2019 CRISPR Year in Review

Science Circle
January 4th 2020

Stephen Gasior, Ph.D.
a.k.a. Stephen Xootfly
Sr. Research Associate
Corteva Agrisciences

Researcher at Corteva some of whose research is presented here. Not representing the company's positions.

Nothing should be construed as investment advice or company forward-looking statements

2019 CRISPR Year in Review

Background

Technology Advances

Prime Editing, Specificity

New Tools

Type I, Transposon insertions, Cas14,

Intellectual Property and Ethics

USPO Revisits UC Berkely, He Jiankui jailed

Target Genes and Modified Organisms

extra DNA oopsie, human trials

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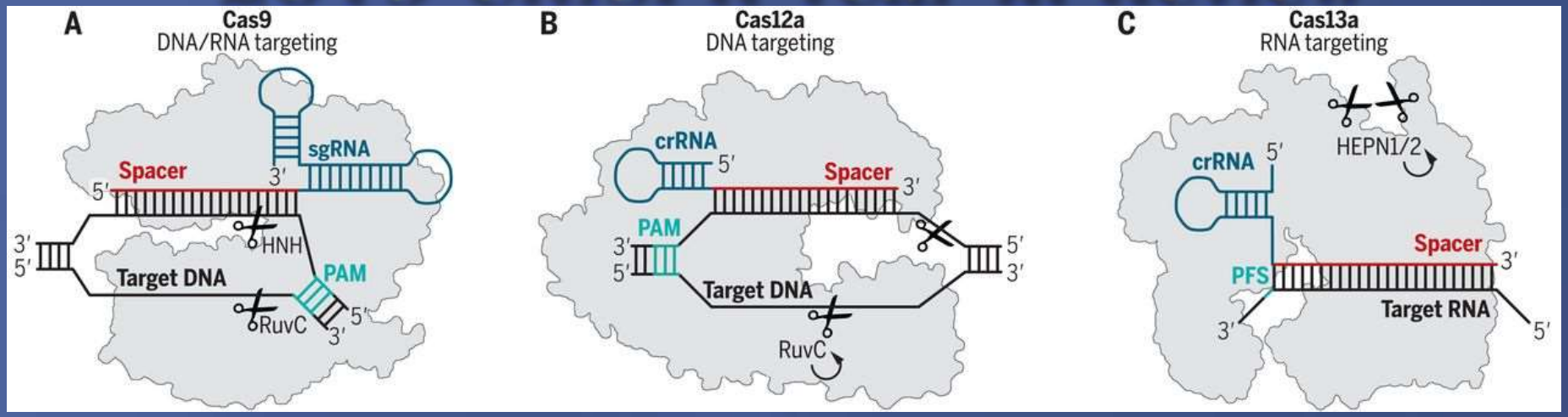

Fig. 2 Schematic of class 2 CRISPR-Cas systems.

(A) Class 2 type II CRISPR-Cas9 shown schematically with an sgRNA (blue) encoding a spacer (red) bound to a target dsDNA (black) proximal to a PAM (teal). Correct base-pairing activates the HNH and RuvC nuclease domains, cleaving both strands (scissors). (B) Class 2 type V CRISPR-Cas12a shown schematically with a crRNA (blue) encoding a spacer (red) bound to a complementary dsDNA target (black) proximal to a PAM (teal). Correct base-pairing activates the RuvC nuclease, cleaving both strands (scissors) with multiple-turnover general ssDNase activity (arrow). (C) Class 2 type VI CRISPR-Cas13a shown schematically with a crRNA (blue) encoding a spacer (red) bound to a complementary RNA target (black). Correct base-pairing activates HEPN nuclease general ssRNase activity (arrow).

Technique allows us to locate a protein to precise locations via RNA homology

Knott, Gavin J., and Jennifer A. Doudna. "CRISPR-Cas guides the future of genetic engineering." *Science* 361.6405 (2018): 866-869.

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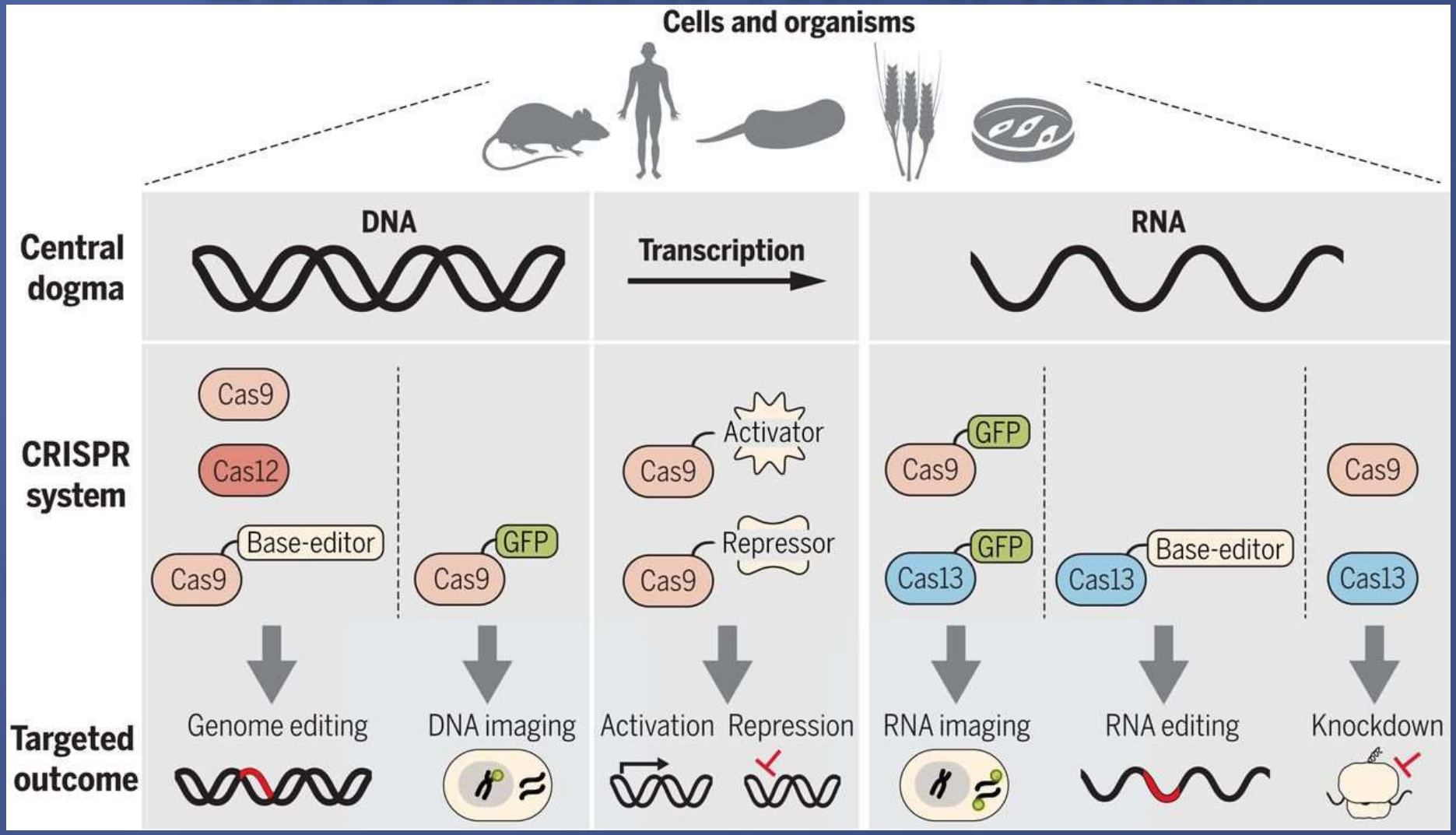

Fig. 3 CRISPR-Cas systems allow genetic manipulation across the central dogma.

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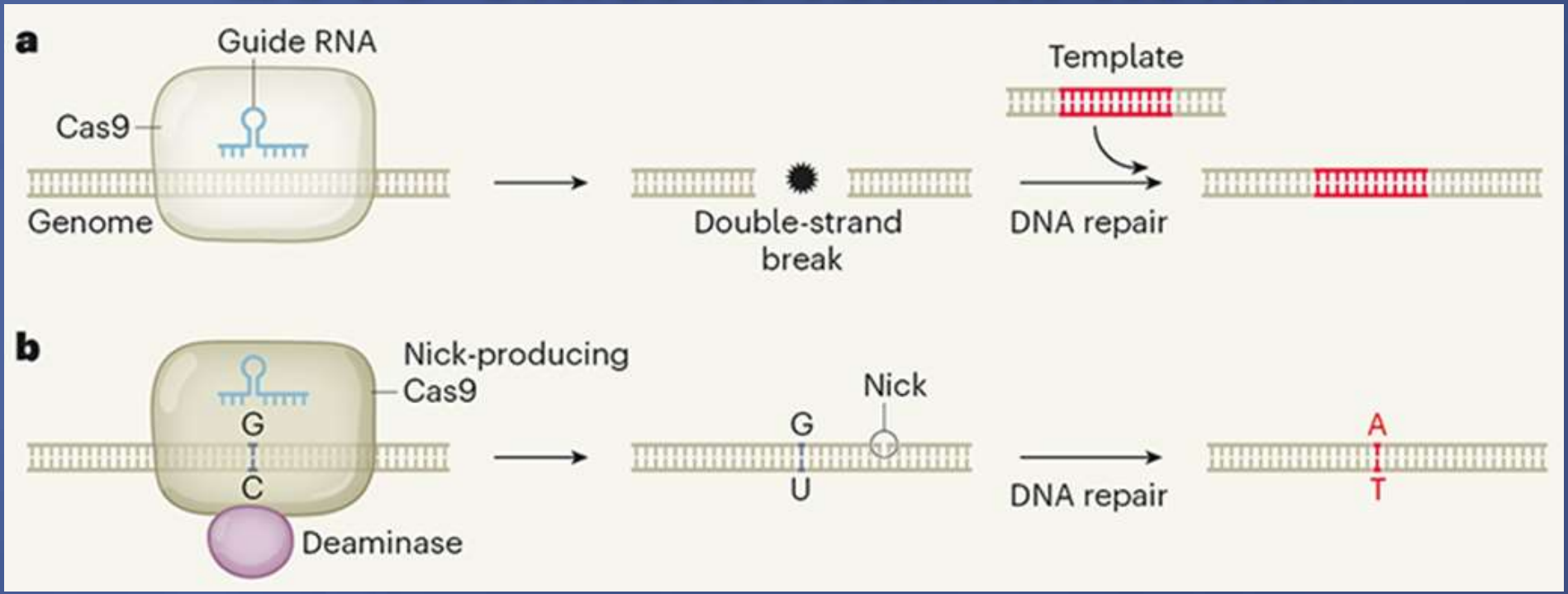

Figure 1 | Evolution of genome editing.

a, In conventional genome editing, a Cas9 enzyme is directed to a position in the genome by a guide RNA, and produces a double-strand break. The host cell's DNA-repair machinery fixes the break, guided by a template DNA, incorporating template sequences into the duplex. **b,** In an approach called base editing, a Cas9 that produces only single-strand breaks (nicks) works with a deaminase enzyme. The deaminase chemically modifies a specific DNA base — here, a cytidine base (C) is converted to uracil (U). DNA repair then fixes the nick and converts a guanine–uracil (G–U) intermediate to an adenine–thymine (A–T) base pair. This method is more precise than a, but makes only single-nucleotide edits

Platt, R. J. "CRISPR tool modifies genes precisely by copying RNA into the genome." Nature 576.7785 (2019): 48.

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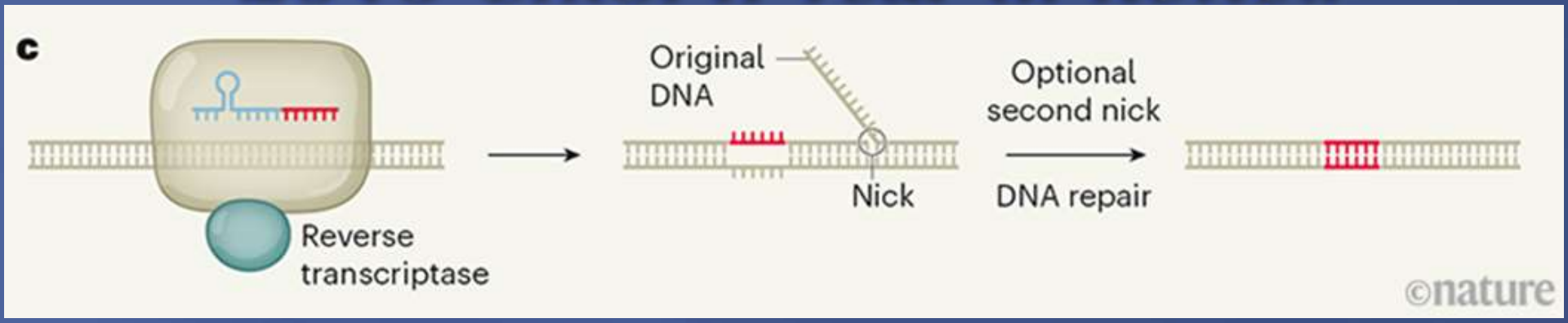

Figure 1 | Evolution of genome editing.

c, Anzalone et al.1 report prime editing, which can precisely edit DNA sequences. A nick-producing Cas9 and a reverse transcriptase enzyme produce nicked DNA into which sequences corresponding to the guide RNA have been incorporated. The original DNA sequence is cut off, and DNA repair then fixes the nicked strand to produce a fully edited duplex. In some cases, another nick is made in the unedited strand of the duplex before the DNA-repair step (not shown).

Technique allows

1) **template sequence for the repair**
2) **small but precise insertions or deletions**

Platt, R. J. "CRISPR tool modifies genes precisely by copying RNA into the genome." Nature 576.7785 (2019): 48.

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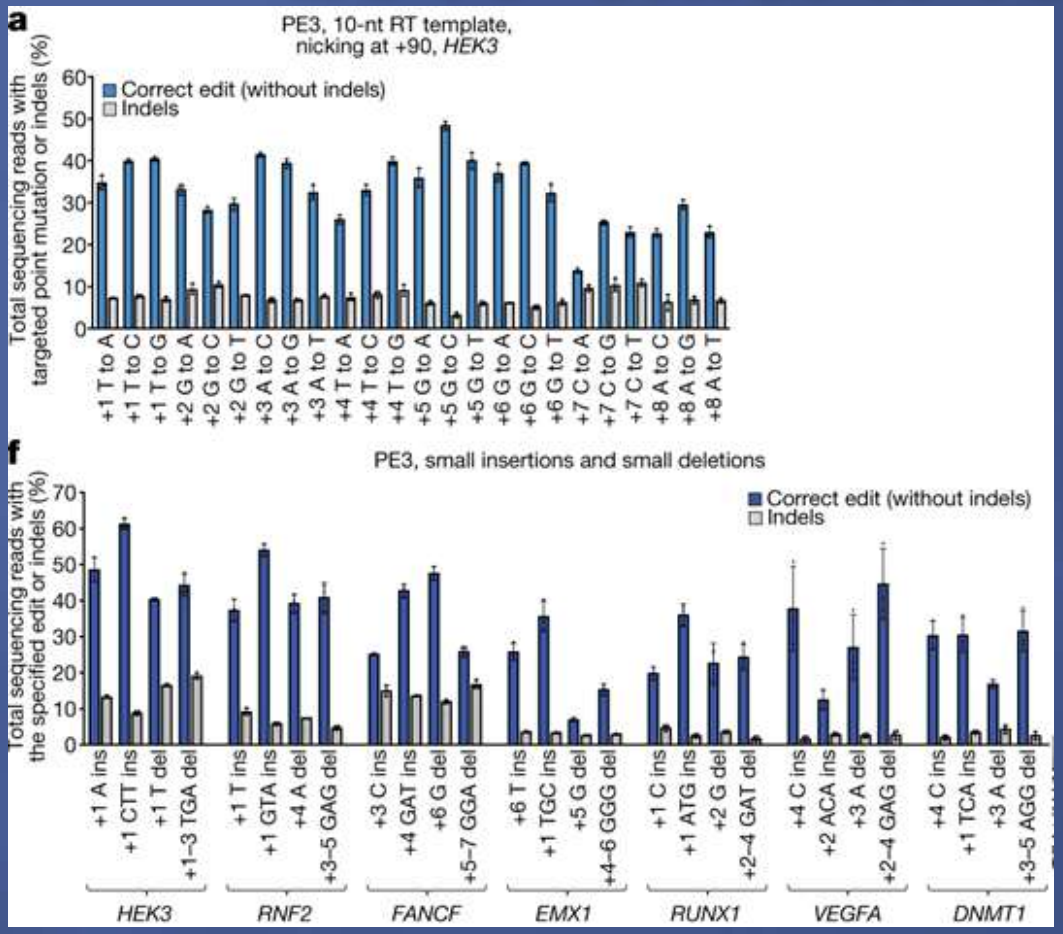

Fig. 4: Targeted insertions, deletions, and all 12 types of point mutation with PE3 at seven endogenous genomic loci in HEK293T cells.

a, All 12 types of single-nucleotide edit from position +1 to +8 of the HEK3 site using a 10-nt RT template, counting the first nucleotide following the pegRNA-induced nick as position +1. b f, Targeted 1- and 3-bp insertions, and 1- and 3-bp deletions with PE3 at seven endogenous genomic loci. Editing efficiencies reflect sequencing reads that contain the intended edit and do not contain indels among all treated cells, with no sorting. Mean ± s.d. of n = 3 independent biological replicates.

Particular C to A|G|T, A to C|G|T, G to A|C|T, T to G|C|A

Anzalone, Andrew V., et al. "Search-and-replace genome editing without double-strand breaks or donor DNA." Nature 576.7785 (2019): 149-157.

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Site	Computational prediction		CLEAVE-Seq reads	*In plant validation (Agro)*	Sequence																							Genomic location
	Mismatch	*Bulge*		*% Mutant Alleles*	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	*0*	*-1*	*-2*	
M1-1	0	0	201	61.3%	G	C	A	C	G	T	A	C	G	T	C	A	C	C	A	T	C	C	C	G	C	*C*	*G*	*G*	Chr1:14702765-14702788
M1-2	2	2	93	0.0%						A														T		*A*			Chr10:138875022-138875045
M1-3	3	1	7	0.0%						A	a					T							A			*G*			Chr10:120497792-120497768
M1-4	3	1	5	0.0%						A	a					T							A			*G*			Chr10:120753156-120753132
M1-5	3	1	3	0.0%						C											A	G				*G*			Chr4:38687989-38688011
M1-6	4	1	4	0.0%			T			A										C		A				*C*			Chr4:170037576-170037599
M1-7	4	1	19	0.0%	C							T	A											T		*G*			Chr4:53255942-53255919
M1-8	4	1	15	0.0%				G	C		a			C		G										*T*			Chr9:14541330-14541354
M1-9	5	1	5	0.0%		G	C			C							G									*G*			Chr1:3497051-3497072
M1-10	5	1	4	0.0%						G		G	T					A		C						*C*			Chr2:114192761-114192783
M1-11	5	1	4	0.0%		a		G				A	A					A		G						*G*			Chr6:32905376-32905399
M1-12	5	1	6	0.0%								T	T	A		T	T									*C*			Chr7:135823779-135823801
M1-13	5	1	8	0.0%		a		T			G		C	A				A								*T*			Chr7:26447133-26447156
M1-14	5	1	9	0.0%		G	T		A									T			A					*C*			Chr8:174780615-174780640
M1-15	5	1	4	0.0%	C			G				A	A											A		*G*			Chr8:53709884-53709861
M1-16	5	2	5	0.0%		G	G			gc	G					G								A		*C*			Chr2:208170984-208170961

Table 2 CLEAVE-seq data and validation of M1 sites in plants.

On-target site M1-1 is shown on the top. Percent mutant allele is number of alleles with mutation/total number of alleles observed. DNA and RNA bulges are shown as gray and black boxes, respectively. Lower case indicates additional nucleotides in RNA. Mismatch is shown as a letter indicating the nucleotide, absence of a nucleotide indicates no mismatch. PAM is italicized.

Young, Joshua, et al. "CRISPR-Cas9 Editing in Maize: Systematic Evaluation of Off-target Activity and Its Relevance in Crop Improvement." Scientific reports 9.1 (2019): 6729.

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Site	Computational prediction		CLEAVE-Seq reads	*In plant validation (Agro)*	Sequence																				Genomic location
	Mismatch	*Bulge*		*% Mutant Alleles*	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	*0*	*-1*	*-2*	
M3-1	0	0	164	94.3%	G	C	G	G	A	G	A	C	T	A	A	G	T	G	G	C	T	G	T	A	*G*	*G*	*G*	Chr2:4233941-4233963
M3-2	3	1	14	0.0%	T								A			a							C		*T*			Chr3:211837283-211837306
M3-3	3	2	7	0.0%							G		G								G				*C*			Chr1:135533537-135533562
M3-4	3	2	11	0.0%							G		A								G				*C*			Chr1:135560571-135560593
M3-5	3	2	19	0.0%	A								A					A							*T*			Chr4:155729295-155729317
M3-6	4	2	7	0.0%	A			T					A											T	*G*			Chr2:234759654-234759632
M3-7	5	1	6	0.0%							G		A								C	A	C	a	*G*			Chr3:74618205-74618227

Table 3 CLEAVE-seq data and validation of M3 sites in plants.

On-target site M3-1 is shown on the top. Percent mutant allele is number of alleles with mutation/total number of alleles observed. DNA and RNA bulges are shown as gray and black boxes, respectively. Lower case in gray box indicates additional nucleotide(s) in RNA. Mismatch is shown as a letter indicating the nucleotide, absence of a nucleotide indicates no mismatch. PAM is italicized.

Young, Joshua, et al. "CRISPR-Cas9 Editing in Maize: Systematic Evaluation of Off-target Activity and Its Relevance in Crop Improvement." Scientific reports 9.1 (2019): 6729.

Similar findings and new techniques below

Wienert, Beeke, et al. "Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq." Science 364.6437 (2019): 286-289.

Zuo, Erwei, et al. "Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos." Science 364.6437 (2019): 289-292.

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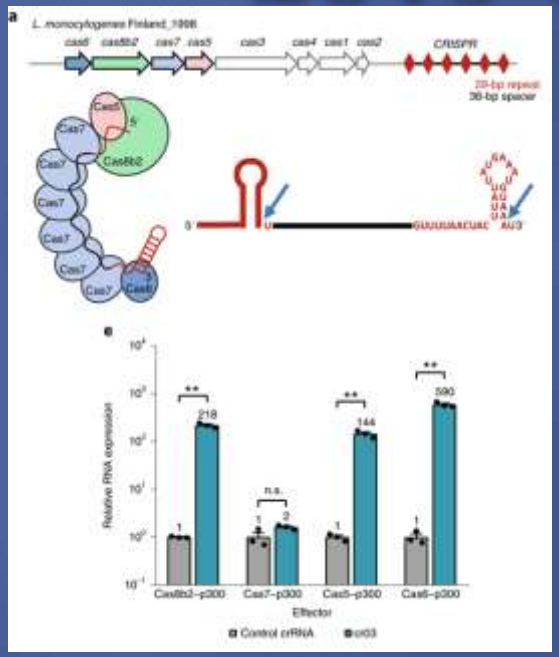
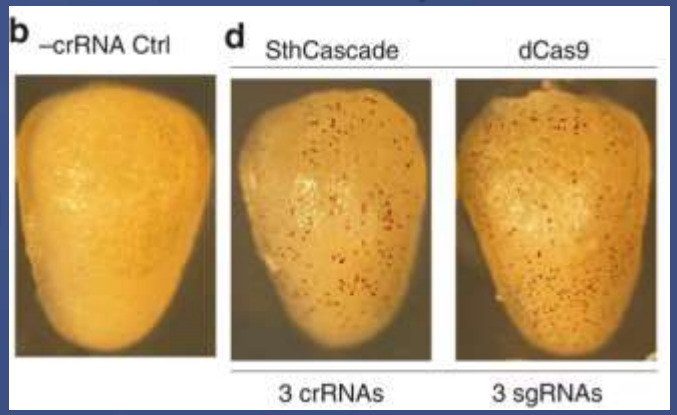


Fig. 4: LmoCascade activates transcription of IL1RN gene in human cells.

a, Schematic of the type I-B CRISPR–Cas system in L. monocytogenes Finland_1998 showing predicted LmoCascade stoichiometry and crRNA processing. Genes encoding proteins that comprise the LmoCascade complex are presented in different colors. e, Relative IL1RN expression following co-transfection of control crRNA or cr03 and LmoCascade with various Cas–p300 effectors. All samples were processed at 3 d post-transfection. Tukey test following log transformation, **$P < 0.001$ compared to control crRNA. Three biological independent samples. Error bars, s.e.m. Numbers above bars indicate mean relative expression.

dCas9 and type I-E Cascade chromosomal gene activation in Zea mays.



d SthCascade (CasA-CBF1, CasB, CasC, CasD-CBF1, and CasE-CBF1) and dCas9-CBF1 anthocyanin phenotype when all 3 SthCascade crRNAs or dCas9 sgRNAs were co-delivered. Photos were taken 48 h post-transformation.

Pickar-Oliver, Adrian, et al. "Targeted transcriptional modulation with type I CRISPR–Cas systems in human cells." Nature biotechnology 37.12 (2019): 1493-1501.

Young, Joshua K., Gasior, Stephen L. et al. "The repurposing of type IE CRISPR-Cascade for gene activation in plants." Communications biology 2.1 (2019): 1-7.

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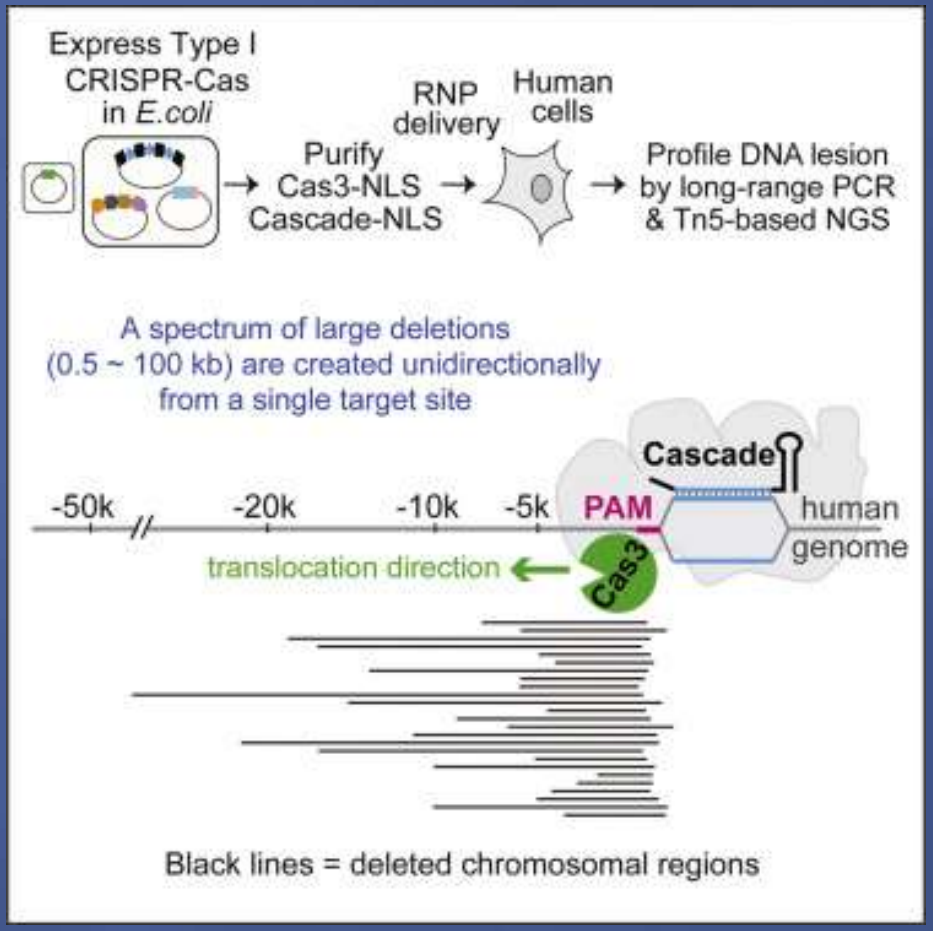
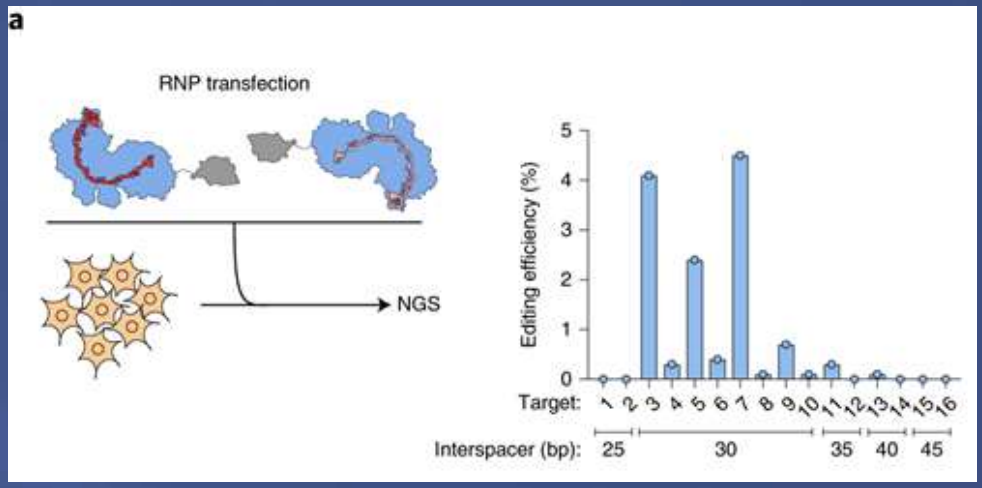


Dolan, Adam E., et al. "Introducing a spectrum of long-range genomic deletions in human embryonic stem cells using type I CRISPR-Cas." Molecular cell 74.5 (2019): 936-950.



Cameron, Peter, et al. "Harnessing type I CRISPR–Cas systems for genome engineering in human cells." Nature biotechnology 37.12 (2019): 1471-1477.

Type I CRISPR systems (multiple proteins) function similarly to single protein systems
--because of size, do offer some expanded utility when compactness is not an issue
--IP is outside of current controversy

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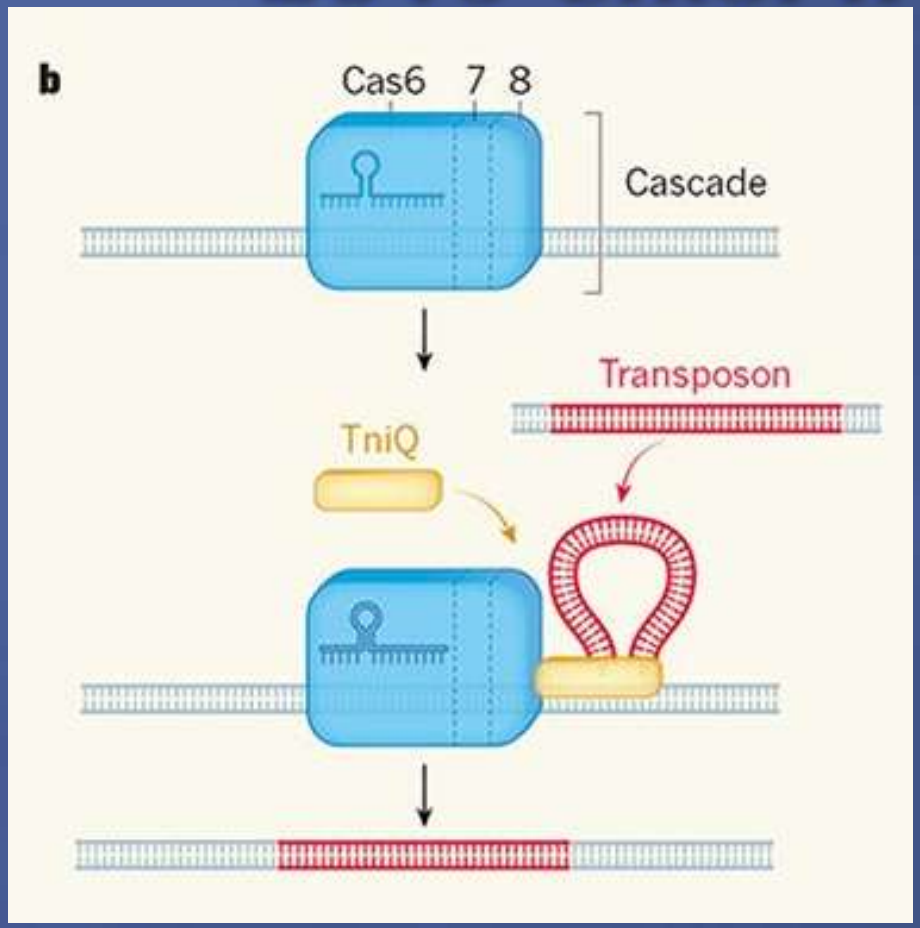

Fig. 1

Klompe et al. report that DNA elements called transposons use CRISPR machinery called Cascade (formed from Cas6, Cas7 and Cas8 proteins) to insert themselves into genomes. Cascade is directed to a chromosome by a guide RNA, but then binds a transposase-associated protein, TniQ, which in turn recruits the transposon and integrates it into the chromosome. This RNA-directed mechanism for DNA transposition avoids the need for double-strand breaks or long flanking sequences, and thus might help to address some of the shortcomings of conventional gene editing.

Inserts with less dangerous lesions than regular Cas9 double strand breaks

Urnov, Fyodor D. "Hijack of CRISPR defences by selfish genes holds clinical promise." (2019).

Klompe, Sanne E., et al. "Transposon-encoded CRISPR–Cas systems direct RNA-guided DNA integration." Nature 571.7764 (2019): 219-225.

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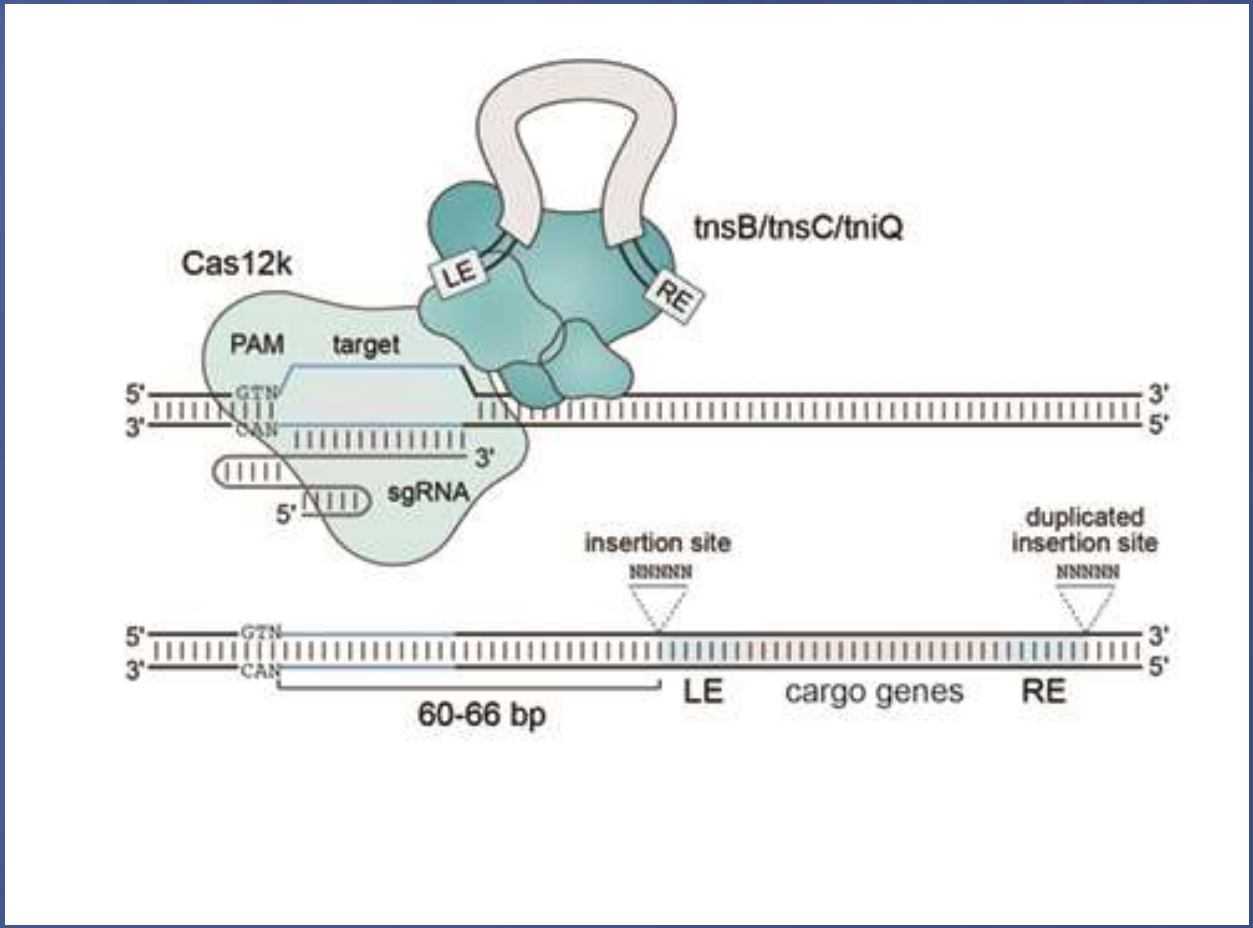

Similar results as previous slide but a less complicated Cas system (Class II)

Strecker, Jonathan, et al. "RNA-guided DNA insertion with CRISPR-associated transposases." Science (2019): eaax9181.

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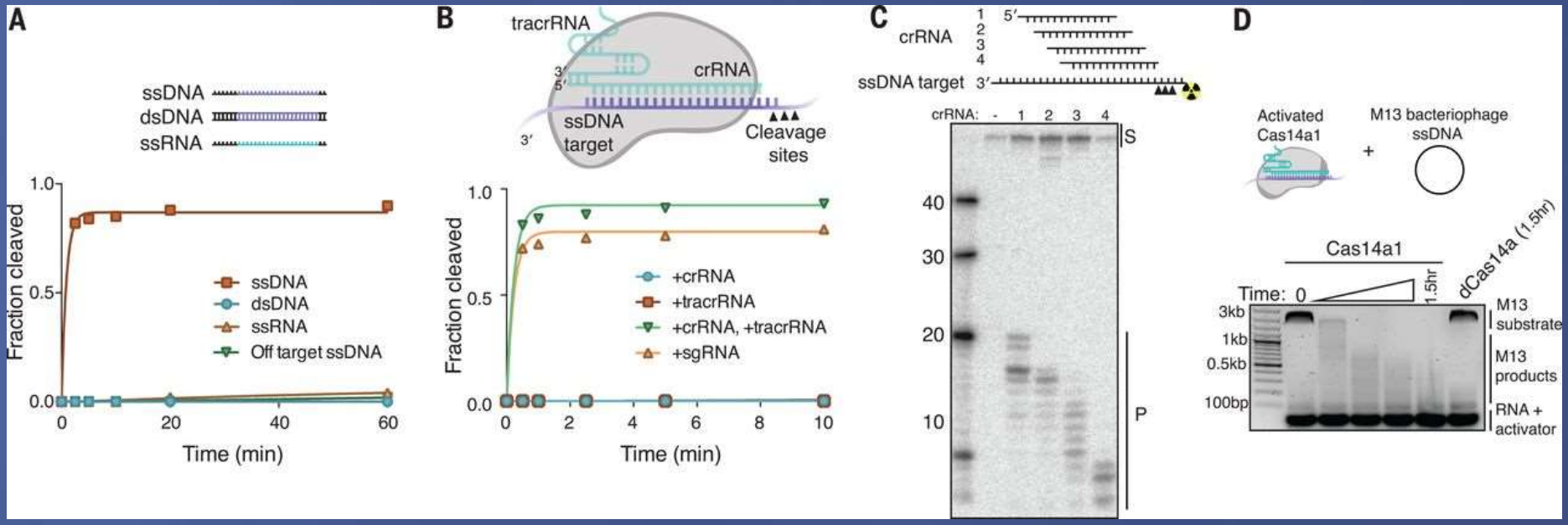

Fig. 3 CRISPR-Cas14a is an RNA-guided DNA endonuclease.

(A) Cleavage kinetics of Cas14a1 targeting ssDNA, dsDNA, ssRNA, and off-target ssDNA. **(B)** Diagram of Cas14a RNP bound to target ssDNA and Cas14a1 cleavage kinetics of radiolabeled ssDNA in the presence of various RNA components. **(C)** Tiling of a ssDNA substrate by Cas14a1 guide sequences. **(D)** Cleavage of the ssDNA viral M13 genome with activated Cas14a1.

1) Guided single-strand DNA cleavage
2) High-fidelity ssDNA SNP detection

Harrington, Lucas B., et al. "Programmed DNA destruction by miniature CRISPR-Cas14 enzymes." Science 362.6416 (2018): 839-842.

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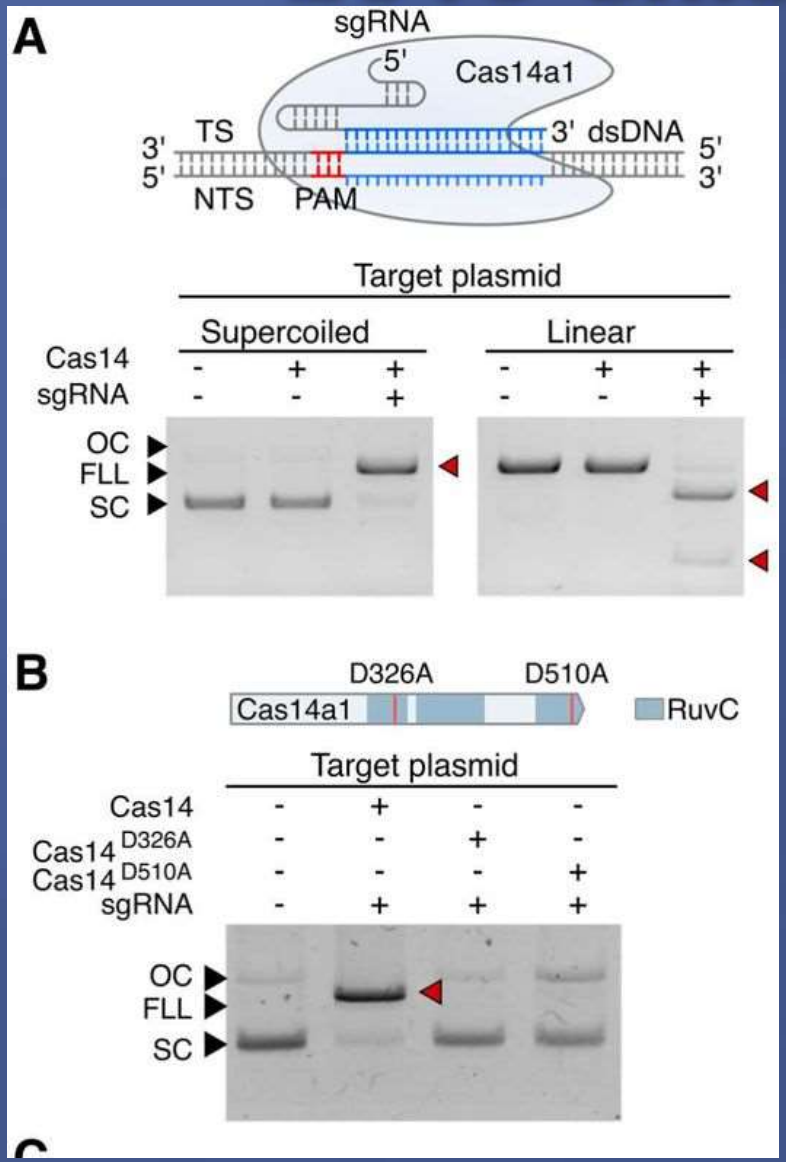

Fig. 2 Cas14a1 RNP complex is a PAM-dependent dsDNA endonuclease.

(A) Cas14a1 RNP complex cleaves plasmid DNA targets in vitro in a PAM-dependent manner. (B) Alanine substitution of two conserved RuvC active site residues completely abolishes Cas14a1 DNA cleavage activity. TS – target strand, NTS – non-target strand, SC – supercoiled, FLL – full length linear, OC – open circular.

1) **Guided double-strand DNA cleavage**
2) **Small size (about 1/3 of Cas9) makes it feasible to use in viral delivery vectors potentially**

Karvelis, Tautvydas, et al. "PAM recognition by miniature CRISPR-Cas14 triggers programmable double-stranded DNA cleavage." bioRxiv (2019): 654897.

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New CRISPR Interference: The Details
By Kevin E. Noonan

On June 24th, the U.S. Patent and Trademark Office declared an interference (No. 106,115) between patents and applications owned between Broad and Berkeley…

Does the prior work by Doudna (of UC Berkeley) in prokaryotes make obvious the application (reduction to practice) of performing the work in eukaryotes in, particular the single guide (cr + tracr) fusion RNA, for which Zhang (of Broad Institute) was awarded a priority patent?

To be decided in 2020? Or beyond…

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Chinese scientist who edited genes of twin babies is jailed for 3 years

Julia Hollingsworth and Isaac Yee
Mon December 30, 2019

On Monday, the Shenzhen Nanshan District People's Court sentenced He to three years behind bars and a 3 million yuan ($430,000) fine.

"The court held that the three defendants failed to obtain a doctor's qualification and pursued profit, deliberately violated the relevant national regulations on scientific research and medical management, crossed the bottom line of scientific and medical ethics, and rashly applied gene-editing technology to human-assisted reproductive medicine, and disrupted the medical treatment,"

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CRISPR Just Created a Hornless Bull, and It's a Step Forward for Gene-Edited Food

By Shelly Fan - Oct 22, 2019

https://singularityhub.com/2019/10/22/crispr-just-created-a-hornless-bull-and-its-a-step-forward-for-gene-edited-food/

Recreating a naturally occurring dominant mutation called *polled*. TALEN

https://recombinetics.com/

https://animalscience.ucdavis.edu/news/alison-van-eenennaam-examines-how-gene-editing-can-enhance-sustainability-plus-animal-health

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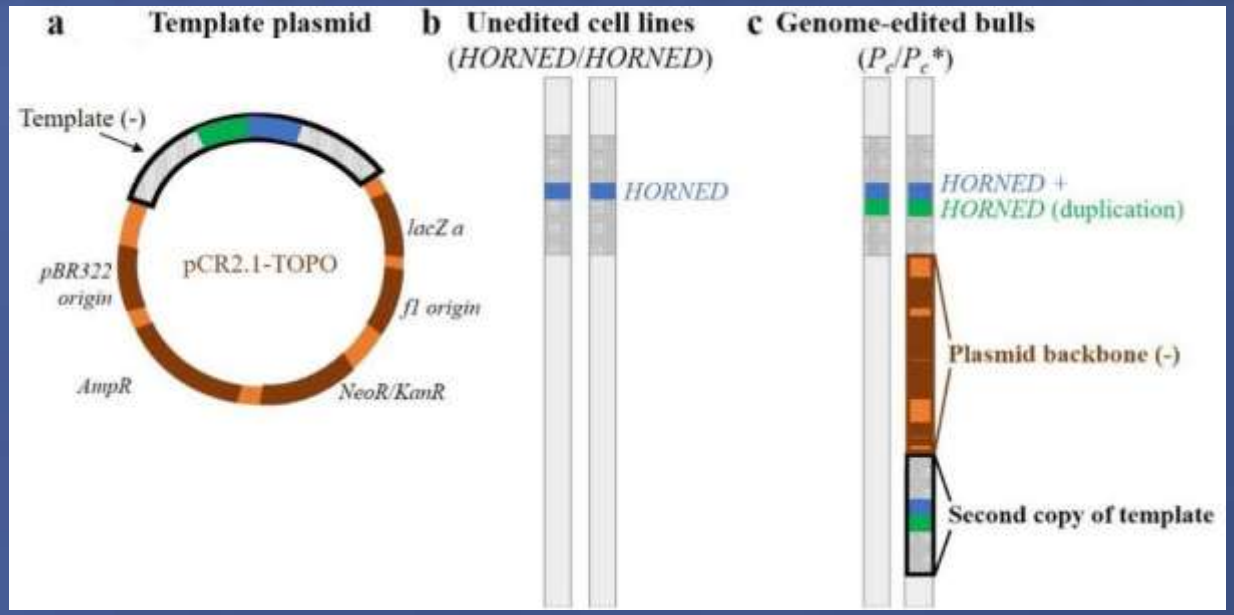

Fig. 1 Template plasmid integration at the target site of genome-edited calves.

Genomic structure of the template plasmid (a), unedited parental cell lines (b), and the genome-edited calves (c). (a) The repair template, containing the Pc sequence and flanking homology arms, is inserted in the pCR2.1 plasmid in an antisense orientation at the TOPO cloning site. (b) The unedited parental cell lines are homozygous for HORNED. (a) The genome-edited calves are heterozygous: one chromosome contains the intended edit (Pc), while the other chromosome harbors template plasmid integration, in addition to the intended edit.

Norris, Alexis L., et al. "Template plasmid integration in germline genome-edited cattle." BioRxiv (2019): 715482.

Young, Amy E., et al. "Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull." Nature biotechnology (2019): 1-8. [UC Davis]

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"First U.S. Patients Treated With CRISPR As Human Gene-Editing Trials Get Underway"
April 16, 2019 (Heard on All Things Considered-NPR)

https://www.npr.org/sections/health-shots/2019/04/16/712402435/first-u-s-patients-treated-with-crispr-as-gene-editing-human-trials-get-underway

Sickle cell anemia and Beta thalassemia (CRISPR Therapeutics and Vertex Pharmaceuticals)

Cancer Immunotherapy (University of Pennsylvania)

Leber congenital amaurosis (Editas Medicine)

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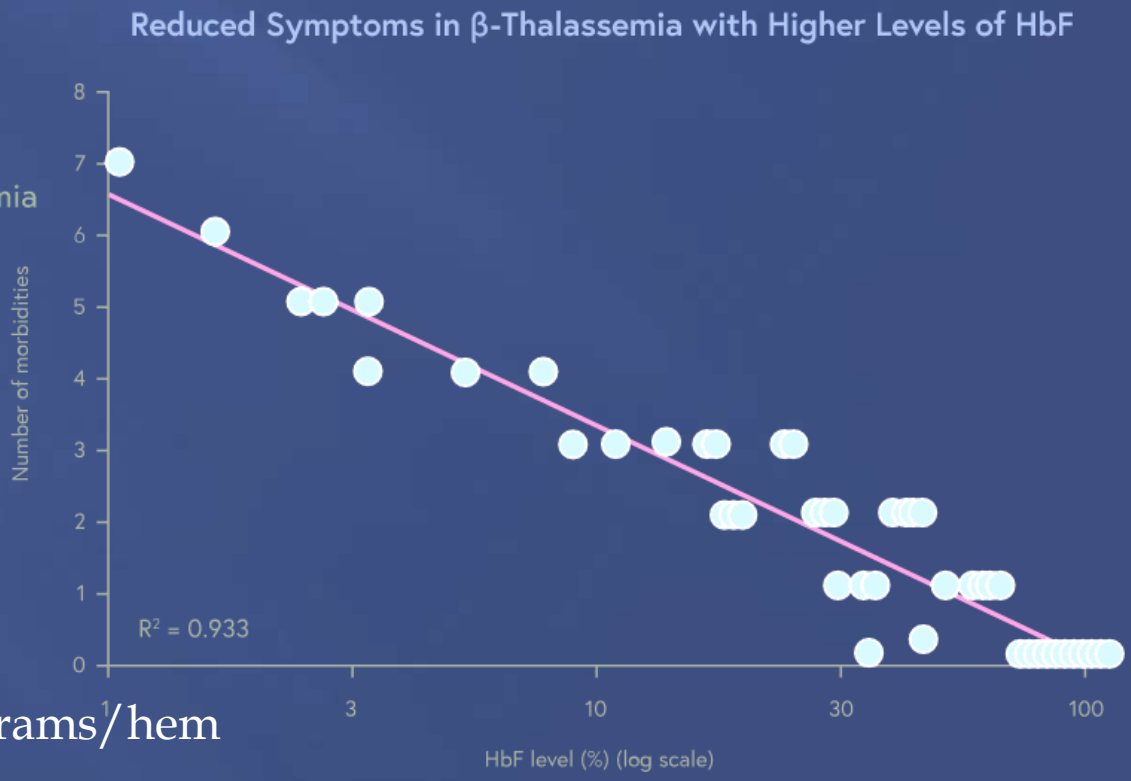
Sickle cell anemia and Beta thalassemia CRISPR Therapeutics and Vertex Pharmaceuticals

Blood disorders caused by mutations in the β-globin gene



http://www.crisprtx.com/programs/hemoglobinopathies

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Sickle cell anemia and Beta thalassemia
CRISPR Therapeutics and Vertex Pharmaceuticals

https://investors.vrtx.com/news-releases/news-release-details/crispr-therapeutics-and-vertex-announce-positive-safety-and

Nov 19, 2019

CRISPR Therapeutics and Vertex Announce Positive Safety and Efficacy Data From First Two Patients Treated With Investigational CRISPR/Cas9 Gene-Editing Therapy CTX001® for Severe Hemoglobinopathies

-Two patients treated with CTX001 successfully engrafted and demonstrated an initial safety profile consistent with myeloablative busulfan conditioning and autologous hematopoietic stem cell transplant-

-Beta thalassemia: Patient is transfusion independent with total hemoglobin level of 11.9 g/dL and 10.1 g/dL fetal hemoglobin at nine months after CTX001 infusion-

-Sickle cell disease: Patient is free of vaso-occlusive crises with total hemoglobin level of 11.3 g/dL and 46.6% fetal hemoglobin at four months after CTX001 infusion-

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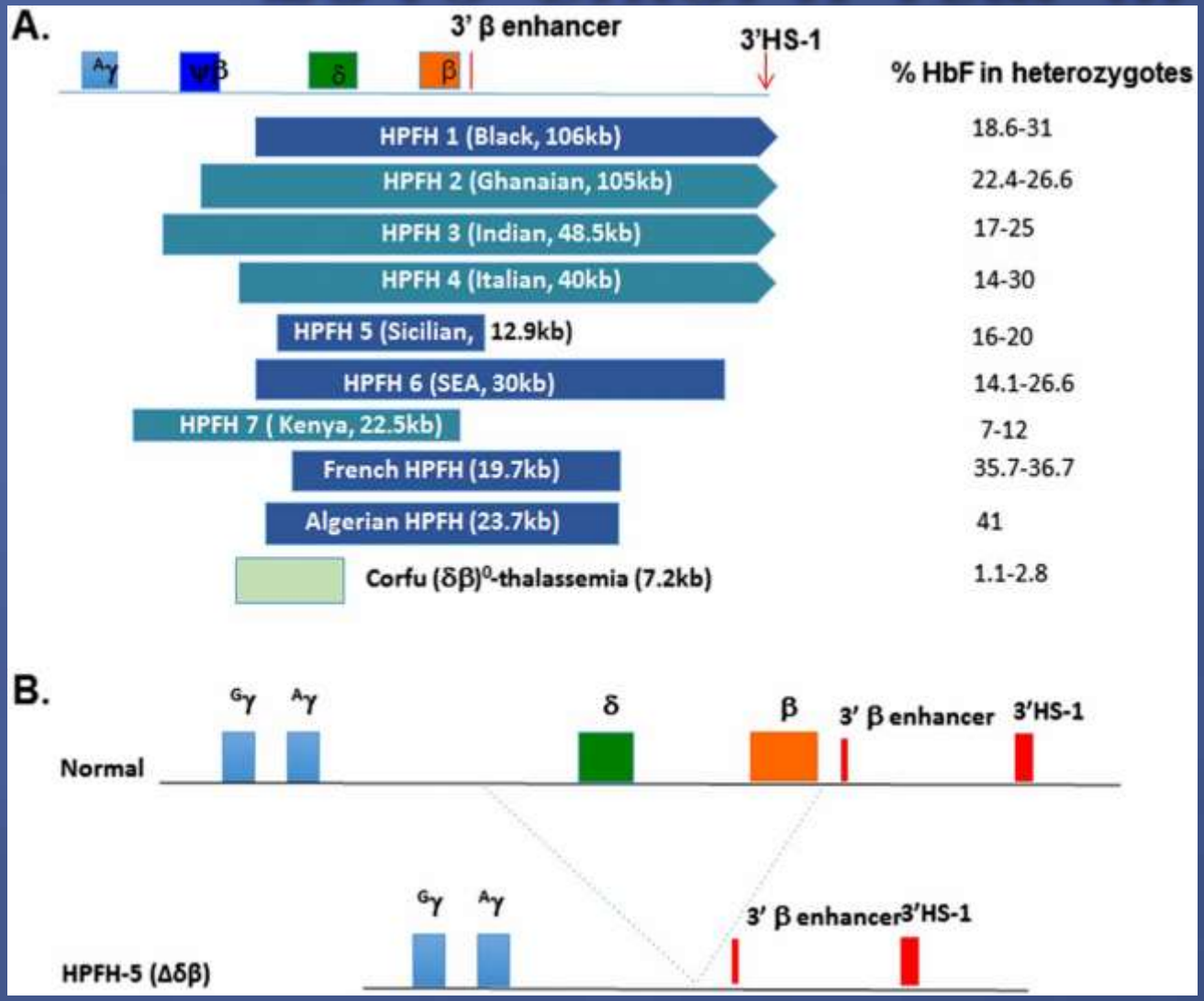

Fig. S1 Deletion type of hereditary persistence of fetal hemoglobin (HPFH).

Ye, Lin, et al. "Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and β-thalassemia." Proceedings of the National Academy of Sciences 113.38 (2016): 10661-10665.

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Cancer

University of Pennsylvania Abramson Cancer Center (Nov 6th Press Release)

Researchers from the Abramson Cancer Center of the University of Pennsylvania have infused three participants in the trial thus far – two with multiple myeloma and one with sarcoma – and have observed the edited T cells expand and bind to their tumor target with no serious side effects related to the investigational approach. Penn is conducting the ongoing study in cooperation with the Parker Institute for Cancer Immunotherapy (PICI) and Tmunity Therapeutics.

The first two edits remove a T cell's natural receptors to make sure the immune cells bind to the right part of the cancer cells. The third edit removes PD-1, a natural checkpoint that sometimes blocks T cells from doing their job. At that point, a lentivirus is used to insert an affinity-enhanced T cell receptor (TCR), which tells the edited T cells to target an antigen called NY-ESO-1.

Two of the patients treated at Penn have an incurable bone marrow cancer called multiple myeloma, while the third has a soft tissue cancer called sarcoma. Their cancers overproduce a protein called NY-ESO-1.

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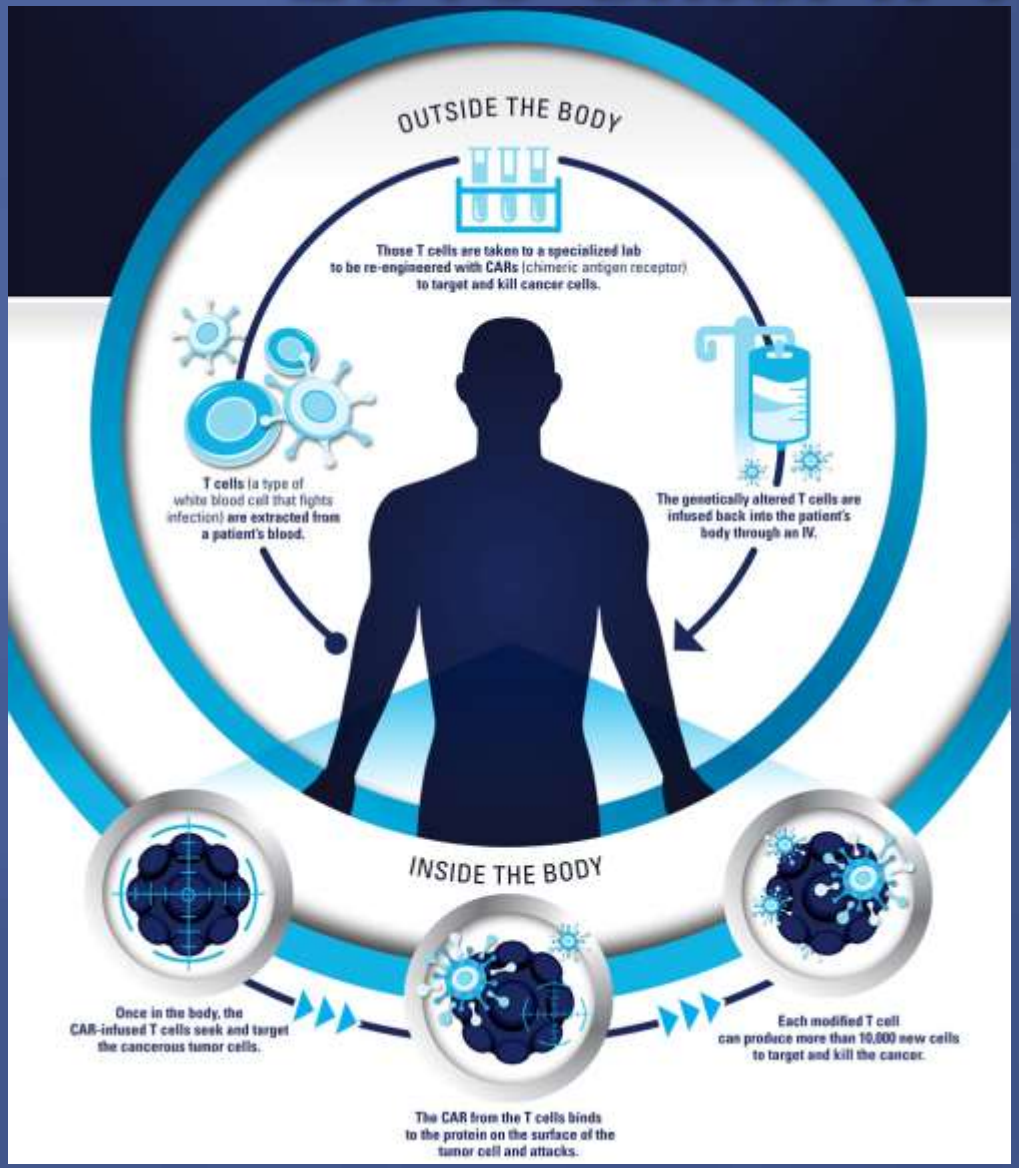

Basic idea is to remove T-cells from patient's body, insert the ability to recognize a cancer epitope and return to patient

CAR-T

https://www.pennmedicine.org/cancer/-/media/images/miscellaneous/infographics/personalized_cellular_therapy_infographic_full.ashx?la=en

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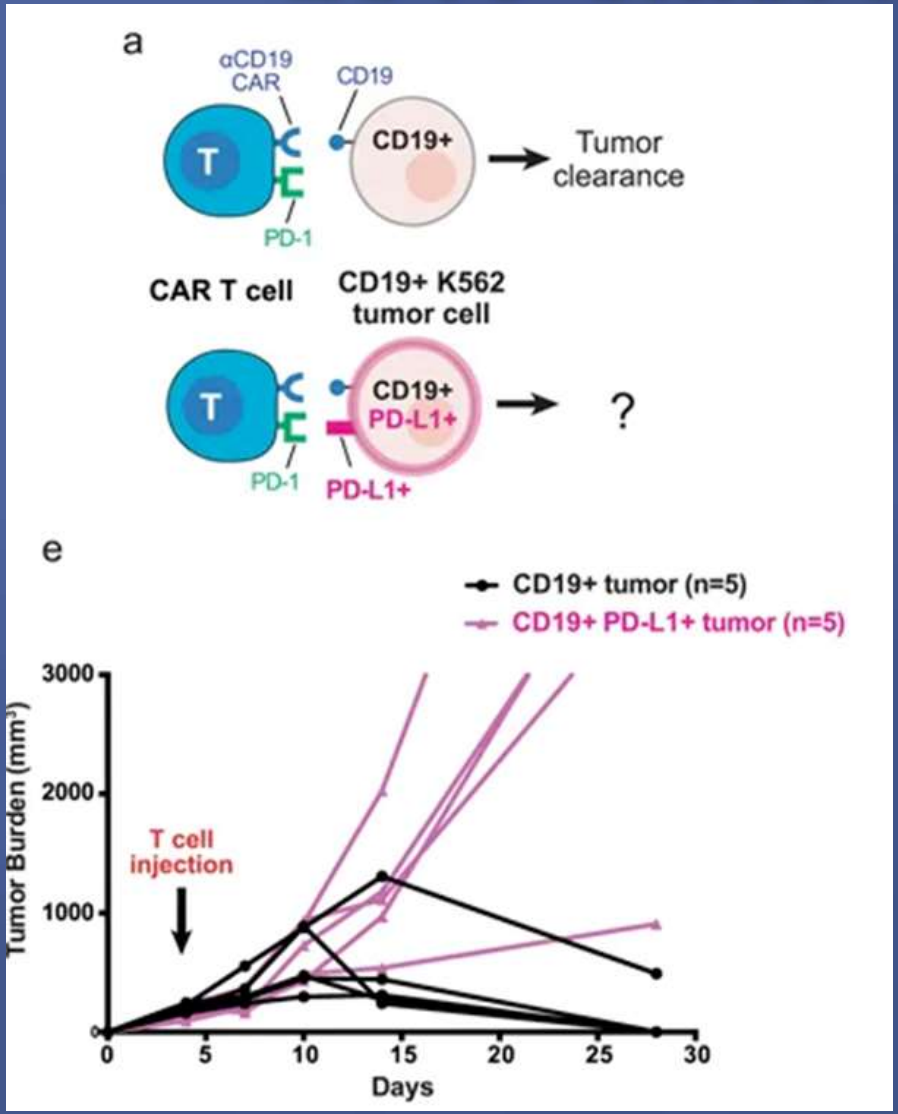

Fig. 1 PD-L1 expression in human K562 myelogenous leukemia cells inhibits anti-CD19 CAR T cell function in vitro and tumor clearance in vivo.

(a) Schematic representation of CAR T cell interaction with either CD19+ or CD19+ PD-L1 K562 tumor cells. … **(e)** CD19+ PD-L1+ subcutaneous xenografts impair anti-CD19 CAR mediated tumor clearance. NOD-scid-IL-2Rγ−/− (NSG) mice were injected with 5 × 106 CD19+ or CD19+ PD-L1+ K562 cells subcutaneously. Mice with established tumors (100–250 mm3) were injected intravenously with 2 × 106 CD4+ and 2 × 106 CD8+ anti-CD19 CAR T cells and tumor burden measured longitudinally by caliper. Shown are tumor burdens for individual mice (n = 5 per tumor type).

Rupp, Levi J., et al. "CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells." Scientific reports 7.1 (2017): 737.

2019 CRISPR Year in Review

Leber congenital amaurosis--Editas Medicine

PRESS RELEASE

Allergan And Editas Medicine Initiate The Brilliance Phase 1/2 Clinical Trial Of AGN-151587 (EDIT-101) For The Treatment Of LCA10

July 25, 2019 at 9:00 AM EDT

About AGN-151587 (EDIT-101)

AGN-151587 (EDIT-101) is a CRISPR-based experimental medicine under investigation for the treatment of Leber congenital amaurosis 10 (LCA10). AGN-151587 is administered via a subretinal injection to reach and deliver the gene editing treatment directly to photoreceptor cells.

About Leber Congenital Amaurosis

Leber congenital amaurosis, or LCA, is a group of inherited retinal degenerative disorders caused by mutations in at least 18 different genes. It is the most common cause of inherited childhood blindness, with an incidence of two to three per 100,000 live births worldwide. Symptoms of LCA appear within the first years of life, resulting in significant vision loss and potentially blindness. The most common form of the disease, LCA10, is a monogenic disorder caused by mutations in the CEP290 gene and is the cause of disease in approximately 20-30 percent of all LCA patients.

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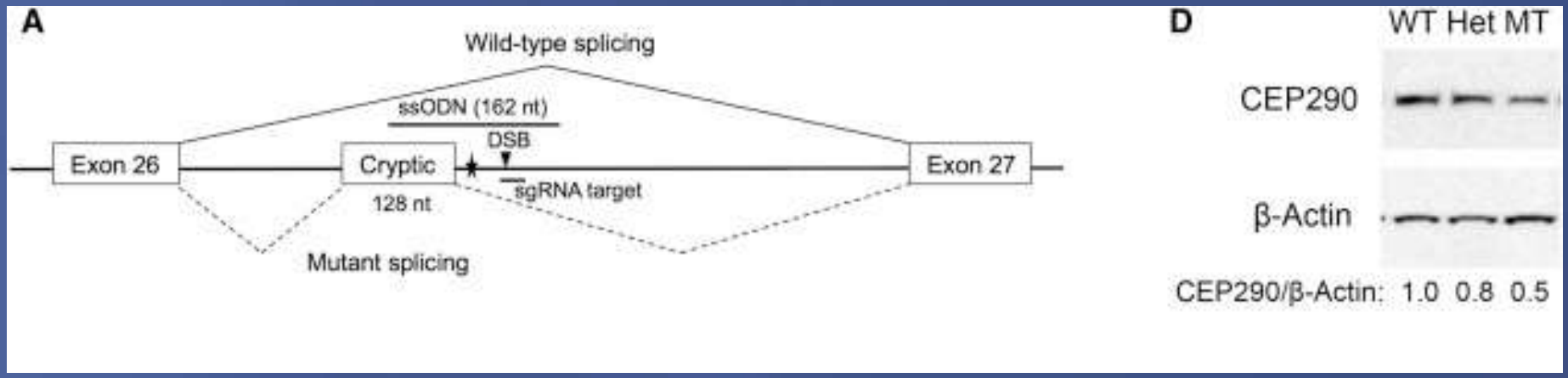

Fig. 1 Generation of an In Vitro Model of LCA10 Using CRISPR/Cas9

(A) A schematic diagram showing the IVS26 mutation (filled star) in the CEP290 gene and the locations of the sgRNA target (antisense strand) and ssODN (sense strand) used for introducing the IVS26 mutation. The DSB site (filled triangle) induced by SpCas9 was located 15 bp downstream of the IVS26 mutation. The splicing patterns for wild-type and mutant are represented by solid and dashed lines, respectively.

Ruan, Guo-Xiang, et al. "CRISPR/Cas9-mediated genome editing as a therapeutic approach for Leber congenital amaurosis 10." Molecular Therapy 25.2 (2017): 331-341.

2019 CRISPR Year in Review

**"Gene-Edited Soybean Oil Makes Restaurant Debut
A Minnesota-based company reports the sale of a soybean oil engineered to have greater stability and no trans-fat."** Mar 13, 2019 CAROLYN WILKE

Calyxt's Calyno high oleic soybean

TALEN edited

https://calyxt.com/products/high-oleic-soybean-oil/

- ✓ Contains approximately 80 percent oleic acid, similar to olive, sunflower and safflower oils
- ✓ 20 percent less saturated fatty acids than commodity soybean oil
- ✓ Zero grams of trans fat and PHOs per serving
- ✓ Fry life up to three times longer than commodity soybean oil
- ✓ Extended shelf life due to high oleic content
- ✓ High stability and smoke point
- ✓ Reduced polymer buildup in fryers

2019 CRISPR Year in Review

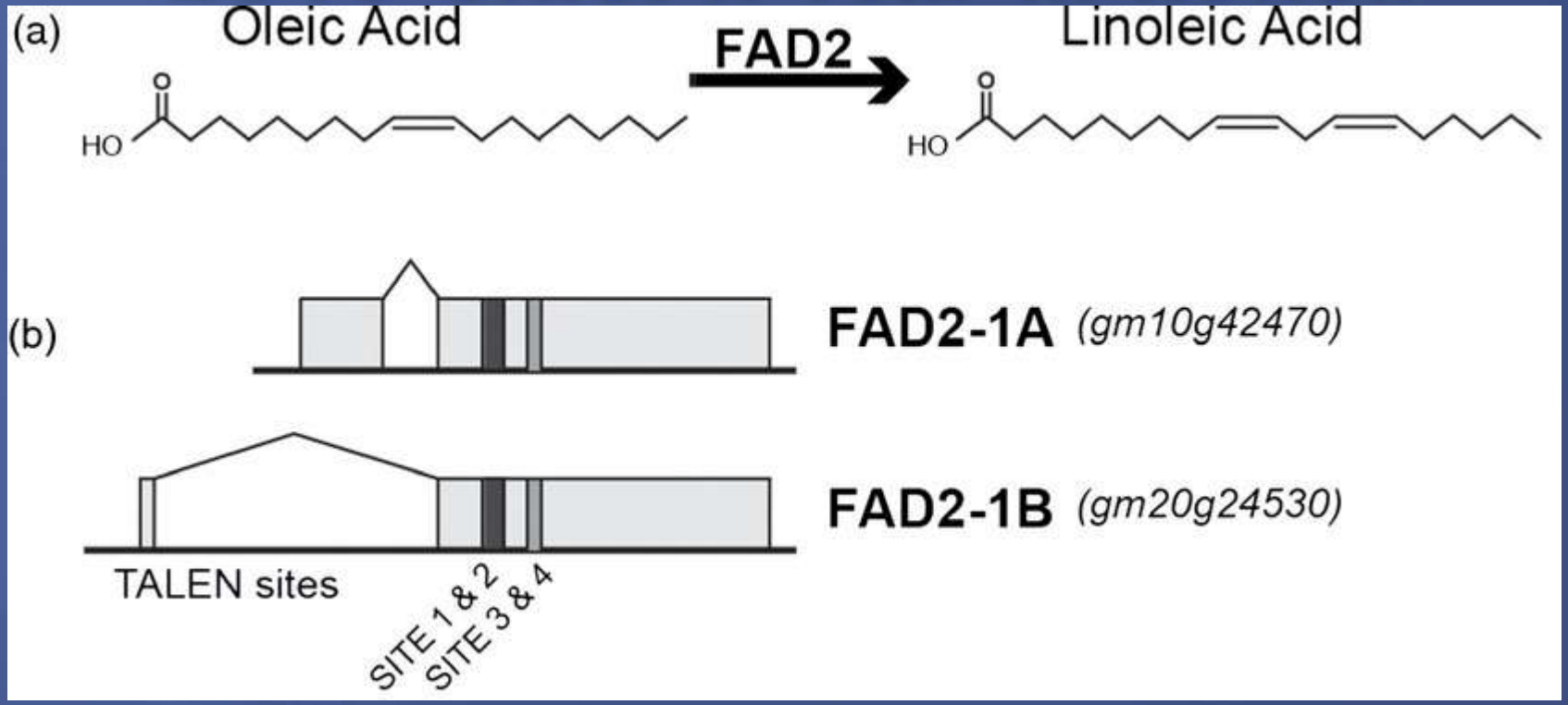

Fig. 1 Transcription activator-like effector nucleases (TALENs) targeting the FAD2-1A and FAD2-1B genes.

(a) FAD2 is responsible for the conversion of oleic acid to linoleic acid. (b) Schematics of the FAD2-1A and FAD2-1B gene structures. The FAD2 genes contain 2 exons, indicated by open boxes, and 1 intron, represented by the angled lines. TALEN target sites are indicated in black (target sites 1 and 2) and grey (target sites 3 and 4

Haun, William, et al. "Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family." Plant biotechnology journal 12.7 (2014): 934-940.

2019 CRISPR Year in Review

A very exciting year for genome editing and the consumer/patient

Questions?

2019 CRISPR Year in Review

Background
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Target Genes and Modified Organisms

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