<u></u>			
> _	15_HBD_unbiaseduniq	17	3,571,871	3,571,876	8		Pass	5	0	19	0	1	1	9	0	0	0	19	0	2	0	8	0
	16_HBD_unbiaseduniq	6	31,515,374	31,515,383	6		Missing																
	17_HBD_unbiaseduniq	6	33,043,583	33,043,593	6		Intermediate																
	18_HBD_unbiaseduniq	6	43,337,577	43,337,581	6		Intermediate																
	19_HBD_unbiaseduniq	7	89,975,854	89,975,859	6		Fail																
	20_HBD_unbiaseduniq	8	17,785,549	17,785,553	6		Fail	_															

HBD Mutation Report



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	///		
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			INDUCE-	sed Brea	ак Da	ta		GIVI24385									MCF10A											
					Break	Misma	Panel		Tre	ated			Cor	ntrol		\square	Trea	ated			Con	trol						
	Off-target name	chr	start	end	Score	tches	Design	indel	mnv	snv	sv	indel	mnv	snv	SV	indel	mnv	snv	sv	indel	mnv	snv	sv					
	01_HBD_common	11	5,255,921	5,255,929	1399	0	Pass	3001	165	452	208	1	0	5	0	5053	275	720	252	0	0	4	0					
C	02_HBD_common	Х	24,521,594	24,521,597	285	3	Pass	1845	69	239	43	0	0	1	0	5972	283	859	220	0	0	3	0					
~	03_HBD_common	10	66,509,508	66,509,511	30	5	Pass	89	0	6	0	1	0	1	0	166	6	21	0	0	0	7	0					
2	04_HBD_common	1	46,987,123	46,987,125	26	4	Fail																					
Ĕ	05_HBD_common	20	21,928,955	21,928,958	19	4	Pass	63	0	18	0	0	0	7	0	112	1	15	1	1	0	5	0					
	06_HBD_common	13	21,300,111	21,300,113	16	3	Pass	146	5	27	0	1	0	1	0	340	16	29	0	1	1	0	0					
<u>o</u> _	07_HBD_common	11	66,610,211	66,610,213	15	3	Intermediate	49	0	3	1	1	0	0	0	411	10	47	6	0	0	3	0					
Ŭ 🗌	08_HBD_common	11	74,935,022	74,935,025	13	4	Pass	6	0	6	0	1	0	5	0	11	1	30	0	0	1	8	0					
	09_HBD_common	3	58,080,494	58,080,498	13	5	Pass	9	1	8	0	0	0	4	0	52	0	11	0	0	0	4	0					
	10_HBD_common	5	138,374,009	138,374,011	8	5	Fail	3	0	2	0	3	0	0	0	2	0	3	0	1	0	1	0					
	11_HBD_common	2	231,737,856	231,737,861	7	5	Pass	81	0	13	0	3	0	4	0	3	1	9	0	0	0	7	0					
										111																		

35 Reproducibility of INDUCE-seq across two labs



Broken String

15 Comparing CRISPR induced break structure with mutational outcome



Mutational outcome at CRISPR on-target site correlates with break structure

1bp read overlap suggests 1bp 'staggered' break rather than expected blunt DSB

Highest frequency mutation detected by targeted deep sequencing is 1bp insertion (37.24%)

Similar 1bp overlapping break pattern and mutational outcome observed at top scoring off-targets:

- OT-1 (18.75%, 1bp insertion)



37 Mutation Frequency of Gene-edited cells



Broken String

38 Mutation Frequency at different time point after gene editing



Broken String

39 INDUCE-seq detects break Structure & Frequency



Broken Strin