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Realizing the Full Potential of CRISPR for Cancer Therapies and Other Precision Medicines

Keynote Speaker: **Rachel Haurwitz**, PhD, President, CEO and Founder, Caribou Biosciences, Inc.

Cancer Progress
New York, NY | May 7 - 8, 2019

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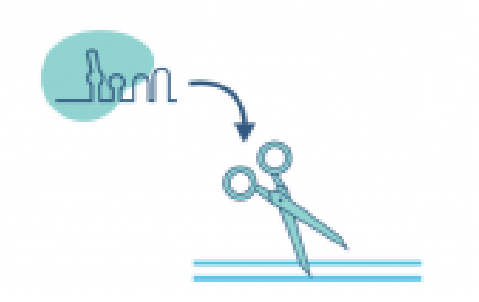
- Evolution of the field
- What opportunities, challenges remain
- Timeline/story telling
- Editing/viral options
- Real world implications – feasibility (auto vs. allo)
- Speed
- Ease of multiple edits (solid tumors esp)
- Accuracy
- Cost
- 45 minutes

Caribou Biosciences, Inc.



Company snapshot
$41.5M financing to date Investors include F-Prime Capital, Novartis, Maverick Own equity of Intellia Therapeutics (NASDAQ: NTLA)
~60 employees ~25 issued US patents

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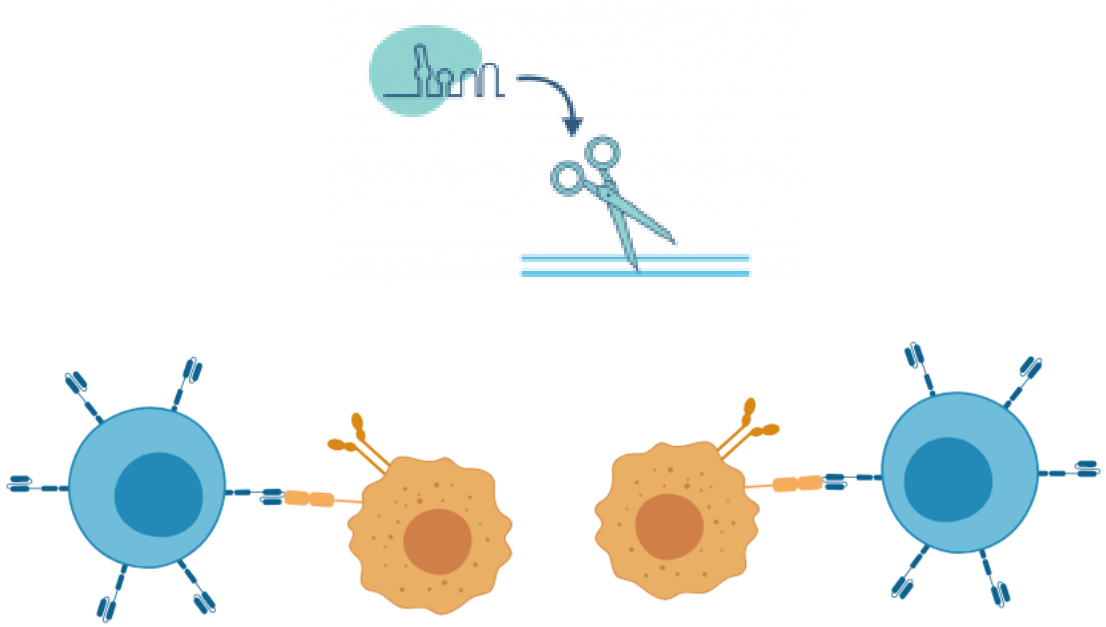

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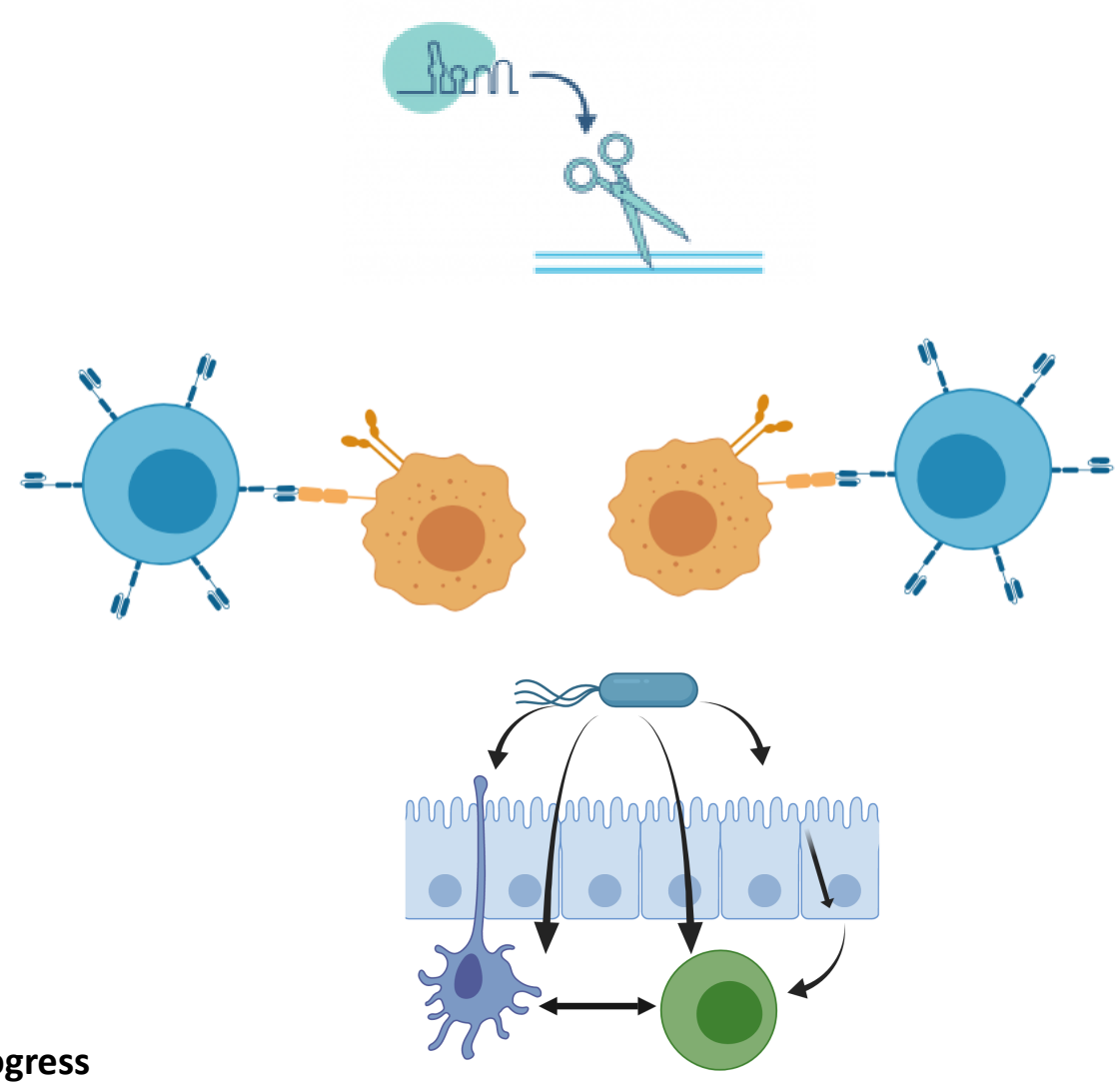

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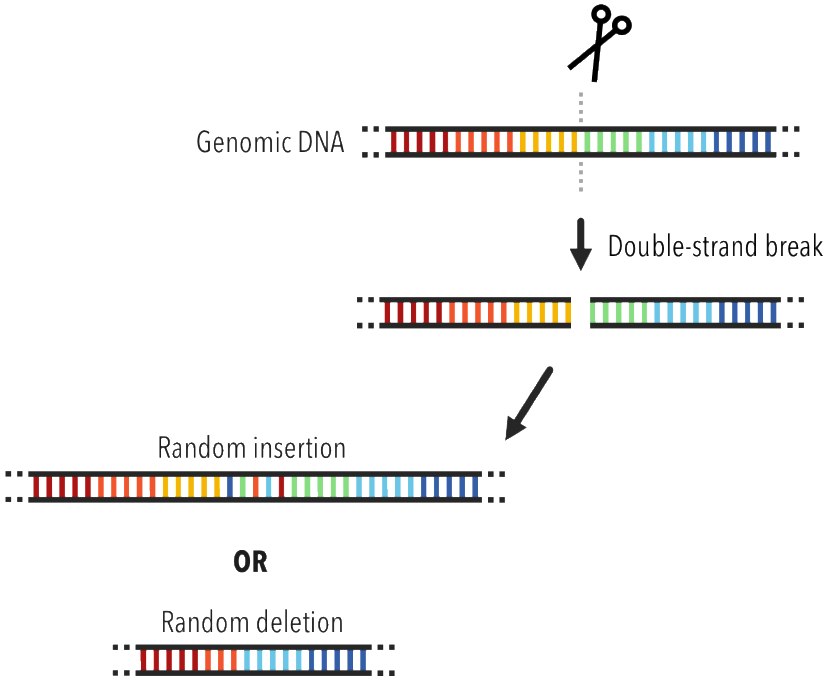
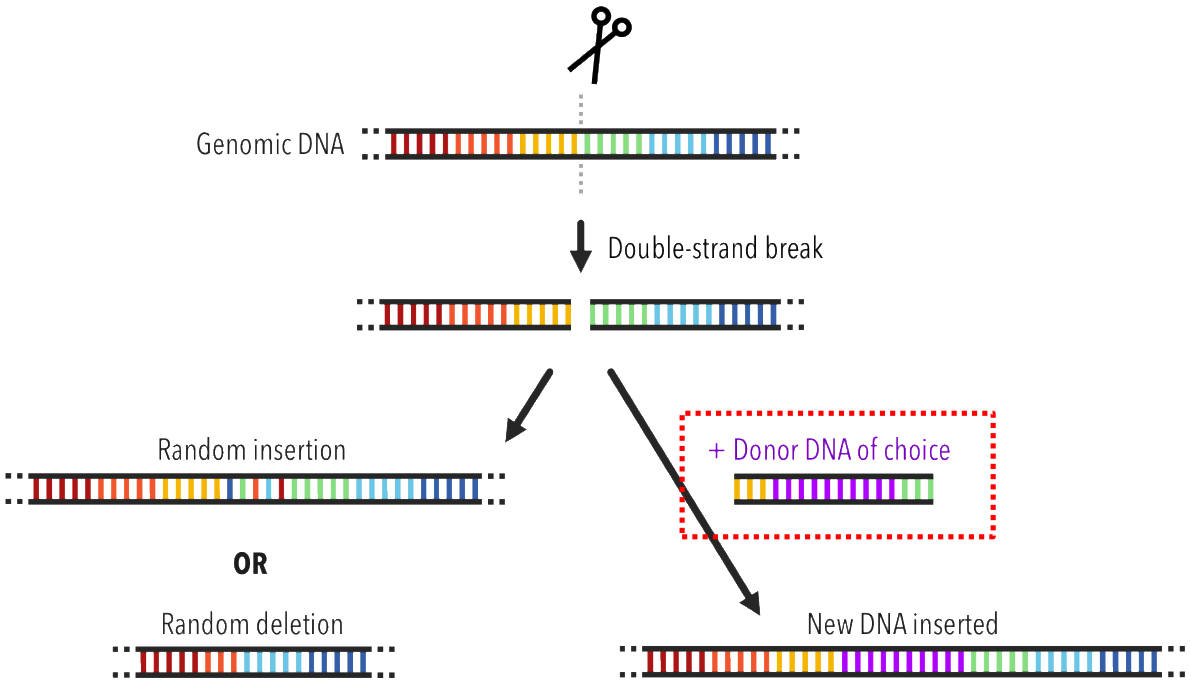
Gene editing is all about specifically cutting DNA



'Indels' lead to loss of gene function 'knock-out'

Gene editing is all about specifically cutting DNA



'Indels' lead to loss of gene function 'knock-out'

The gene editing toolbox has evolved over time

meganucleases

Image adapted from Adli et al,
Nature Comm 2018

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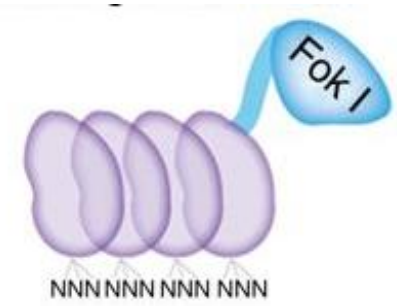
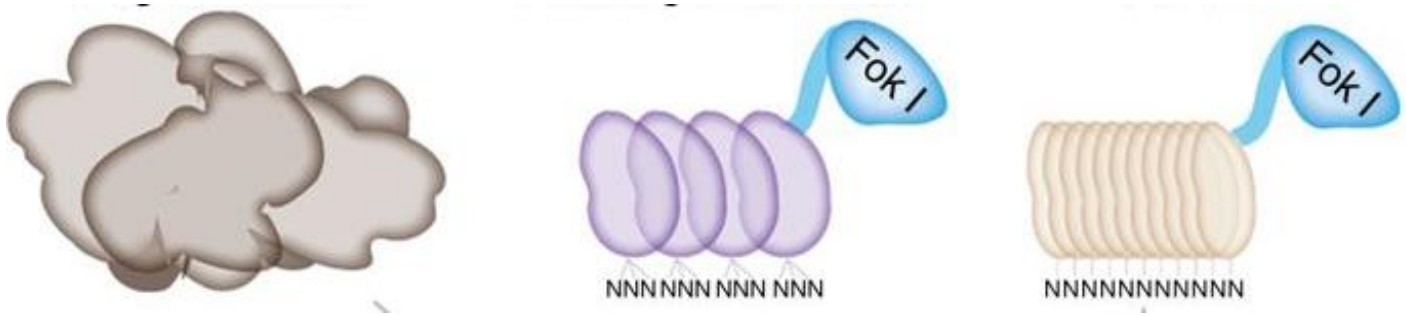


ZFNs

Image adapted from Adli et al, Nature Comm 2018

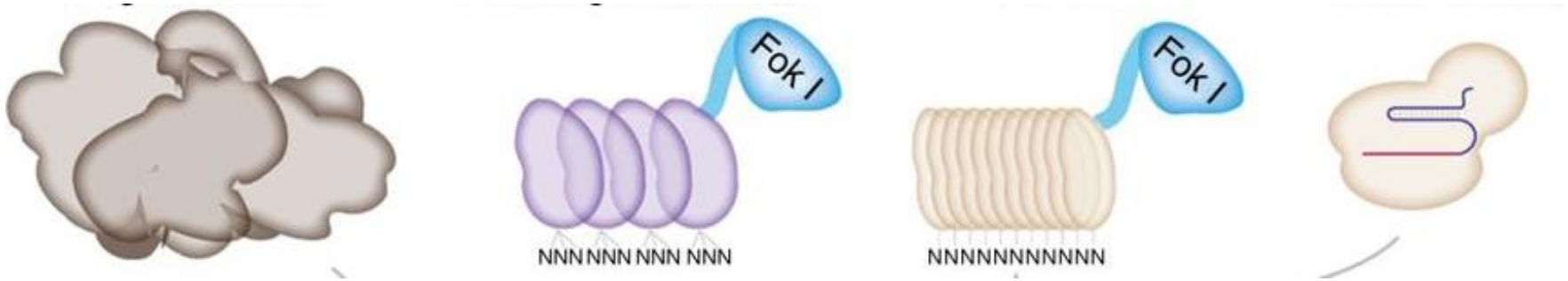
The gene editing toolbox has evolved over time



meganucleases ZFNs TALENs

Image adapted from Adli et al, Nature Comm 2018

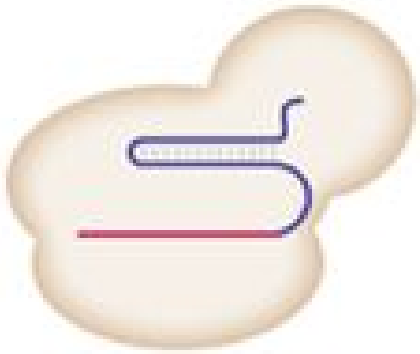
The gene editing toolbox has evolved over time



meganucleases ZFNs TALENs CRISPR

Image adapted from Adli et al, Nature Comm 2018

Gene editing vs. gene therapy



- Deletion or alteration of existing gene(s)
- Addition of new genetic material to chromosome(s)
- Edits are heritable and propagated through cell division

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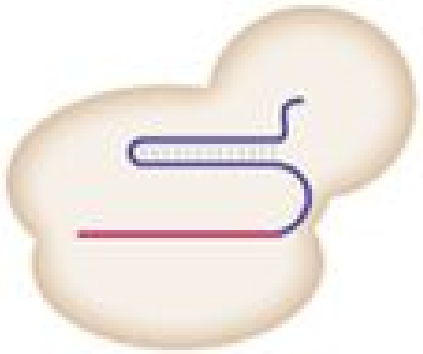
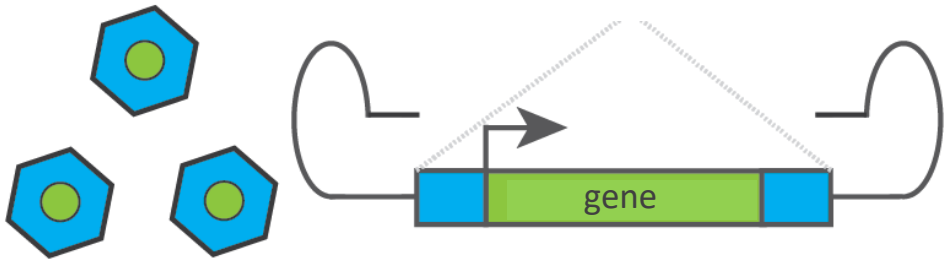
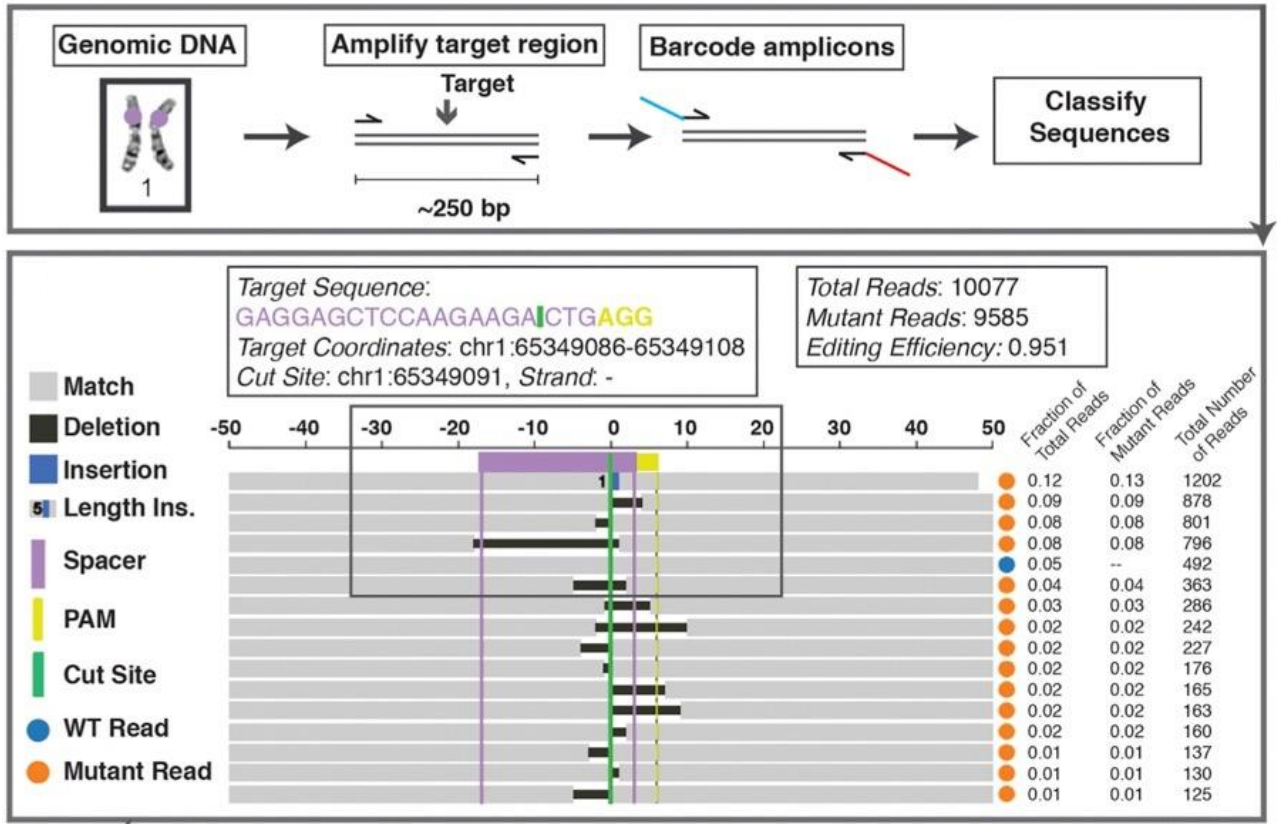




- Deletion or alteration of existing gene(s)
- Addition of new genetic material to chromosome(s)
- Edits are heritable and propagated through cell division

- Addition of new genetic material to chromosome (random locations) or extrachromosomally
- If not genomically integrated, will be diluted through cell division

Understanding editing and its unexpected reproducibility



van Overbeek et al, Mol Cell 2016

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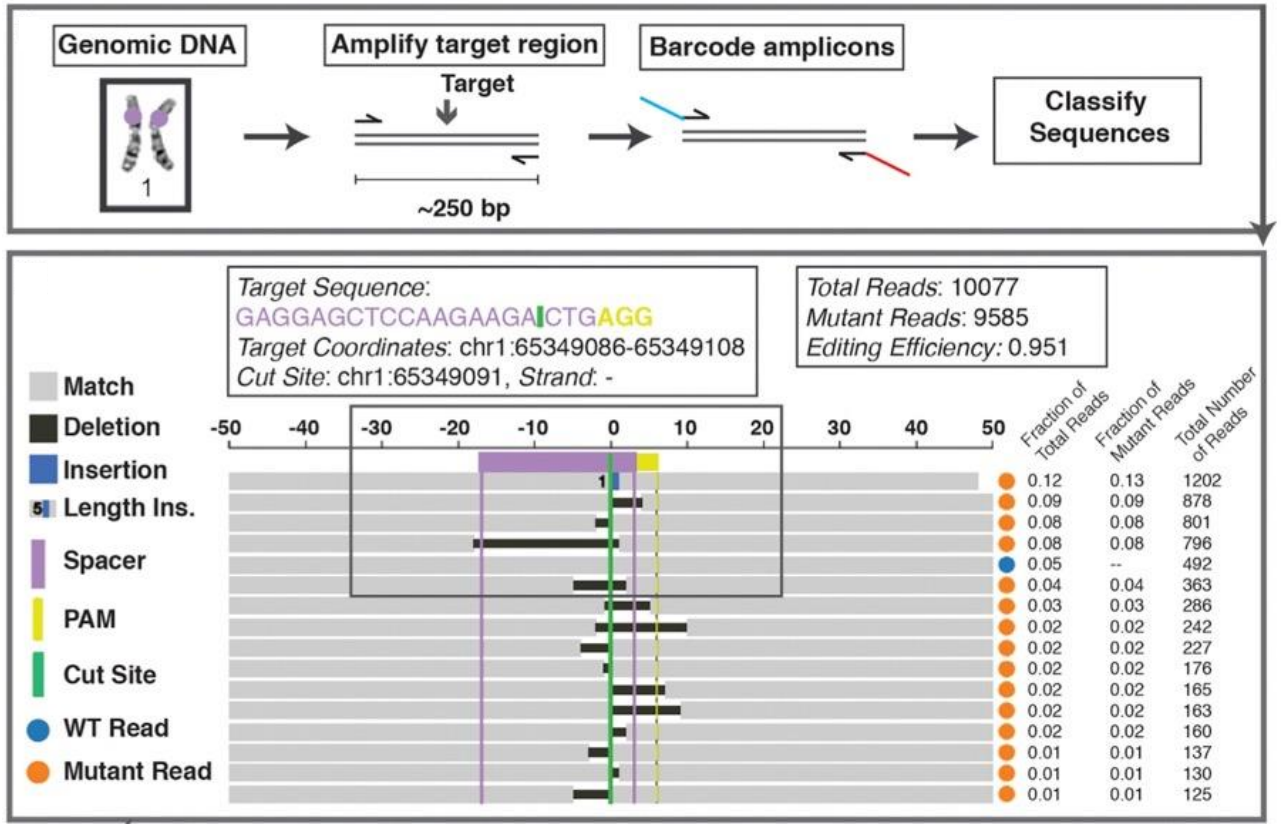

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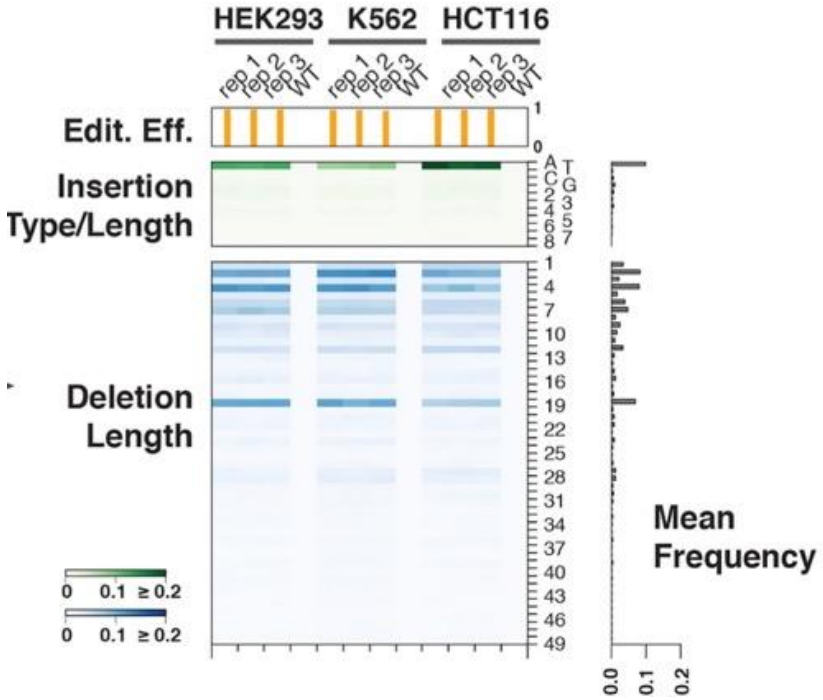

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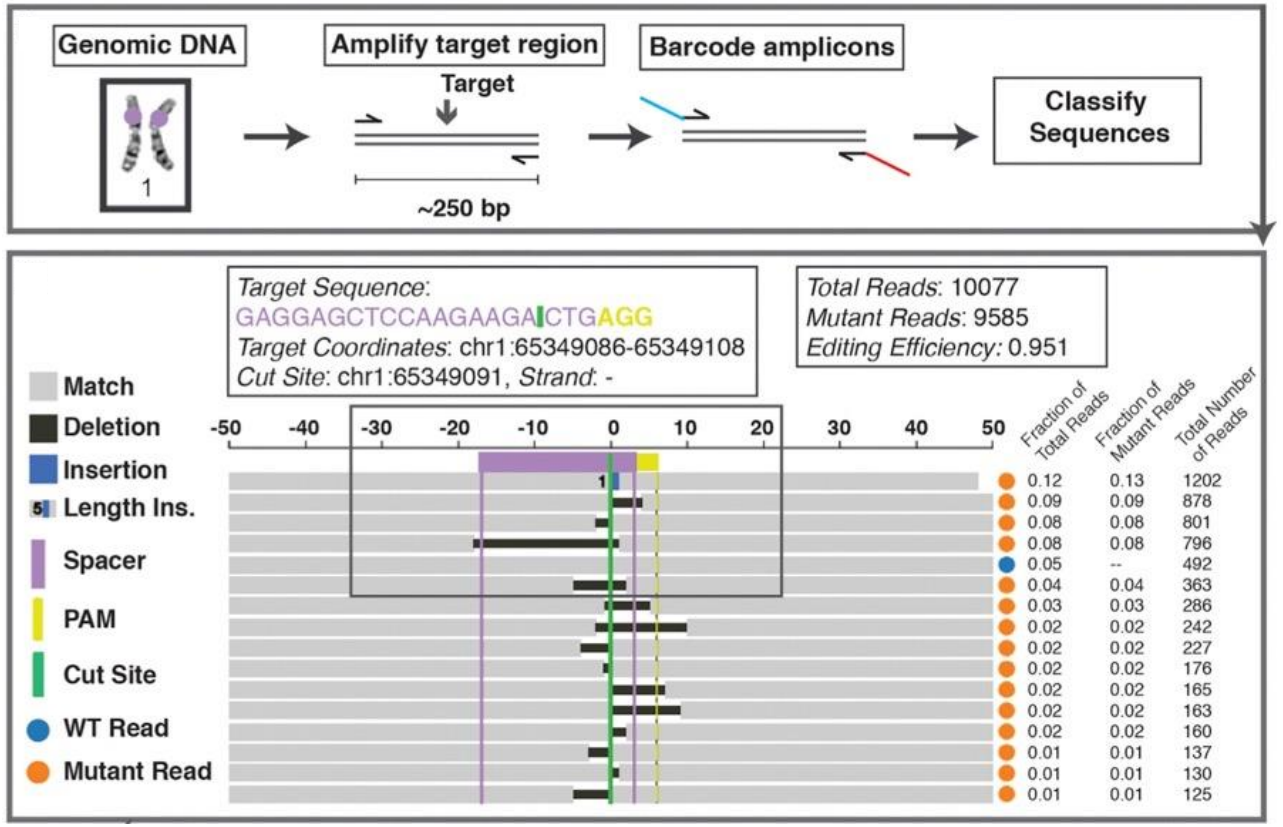
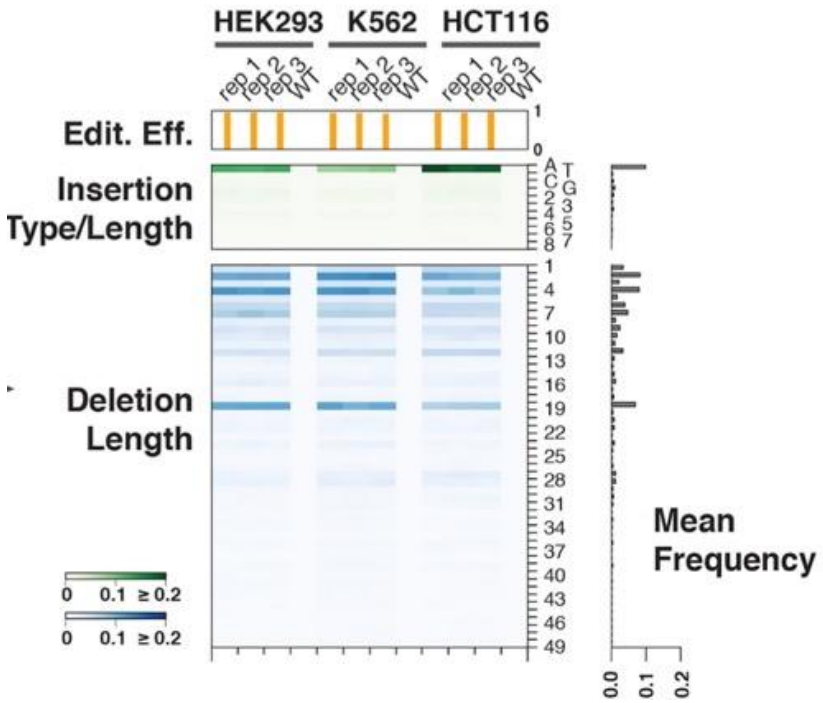
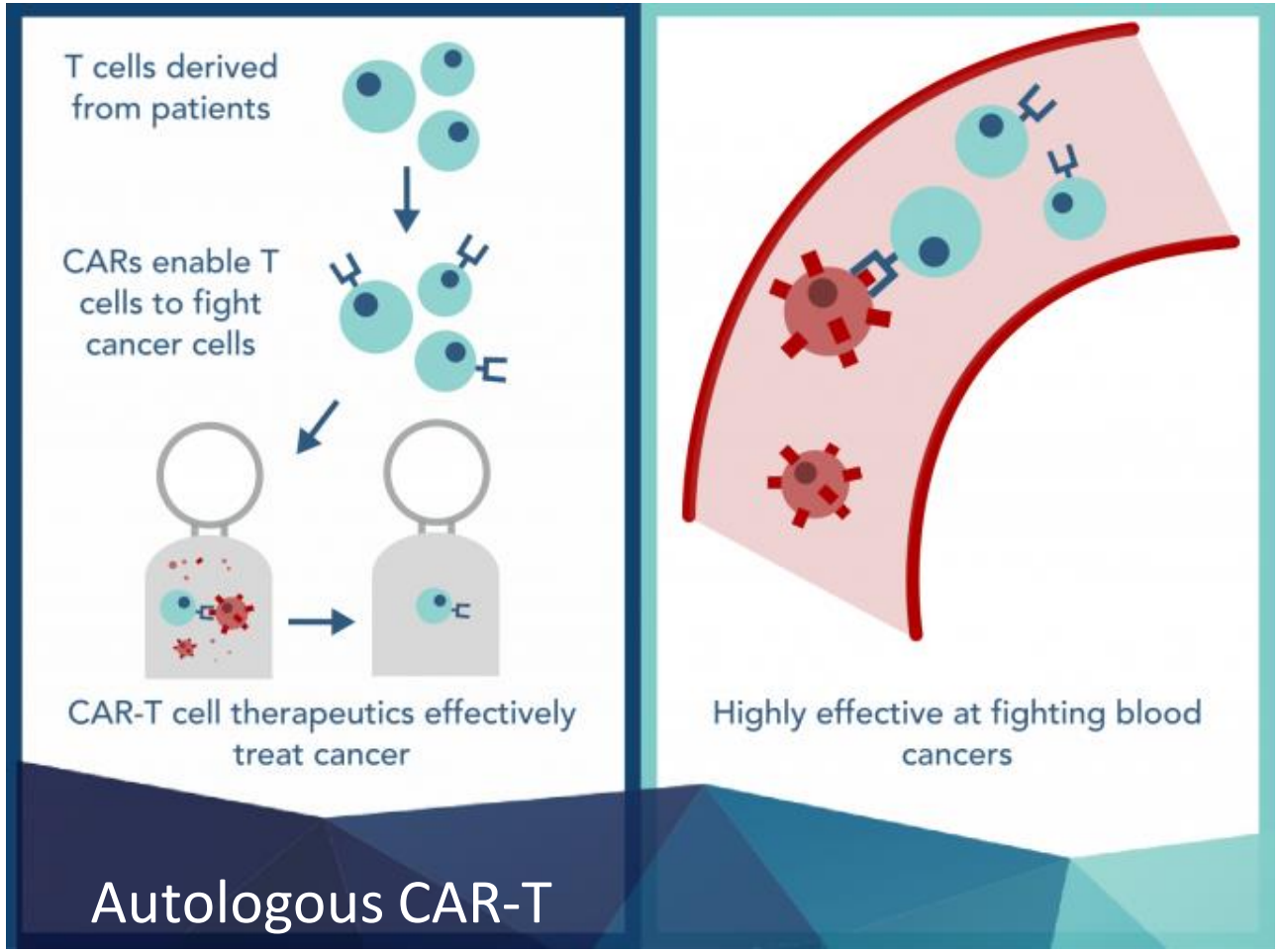




DNA repair outcomes are consistent across replicates and cell types

van Overbeek et al, Mol Cell 2016

Gene-edited CAR-T cell therapies to treat cancer



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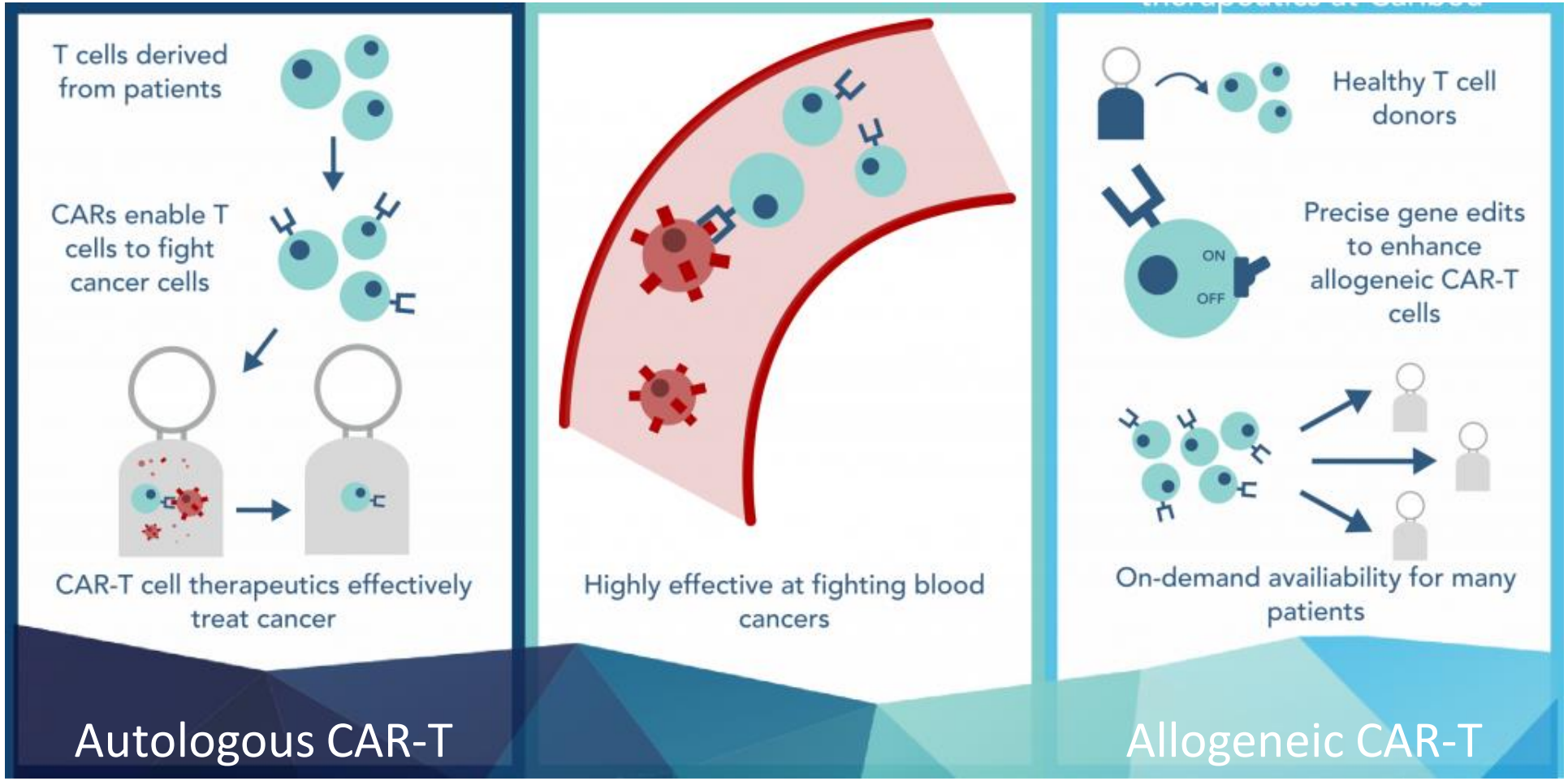
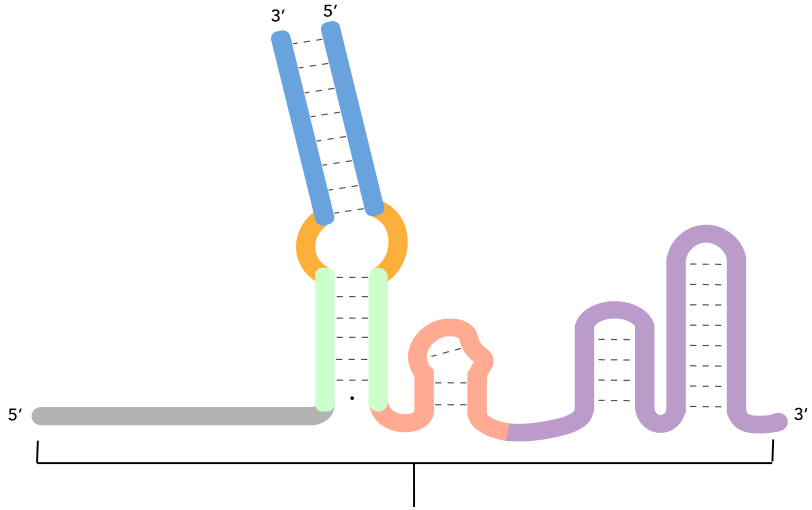


Caribou's next-gen editing platform substantially improves specificity

CRISPR hybrid RNA-DNA (chRDNAs)



DNA nucleotides at multiple sites in the guide

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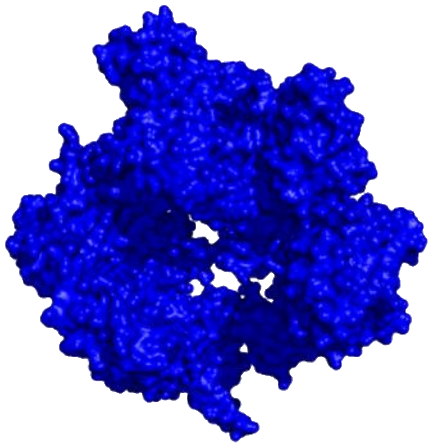
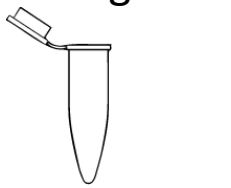


Cas9

While retaining high on-target editing efficiency, chRDNAs significantly reduce off-target editing relative to first-gen CRISPR in human primary cells

Specificity is critical for therapeutic development

Biochemical cleavage



T cell genomic DNA

Cameron et al, Nat Methods 2017

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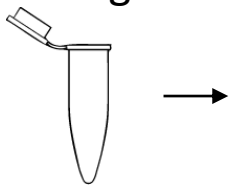
Specificity is critical for therapeutic development

Comprehensive list of
biochemical off-targets

Biochemical
cleavage



T cell genomic DNA

GGTGGACAAGCGGCAGATAGCGG
GGAGAACAAGTGGCAGATAGAGG
AGGTGGGAAGCAGCAGATAGAGG
GGTGAACAAACAGCAGATAGAAG
GGTGGACAAACAGCAGATTGTGG
AGGTGGACATGAGCAGATAGAGG
AGGTGGACAGCCCCAGATAGGGG
GGTGGATAAGCAGAAGATAGTGG
AATGGACAAAAGGCAGAAAGGGG
GGTGGTCAAGAGGCAGATAGGAG
CGTGGACAAGCAGCAAATATTGG
AGTGGAACTTAGGCAGATAGCGG
GGAGGAACAAGAGGAGATAGAGG
GGAAGACAAACGGCAGAAGTGGA
AGTGGACACCCCGCAGATAGGGG
GGTGGATAAGAGGCAGAAGTGGA
TGTGGACAAGTGGCAATAGGGGA
GGGGGTCAAGAGGCAGTAGAGGA
GGGGGACAAGCAGCAGCCAGGGG
GGGAGAGAAGCGGCAGATGAGGG
GGTGGACAAGAGCCAGATAGTAG
GTGGGTAGACAAACAGATAGAGG
GTGTGGACAGAGGCAGATTGGGG
AGGTGGACAAACAGAGATAGGGG
GGTGGACTAGGGGCAGATGGAGG

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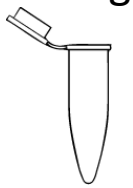

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GGTGGACAAGCGGCAGATAGCGG
GGAGAACAAGTGGCAGATAGAGG
AGGTGGGAAGCAGCAGATAGAGG
GGTGAACAAACAGCAGATAGAAG
GGTGGACAAACAGCAGATTGTGG
AGGTGGACATGAGCAGATAGAGG
AGGTGGACAGCCCCAGATAGGGG
GGTGGATAAGCAGAAGATAGTGG
AATGGACAAAAGGCAGAAAGGGG
GGTGGTCAAGAGGCAGATAGGAG
CGTGGACAAGCAGCAAATATTGG
AGTGGAACTTAGGCAGATAGCGG
GGAGGAACAAGAGGAGATAGAGG
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AGTGGACACCCCGCAGATAGGGG
GGTGGATAAGAGGCAGAAGTGGA
TGTGGACAAGTGGCAATAGGGGA
GGGGGTCAAGAGGCAGTAGAGGA
GGGGGACAAGCAGCAGCCAGGGG
GGGAGAGAAGCGGCAGATGAGGG
GGTGGACAAGAGCCAGATAGTAG
GTGGGTAGACAAACAGATAGAGG
GTGTGGACAGAGGCAGATTGGGG
AGGTGGACAAACAGAGATAGGGG
GGTGGACTAGGGGCAGATGGAGG

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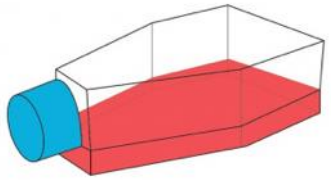
Cell-based
validation



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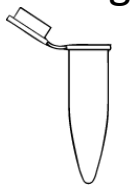
Biochemical cleavage



T cell genomic DNA

Comprehensive list of **biochemical** off-targets

GGTGGACAAGCGGCAGATAGCGG
GGAGAACAAGTGGCAGATAGAGG
AGGTGGGAAGCAGCAGATAGAGG
GGTGAACAAACAGCAGATAGAAG
GGTGGACAAACAGCAGATTGTGG
AGGTGGACATGAGCAGATAGAGG
AGGTGGACAGCCCCAGATAGGGG
GGTGGATAAGCAGAAGATAGTGG
AATGGACAAAAGGCAGAAAGGGG
GGTGGTCAAGAGGCAGATAGGAG
CGTGGACAAGCAGCAAATATTGG
AGTGGAACTTAGGCAGATAGCGG
GGAGGAACAAGAGGAGATAGAGG
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AGTGGACACCCCGCAGATAGGGG
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GGTGGACAAGAGCCAGATAGTAG
GTGGGTAGACAAACAGATAGAGG
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AGGTGGACAAACAGAGATAGGGG
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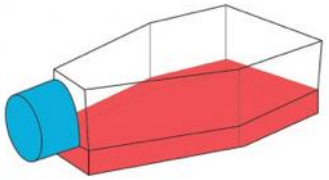
Cell-based validation



T cells

Subset of edited sites

GGTGGACAAGCGGCAGATAGCGG
GGAGAACAAGTGGCAGATAGAGG
AGGTGGGAAGCAGCAGATAGAGG
GGTGAACAAACAGCAGATAGAAG
GGTGGACAAACAGCAGATTGTGG
AGGTGGACATGAGCAGATAGAGG
AGGTGGACAGCCCCAGATAGGGG
GGTGGATAAGCAGAAGATAGTGG
AATGGACAAAAGGCAGAAAGGGG
GGTGGTCAAGAGGCAGATAGGAG
CGTGGACAAGCAGCAAATATTGG
AGTGGAACTTAGGCAGATAGCGG
GGAGGAACAAGAGGAGATAGAGG
GGAAGACAAACGGCAGAAGTGGA
AGTGGACACCCCGCAGATAGGGG
GGTGGATAAGAGGCAGAAGTGGA
TGTGGACAAGTGGCAATAGGGGA
GGGGGTCAAGAGGCAGTAGAGGA
GGGGGACAAGCAGCAGCCAGGGG
GGGAGAGAAGCGGCAGATGAGGG
GGTGGACAAGAGCCAGATAGTAG
GTGGGTAGACAAACAGATAGAGG
GTGTGGACAGAGGCAGATTGGGG
AGGTGGACAAACAGAGATAGGGG
GGTGGACTAGGGGCAGATGGAGG

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cellular off-target

Comprehensive list of **cellular** off-targets

GGAGAACAAGTGGCAGATAGAGG
GGTGGACAAACAGCAGATTGTGG
GGTGGTCAAGAGGCAGATAGGAG
GGAAGACAAACGGCAGAAGTGGA
GGTGGATAAGAGGCAGAAGTGGA
GGGGGTCAAGAGGCAGTAGAGGA
GGTGGACAAGAGCCAGATAGTAG
AGGTGGACAAACAGAGATAGGGG

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Cameron et al, Nat Methods 2017

chRDNAs consistently improve specificity across multiple targets

	AAVS1 target-1		TRAC target-1		TRAC target-2		Gene A target-1		PDCD1 target-1	
	crRNA	chRDNA	crRNA	chRDNA	crRNA	chRDNA	crRNA	chRDNA	crRNA	chRDNA
On-targets	84.1%	79.3%	94.6%	93.3%	98.6%	92.4%	90.9%	83.2%	89.5%	88.9%
Off-targets	63.0%	< 0.1%	1.6%	< 0.1%	4.0%	< 0.1%	1.1%	< 0.1%	14.8%	< 0.1%
	25.3%	< 0.1%	0.4%	< 0.1%	0.9%	< 0.1%			0.4%	< 0.1%
	8.5%	< 0.1%	1.0%	< 0.1%	0.4%	< 0.1%			0.2%	< 0.1%
	8.4%	< 0.1%	0.1%	< 0.1%	0.2%	< 0.1%				
	5.5%	< 0.1%								

0 — 100

% editing efficiency

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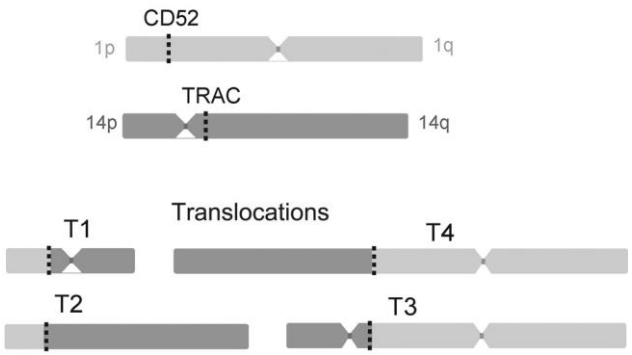
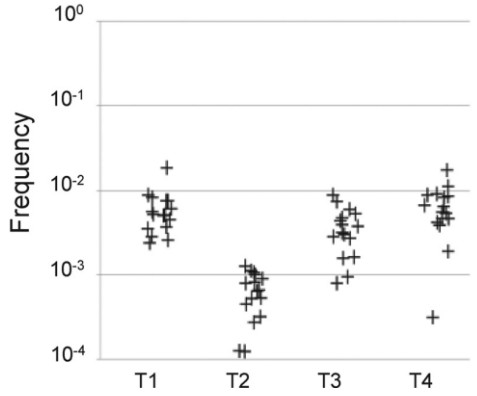
Translocations in a multiplex setting



Translocation frequency using TALEN gene editing technology determined with qPCR assay

Copy numbers determined using plasmids with expected translocations

Acentric T2 unstable



1% of total cells (3% of edited cells)

0.1% of total cells (0.3% of edited cells)

Translocation rate ~4.8% of edited cells

(Poirot et al, 2015)

~4% translocation rates with TALEN gene editing technology

chRDNAs achieve high efficiency multiplex editing with fewer translocations

Multiplex editing (TRAC + PDCD1)

	Standard delivery		**Proprietary delivery**	
	Editing	Translocation	Editing	Translocation
TRAC	80%	3.5%	90%	0.1%
PDCD1	66%		70%	

Proprietary delivery approach for chRDNAs results in robust editing efficiency and significantly decreases translocation frequency

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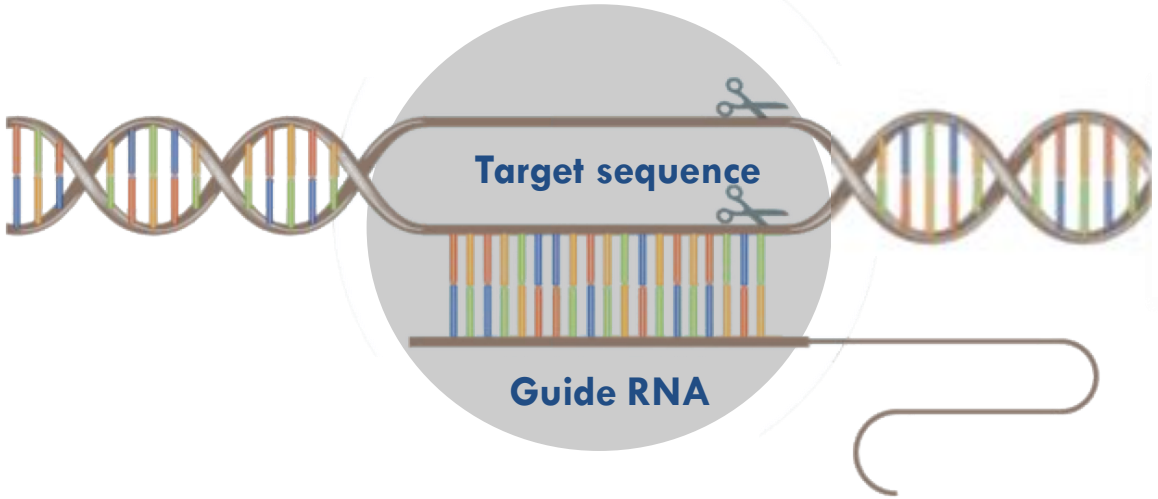
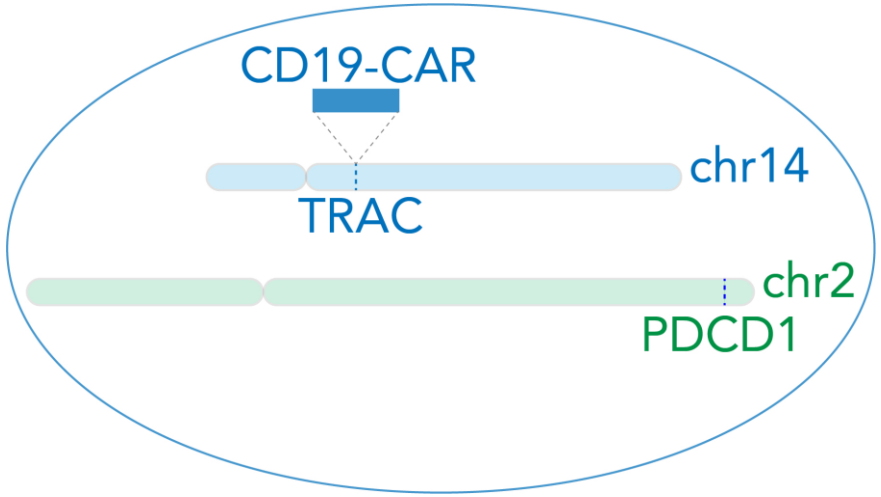
Gene editing capabilities and challenges

- Gene knockouts
  - Single gene
  - Multiple genes
- Gene insertion
  - Single gene
  - Multiple genes
  - Donor DNA options
- Delivery
  - *ex vivo*
  - *in vivo*
- Manufacturing



CB-010: anti-CD19 allo CAR-T



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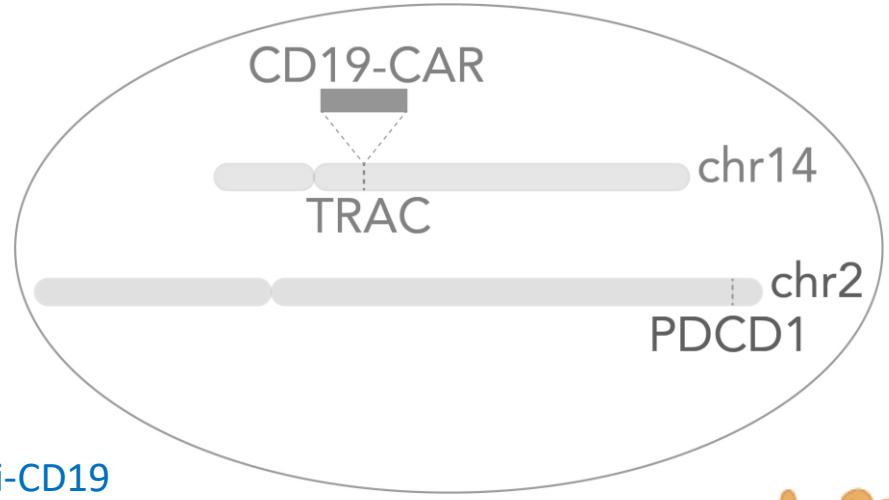
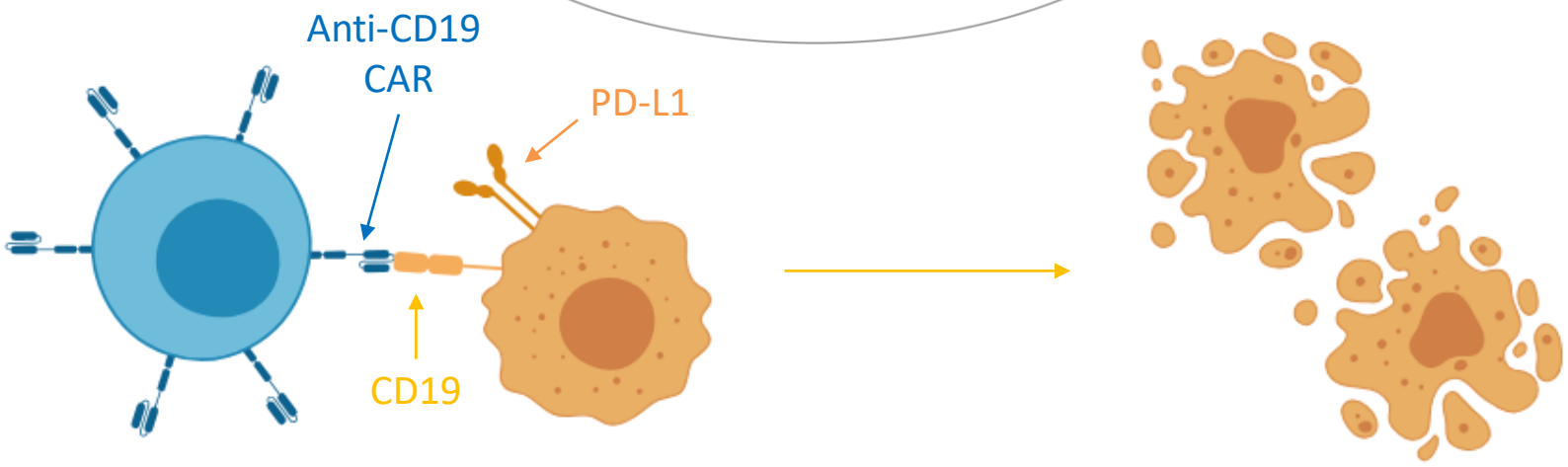
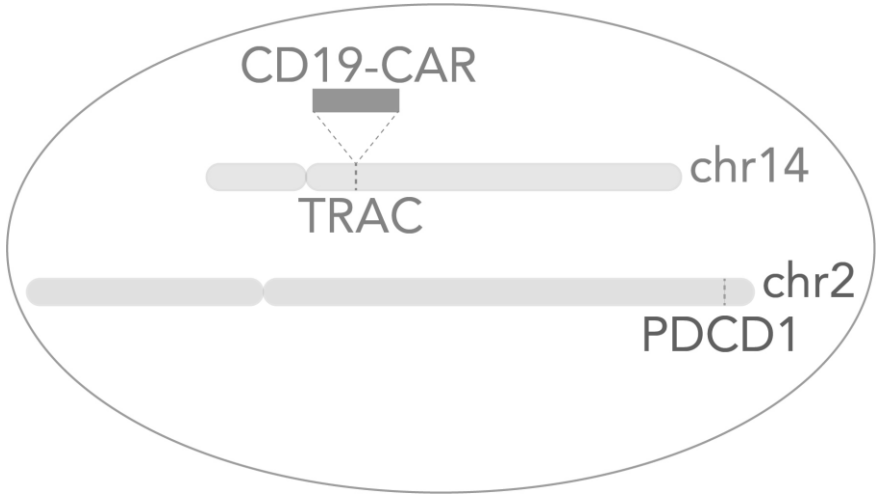




CB-010 Malignant B cell B cell death

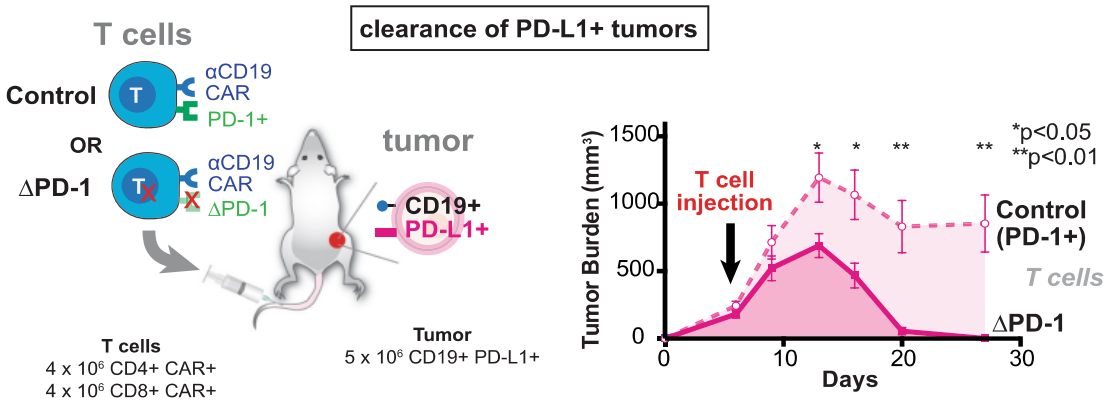
CB-010: anti-CD19 allo CAR-T



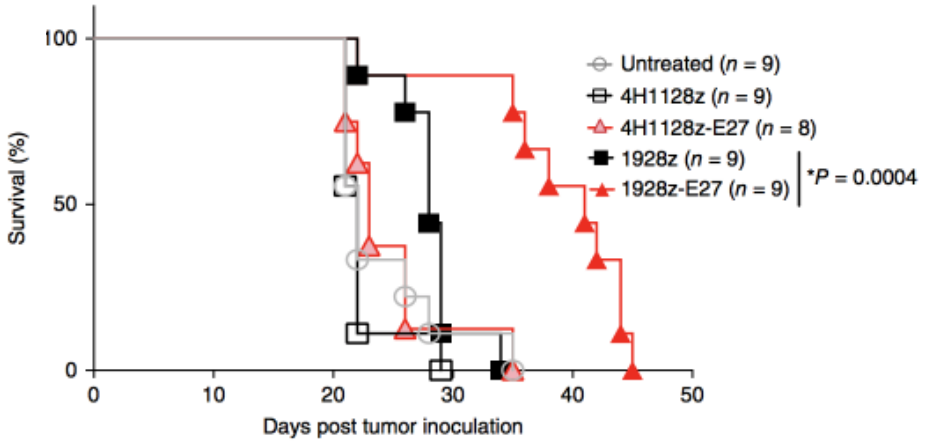
- AAV6-mediated site-specific insertion of anti-CD19 CAR into TRAC locus
  - Knockout of the TCR to prevent graft vs host disease
- PDCD1 knockout
  - Increased persistence of T cell polyfunctionality during tumor cell interaction

Disruption of PD-1/PD-L1 axis extends persistence of antitumor activity

- CRISPR-Cas9 mediated PDCD1 knockout in CD19-specific CAR-T cells (left)
- Enforced expression of anti-PD-1 blocking scFv in CD19-specific CAR-T cells (right)



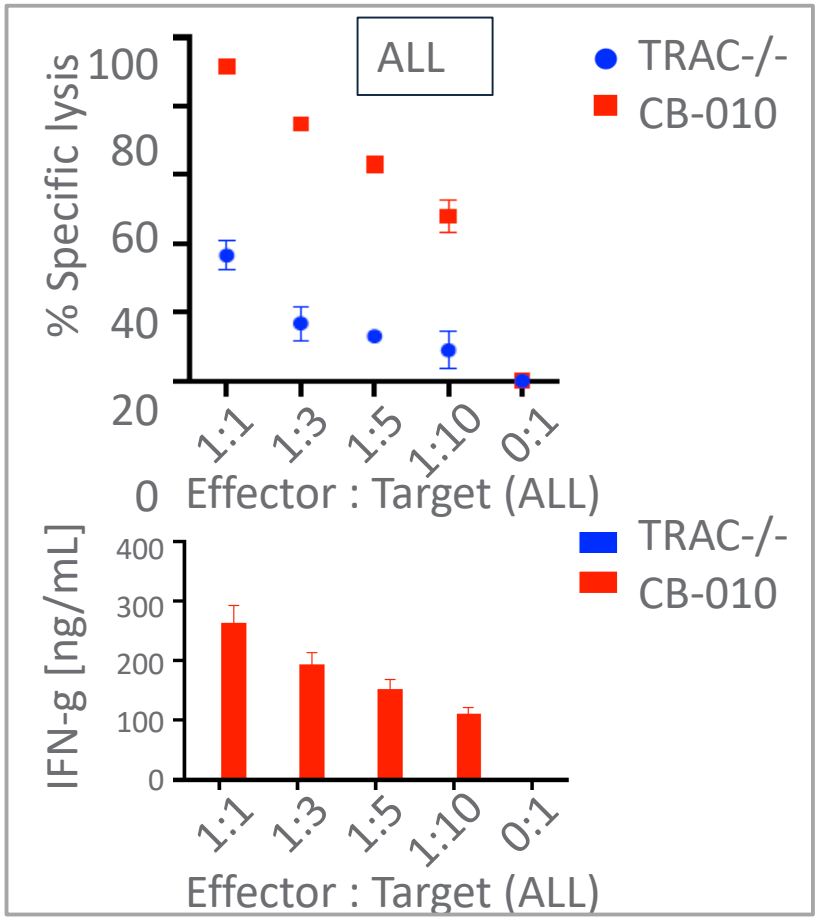
Rupp, LJ, et al. (2017) *Scientific Reports* 7:737

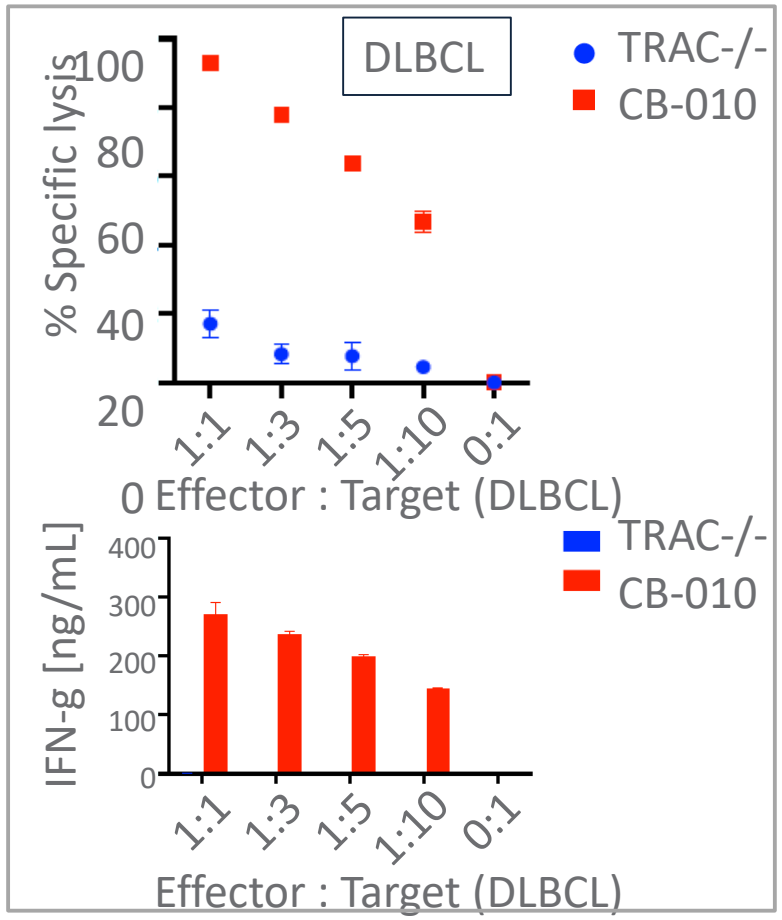


Rafiq, S, et al. (2018) *Nature Biotechnology* 36:847

Both approaches disrupt the PD-1/PD-L1 axis leading to enhanced persistence of CD19-specific CAR-T antitumor activity targeting PD-L1⁺ B cell tumors

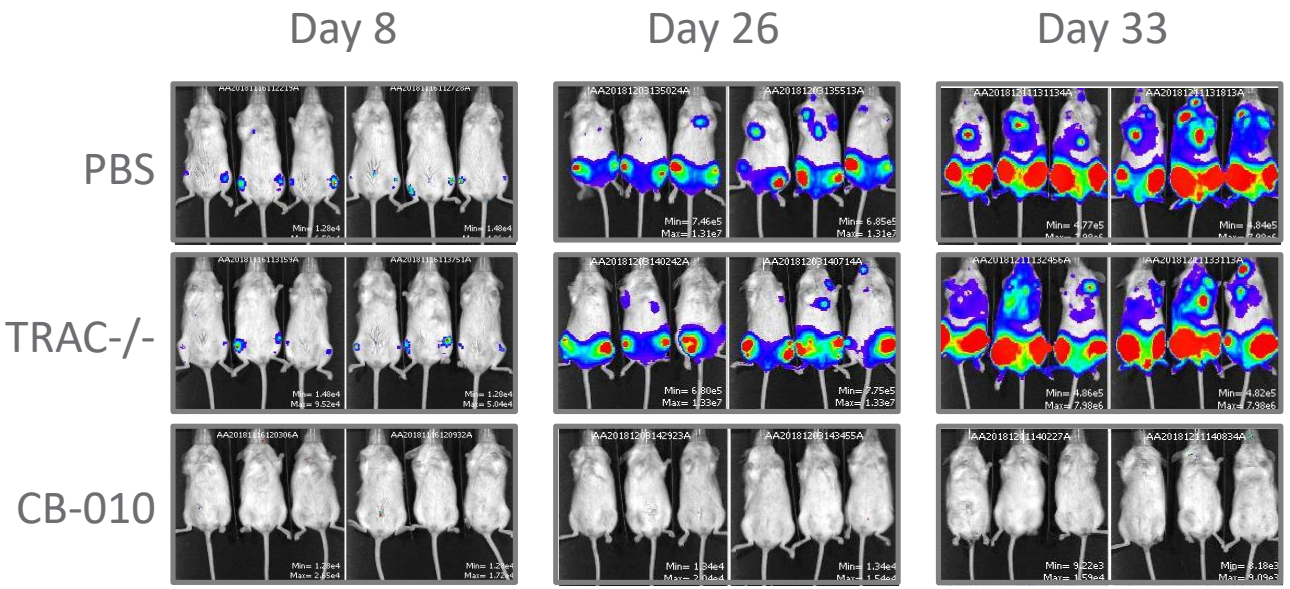
CB-010 specific *in vitro* cytotoxicity

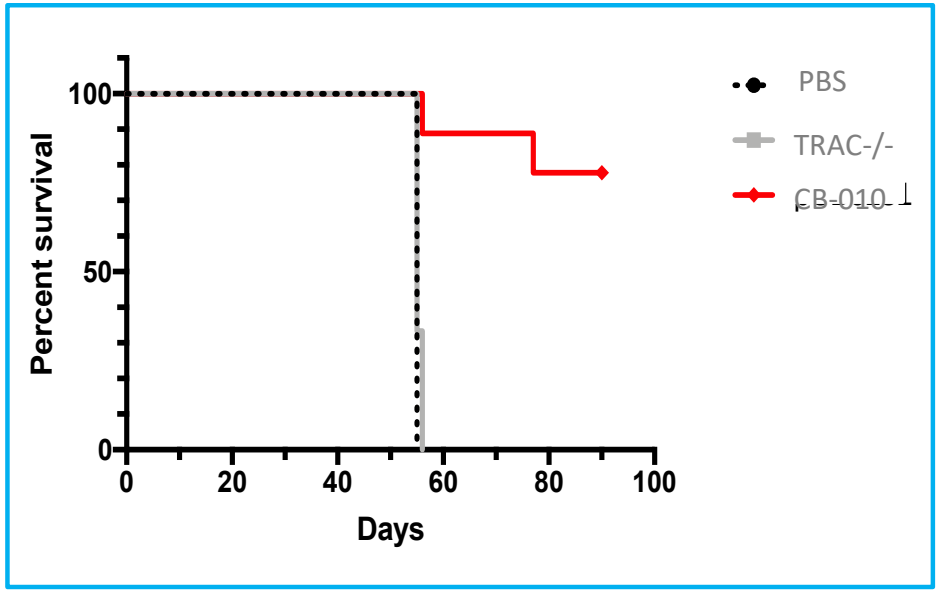




CB-010 leads to regression of an established B-ALL xenograft

NALM6-PDL1 tumor cells were implanted orthotopically 5 days prior to CAR-T treatment





Reduction of tumor cell burden observed after a single injection of allogeneic CB-010 CAR-T cells

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Caribou pipeline overview

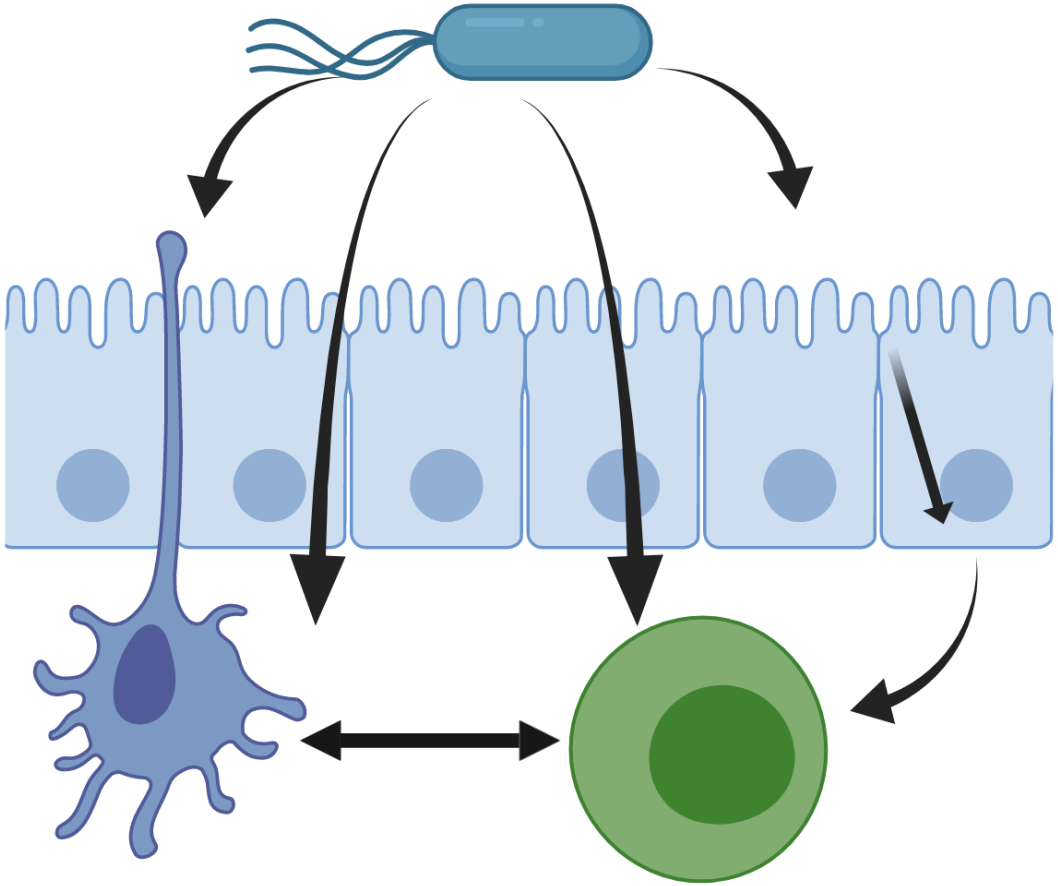
Program	Cell type	Target	Edit type	Discovery	IND-enabling
CB-010	T cell	CD19	CAR into TRAC, PDCD1 KO		
CB-011	T cell	Undisclosed	Multiplex: 2 knock-ins		
CB-020	Immune cell	Undisclosed	Undisclosed		
CB-031	Gut microbe	Undisclosed	Undisclosed		

Caribou has retained commercial rights to all programs

CRISPR-based microbial engineering

Immune interactions

Commensal gut bacteria closely interact with the immune system and regulate strength of immune activation

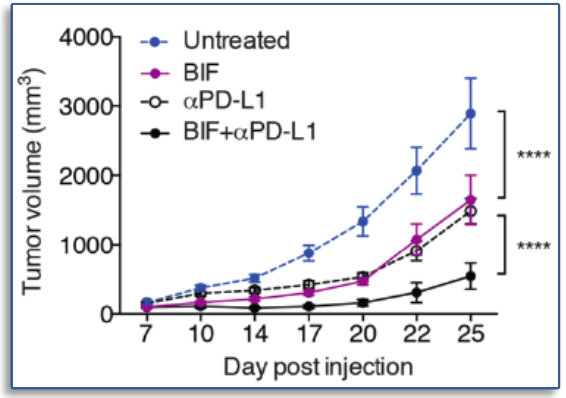


Microbial-immune interactions

CANCER IMMUNOTHERAPY

Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy

Ayelet Sivan,[1*] Leticia Corrales,[1*] Nathaniel Hubert,[2] Jason B. Williams,[1] Keston Aquino-Michaels,[3] Zachary M. Earley,[2] Franco W. Benyamin,[1] Yuk Man Lei,[2] Bana Jabri,[2] Maria-Luisa Alegre,[2] Eugene B. Chang,[2] Thomas F. Gajewski[1,2]†

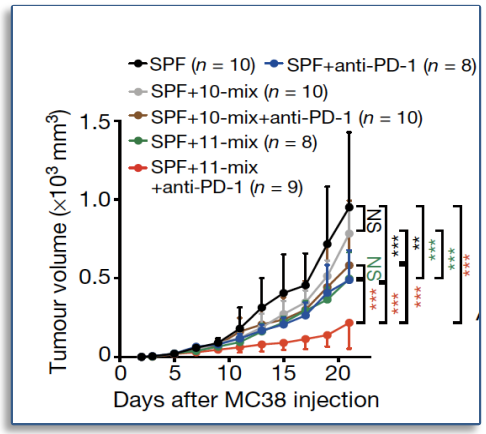


ARTICLE

https://doi.org/10.1038/s41586-019-0878-z

A defined commensal consortium elicits CD8 T cells and anti-cancer immunity

Takeshi Tanoue[1,2,3,16], Satoru Morita[1,16], Damian R. Plichta[4], Ashwin N. Skelly[1], Wataru Suda[3,5,6], Yuki Sugiura[7], Seiko Narushima[1,3], Hera Vlamakis[4], Jiri Motoo[3], Kayoko Sugita[1], Atsushi Shiota[1,2], Kozue Takeshita[1], Keiko Yasuma-Mitobe[1], Dieter Rhethmacher[8], Tsuneyasu Kaisho[9], Jason M. Norman[10], Daniel Macida[11], Makoto Suematsu[7], Tomonori Yaguchi[12], Vanni Bucci[13], Takashi Inoue[14], Yutaka Kawakami[12], Bernat Olle[10], Bruce Roberts[10], Masahira Hattori[3,5,6], Ramnik J. Xavier[4,15], Koji Atarashi[1,2,3] & Kenya Honda[1,2,3*]



LETTER

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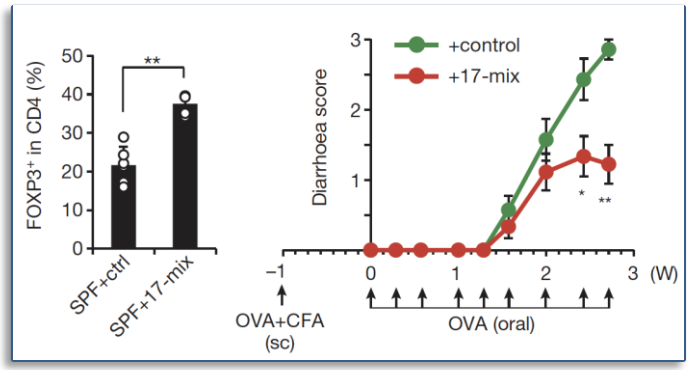
T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota

Koji Atarashi[1,2,3*], Takeshi Tanoue[1,2*], Kenshiro Oshima[4,5*], Wataru Suda[5], Yuji Nagano[1,2], Hiroyoshi Nishikawa[6], Shinji Fukuda[1,7], Takuro Saito[6], Seiko Narushima[1], Koji Hase[1,3], Sangwan Kim[5], Joëlle V. Fritz[8], Paul Wilmes[8], Satoshi Ueha[9], Kouji Matsushima[9], Hiroshi Ohno[1], Bernat Olle[10], Shimon Sakaguchi[6], Tadatsugu Taniguchi[2], Hidetoshi Morita[4,11], Masahira Hattori[5] & Kenya Honda[1,2,4]



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Advantage of Cas9

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- Others found *Akkermansia* and *E. hirae*

Parabacteroides distasonis	99.3	82G9
Parabacteroides gordonii	97.4	81H9
Alistipes senegalensis	99.5	81E7
Parabacteroides johnsonii	99.9	82F11
Paraprevotella xylaniphila	99.2	82A6
Bacteroides dorei	99.7	81B11
Bacteroides uniformis JCM 5828	99.7	82G1
Eubacterium limosum	99.9	81C1
Ruminococcaceae bacterium cv2	99.8	82B1
Phascolarctobacterium faecium	99.8	82G5
Fusobacterium ulcerans	99.6	81A6

Clostridium asparagiforme	St 15
Anaerotruncus colihominis	St 13
Clostridiaceae JC13	St 8
Clostridium bolteae	St 7
Clostridiales 1_7_47FAA	St 28
Lachnospiraceae 7 1 58FAA	St 3
Clostridium scindens	St 26
Clostridium 7 3 54FAA	St 16
Ruminococcus sp. ID8	St 14
Clostridium indolis	St 9
Eubacterium fissicatena	St 21
Clostridium ramosum	St 18
Lachnospiraceae 3_1_57FAA	St 29
Clostridium sp. 14774	St 1
Lachnospiraceae 3_1_57FAA	St 27
Blautia producta	St 6
Clostridium hathewayi	St 4

Cas9 is the only tool that enables rapid and robust engineering of these diverse organisms

CRISPR-based editing differs in prokaryotes vs. eukaryotes

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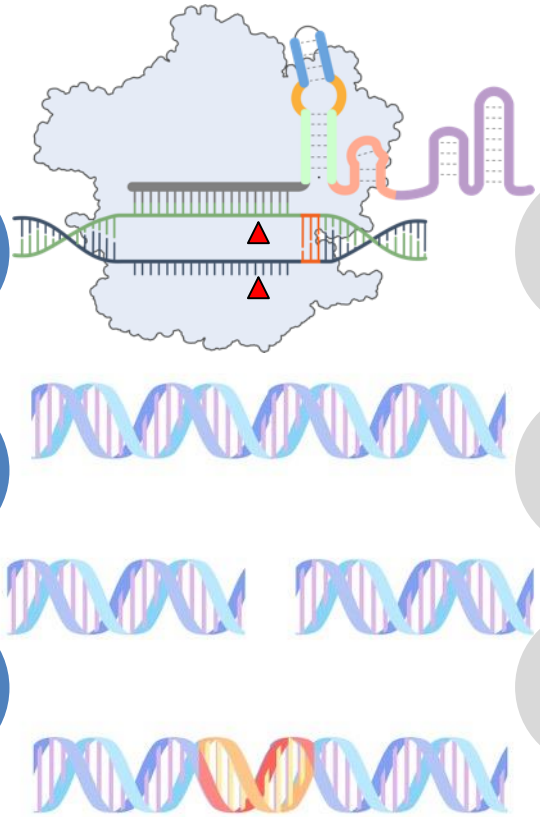
Eukaryotes

Cas9 makes site-specific double-strand DNA break

NHEJ or HDR generates deletions or insertions

Successfully edited genome



Prokaryotes

Provide a template for microbial genome editing

Cas9 kills unedited cell
nCas9 improves efficiency
dCas9 modulates activity

Successfully edited genome

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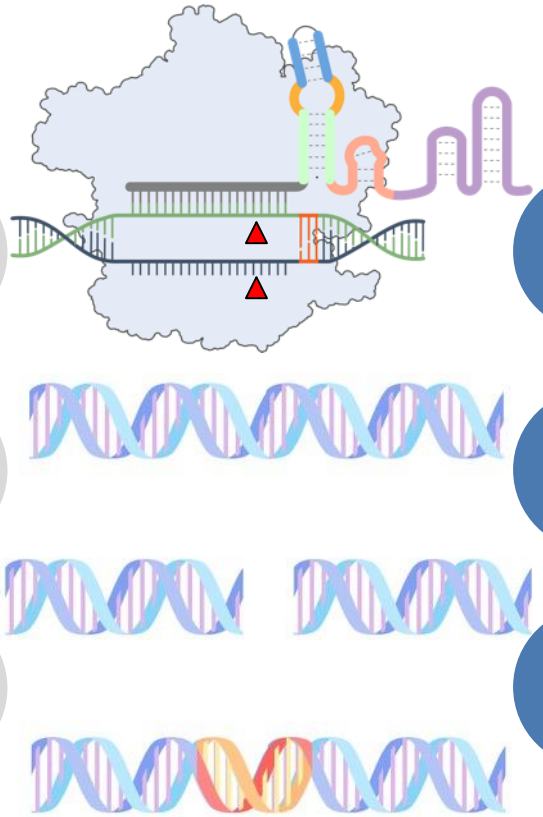

Prokaryotes

Provide a template for microbial genome editing

Cas9 kills unedited cell
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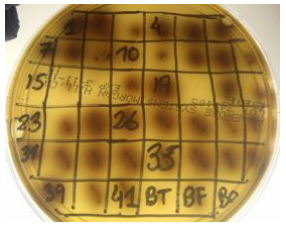
Successfully edited genome

Engineering *B. thetaiotaomicron*, *Lactobacillus* spp.

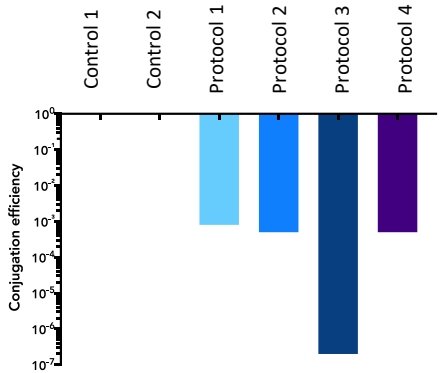
Isolate and characterize strains from fecal samples

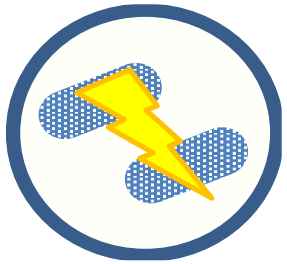
- Verify identity
- Sequence and de novo assemble



DNA delivery via conjugation

- Determine conjugation efficiency

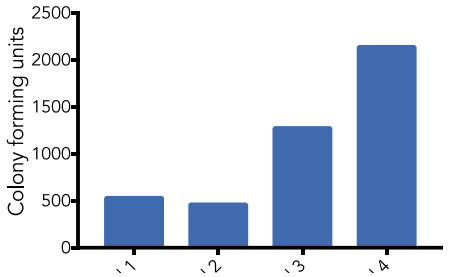




DNA delivery via electroporation

- Determine electroporation efficiency

Transformation of Lactobacillus spp.



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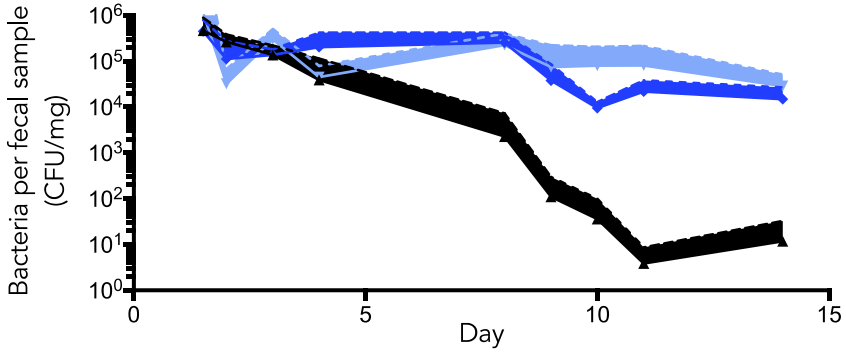
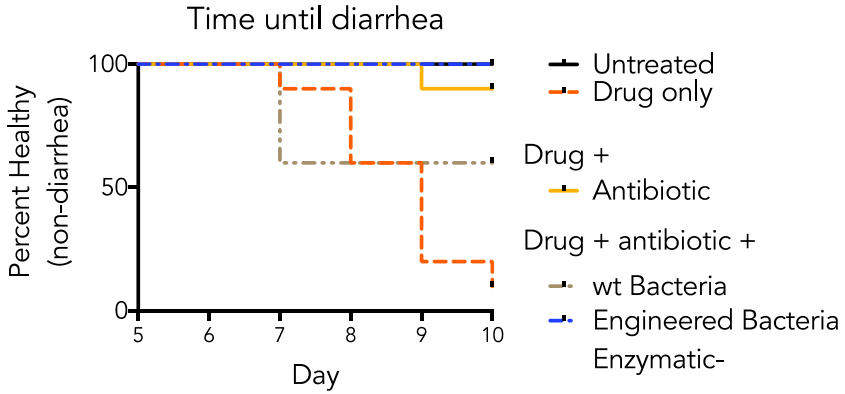
Edited microbe – *in vivo* characterization

Transit and residency of edited microbes through mouse GI tract



In vivo evaluation of therapeutic potential of edited microbe



- Edited microbes can be administered to relevant mouse models
- Residence location and time through the GI tract as well as metabolic activity per dose can be evaluated
- Systemic health metrics inform therapeutic potential of edited microbes

Example clinical applications of gene editing in oncology

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cellectis EDITING LIFE

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Gene editing landscape

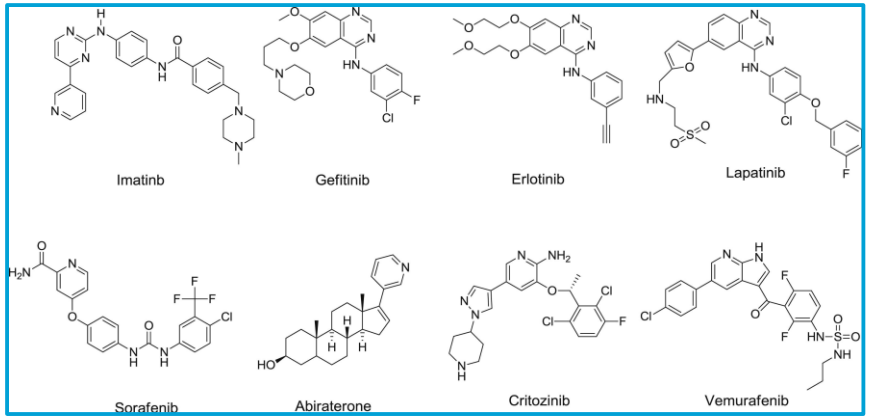
THERAPEUTICS

AGRICULTURAL BIOTECH

BIOLOGICAL RESEARCH

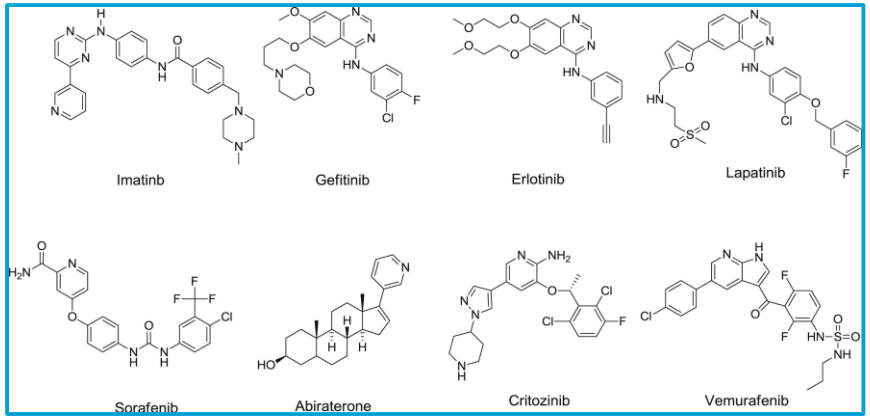
INDUSTRIAL BIOTECH

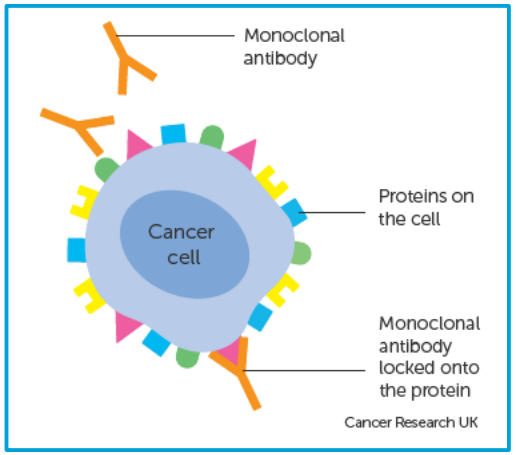
Future of oncology therapies



Hoelder et al, Mol Onc 2012

Future of oncology therapies





Hoelder et al, Mol Onc 2012; Cancer Research UK

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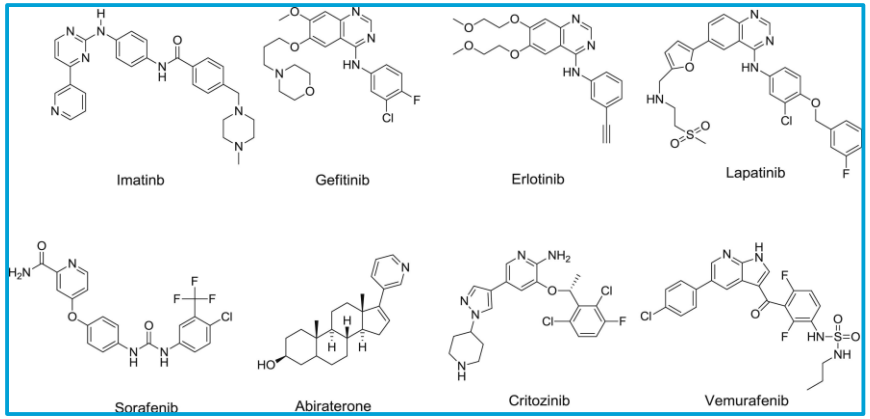

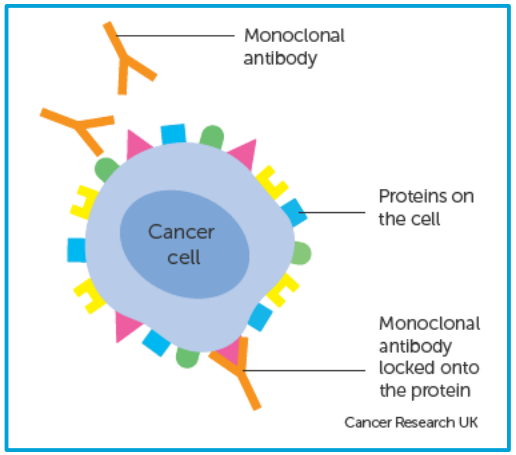

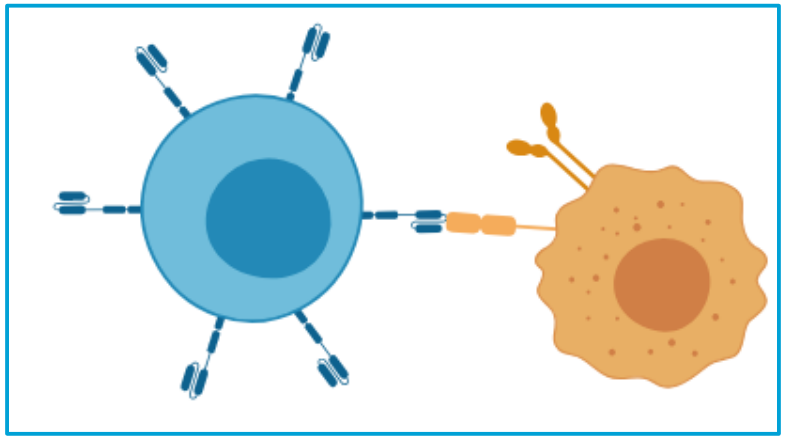

Cancer Progress
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