Gene Therapy for HIV Cure

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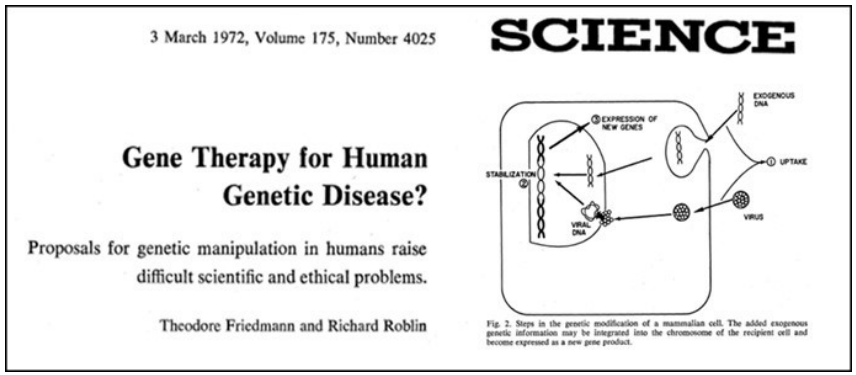
AETC AIDS Education & Training Center Program Mountain West

Gene therapy: a 'good idea' since 1972, clinical trials since 1990



3 March 1972, Volume 175, Number 4025

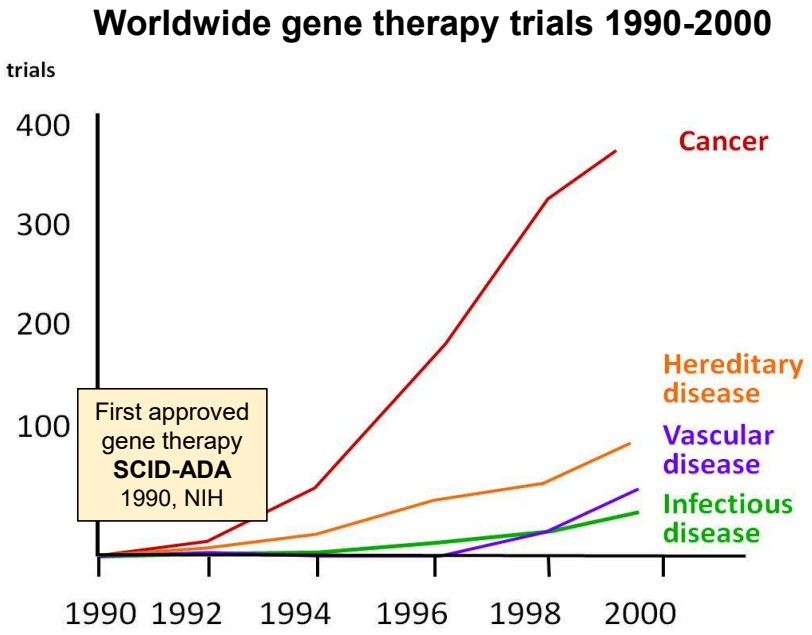
SCIENCE

Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

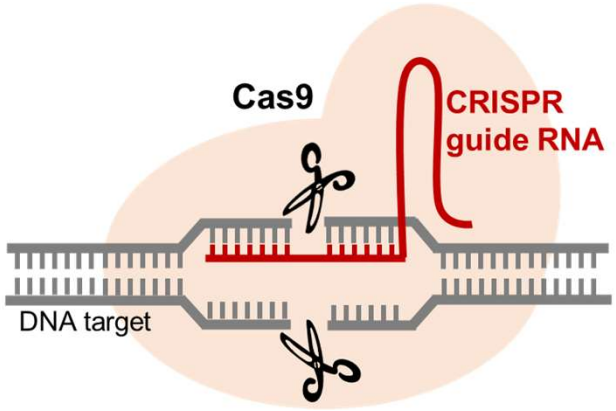
Fig. 2. Steps in the genetic modification of a mammalian cell. The added exogenous genetic information may be integrated into the chromosome of the recipient cell and become expressed as a new gene product.



Current gene therapies (not incl. cancer), 2021

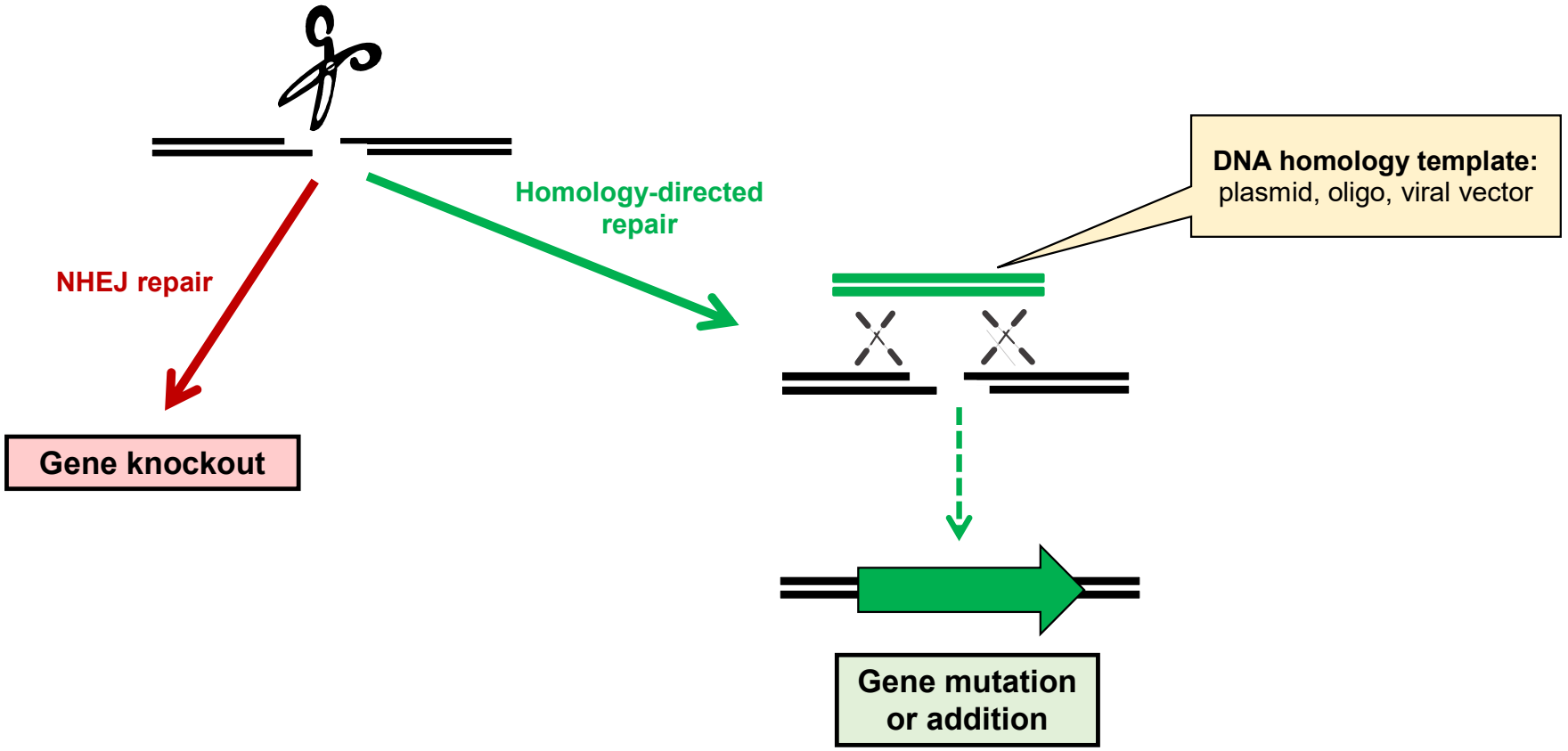
Disease	Gene of interest	Company pursuing gene therapy
AADC deficiency (CNS)	AADC	PTC Therapeutics (GT-AADC)
ADA-SCID	adenosine deaminase	Orchard Therapeutics, (Strimvelis, EMA approved)
Alpha-1 antitrypsin deficiency	A1AT	Adverum
β-Thalassemia (severe sickle cell)	Hemoglobin (β-chain)	Bluebird Bio (Zynteglo, EMA approved)
Cerebral ALD	ABCD1	Bluebird Bio (Lenti-D)
Choroideremia	CHM	Biogen/Nightstar, Spark
Congestive heart failure	Adenyl cyclase 6	Renova (RT-100)
Cystic Fibrosis	CTFR	Vertex, Boehringer Ingelheim
Duchenne muscular dystrophy (DMD)	Dystrophin	Sarepta, Pfizer, Audentes, Solid
Fabry disease	alpha-galactosidase A	UniQure, Sangamo
Glaucoma	BDNF pathway	Astellas
Glioma (cancer)	RRVs deliver cytosine deaminase	Tocagen (Toca511 & TocaFC)
Hemophilia A	Factor VIII	BioMarin, Spark, Shire, Sangamo, UniQure
Hemophilia B	Factor IX	Spark/Pfizer, UniQure, Sangamo, Freeline
HIV	CCR5 negative CD4 cells	American Gene Technology
HoFH (hypercholesterolemia)	LDLR	RegenxBio
Huntington's Disease	huntingtin	UniQure
Lipoprotein lipase deficiency	Lipoprotein lipase	UniQure (Glybera, EMA approval)
Leber's hereditary optic neuropathy (LHON)	ND4	GenSight Biologics
Leber's congenital amaurosis (LCA)	CEP290	ProQR
Metachromatic leukodystrophy	ARSA	Orchard
MPS III (Sanfilippo Syndrome)	SGSH	Abeona
Parkinson's disease	AADC	Voyager
Pompe Disease	acid alpha-glucosidase	Sarepta, Audentes
Recessive Dystrophic Epidermolysis Bullosa	Colagen C7	Abeona (EB-101)
RPE65 deficiency (vision loss)	RPE65	Spark (Luxturna, FDA approved)
Spinal Muscular Atrophy (SMA I)	SMN1	Novartis (Zolgensma, FDA approved)
Wet AMD (retinal disease)	anti-VEGF	RegenexBio
Wiskott Aldrich syndrome (WAS)	WAS	Orchard
X-linked myotubular myopathy	MTM1	Audentes
X-linked retinitis pigmentosa	RPGR	Biogen/Nightstar
X-linked SCID	IL2RG	Mustang Bio

Gene editing (**CRISPR/Cas9**) is bringing new capabilities to gene therapy



- Cas9 cuts DNA at a site determined by its partner CRISPR guide RNA
- Guide RNAs are easy to synthesize to match specific DNA targets

Repair of DNA breaks allows site-specific modification of genes

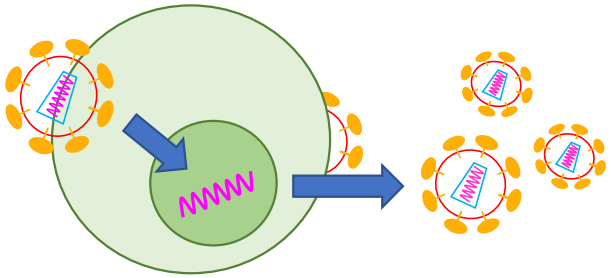


Gene editing trials, 2021

Disease	Group / strategy / status
Cancer (PD-1 knockout)	**Hangzhou Cancer Hospital** •T cells modified (ex vivo) with CRISPR, for advanced esophageal cancer, Phase 2 trial active **Sichuan University** •T cells modified (ex vivo) with CRISPR, for metastatic non-small cell lung cancer, Phase 1 trial active **U Penn/Parker Institute** •T cells modified (ex vivo) with CRISPR, TCR and PD-1 removed, NY-ESO-1 added •trial at U Penn aimed at late-stage cancer patients (multiple myeloma, melanoma, sarcoma)
Cancer (multiple myeloma)	**CRISPR Therapeutics** •allogeneic CRISPR gene edited CAR-T cell therapy, CAR targeting BCMA antigen is inserted into T cells (*ex vivo*) •native TCR removed to decrease chance of immune rejection (GvHD), native MHC-1 removed to increase T cell persistence
Cancer (lymphoma)	**CRISPR Therapeutics** •allogeneic CRISPR gene edited CAR-T cell therapy, CAR targeting CD19 antigen is inserted into T cells (*ex vivo*) •native TCR removed to decrease chance of immune rejection (GvHD), native MHC-1 removed to increase T cell persistence
Hemoglobinopathies (β-thalassemia, sickle cell disease)	**Vertex Pharmaceutical/CRISPR Therapeutics** •CD34+ stem cells modified (ex vivo) with CRISPR, *BCL11A* is cut which increases fetal hemoglobin, trials in progress
Hemophilia B	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert missing F9 gene under albumin promoter in patient's liver cells, Phase 1/2
HIV	**Affiliated Hospital to Academy of Military Medical Sciences** •CD34+ stem cells treated with CRISPR to eliminate CCR5, resulting T cells should be immune to HIV, Trial in Beijing, China. **Sangamo** •HSC cells and T cells (separate trials) modified with ZFN to remove CCR5
Leber congenital amaurosis 10 (LCA10, hereditary blindness)	**Allergan/Editas** •First time CRISPR delivered into the body (*in vivo*), gene editing to fix a mutation in centrosomal protein 290 gene •Virus carrying CRISPR delivered with injection into subretinal area of the eye, human trials in progress
MPS I (Hurler syndrome)	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert IDUA gene under albumin promoter in patient's liver cells, Phase 1/2
MPS II (Hunter's syndrome)	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert IDS under albumin promoter in patient's liver cells, Phase 1/2

Is HIV a good candidate for gene therapy?

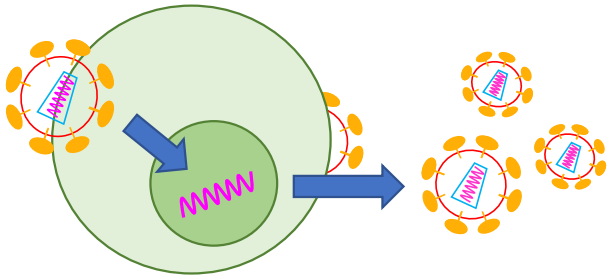
(1) HIV integrates permanently into host chromosomes



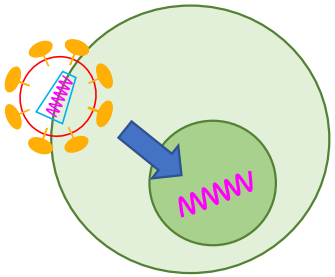
- Its like an “infectious genetic disease”

(2) HIV can become latent and persist, so ART doesn't cure

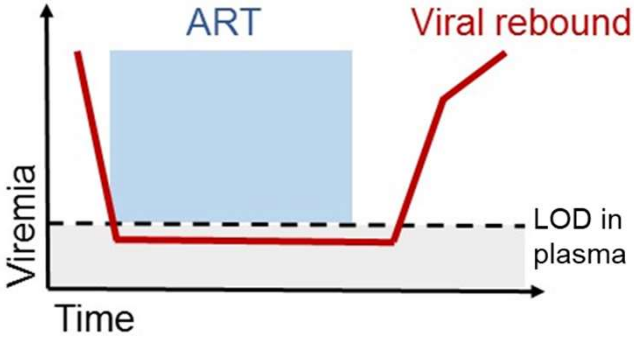
- HIV infects activated T cells and becomes latent as cells transition to memory T cells
- Latent virus persists, including if T cell clone expands



Productively infected cell
- can be seen by immune system



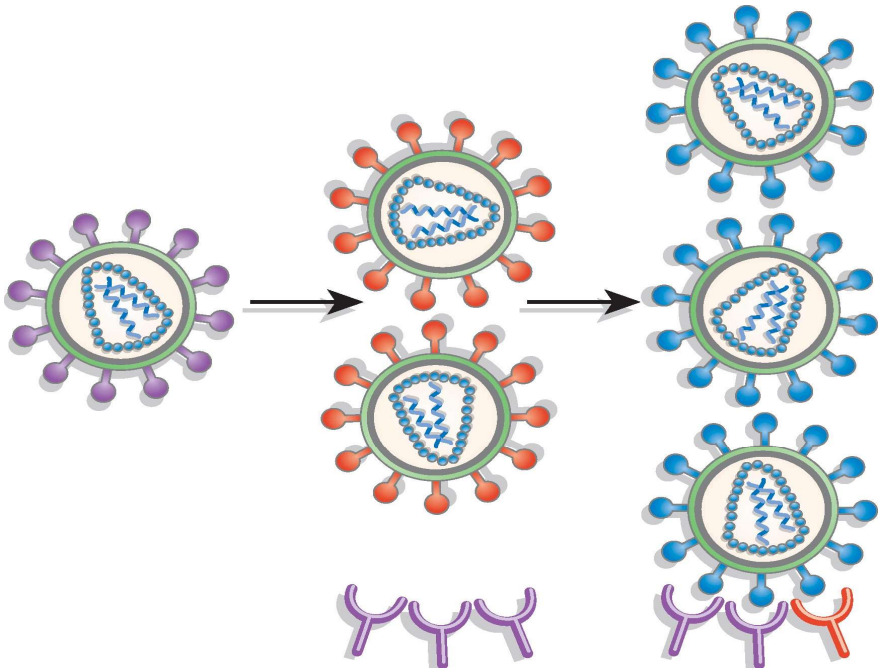
Latently infected cell
- invisible to immune system



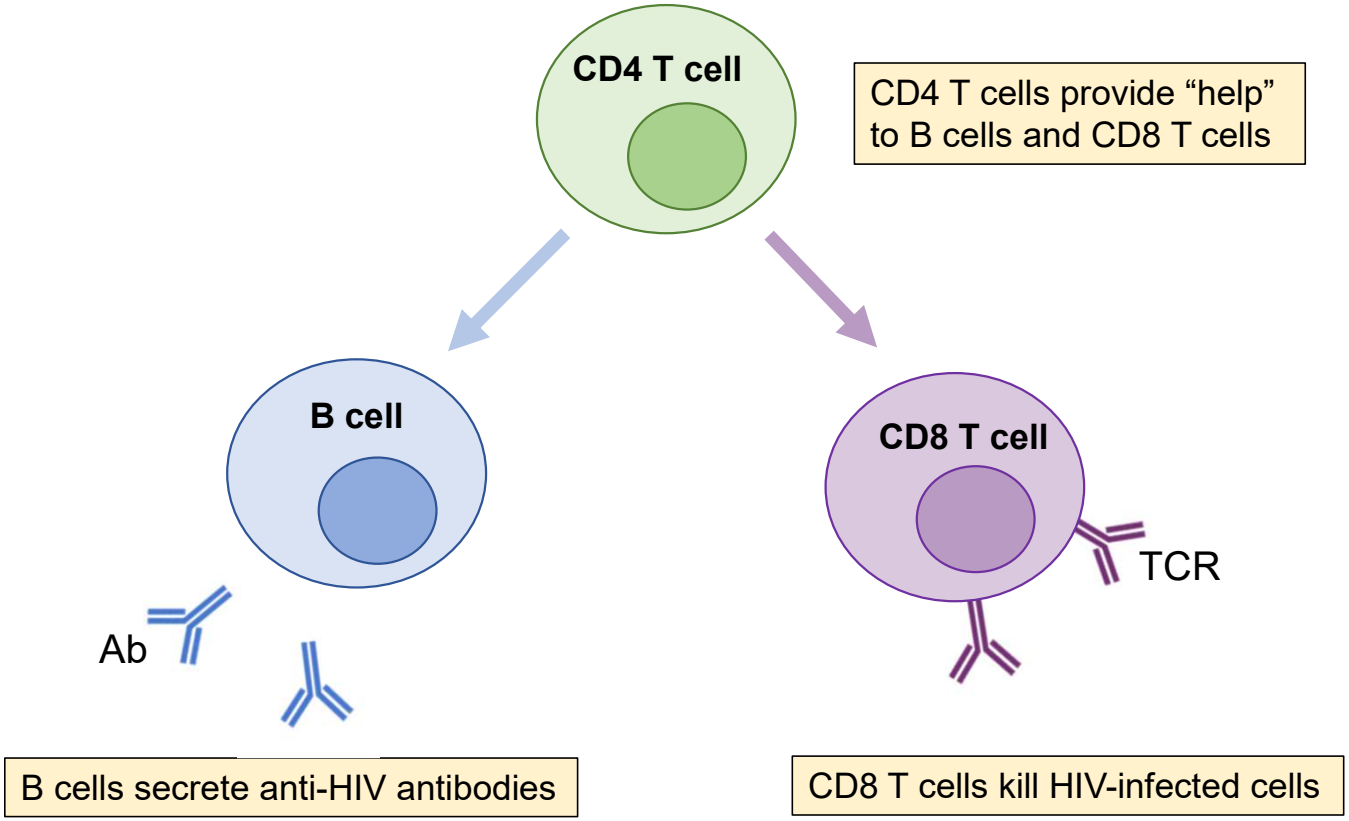
- Latently infected cells are rare (1 in 10^6 memory CD4 T cells) but can reactivate and cause viral rebound if ART stopped

(3) HIV mutates and evolves away from adaptive immune responses

- Keeps one-step ahead of both antibodies and CD8 T cells



(4) HIV thwarts immune responses by targeting CD4 helper T cells



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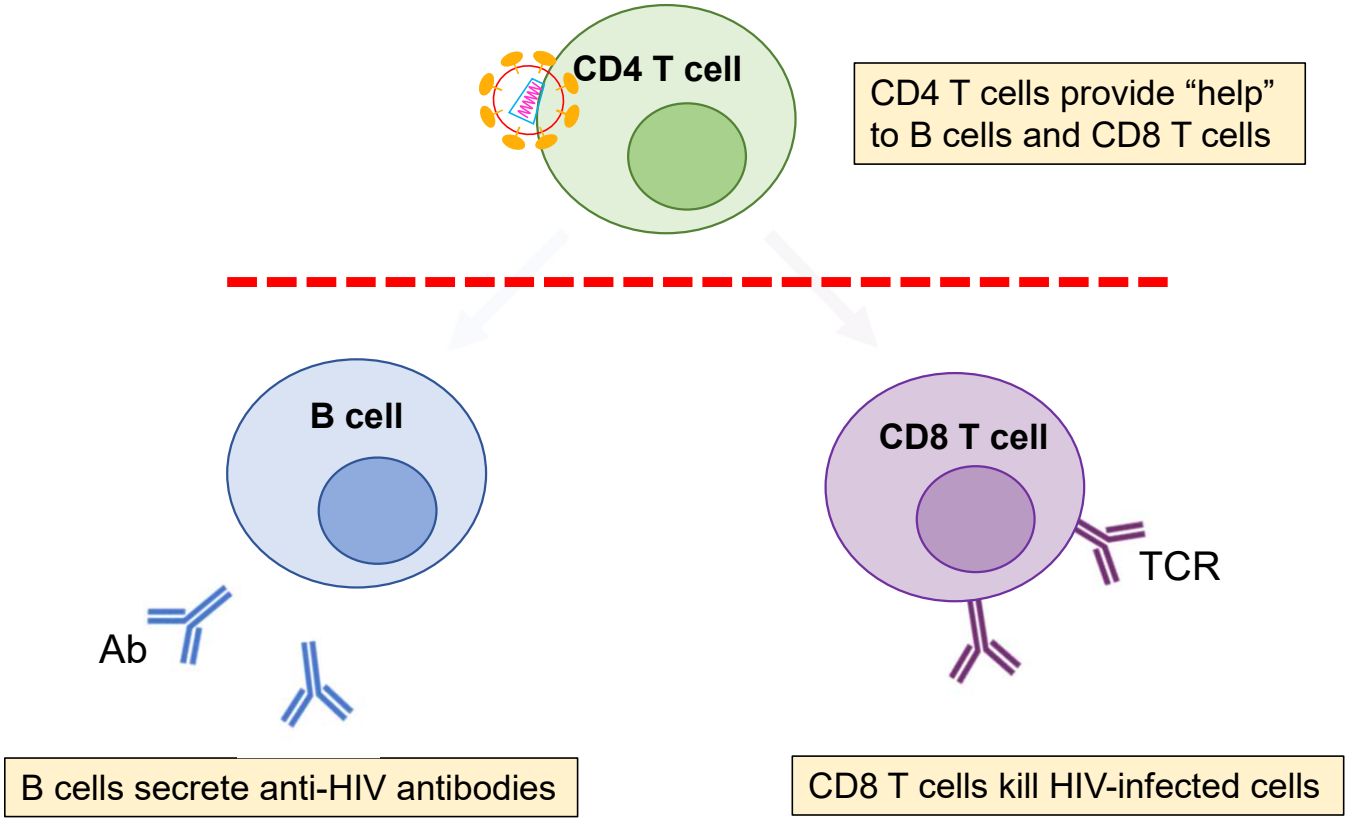


Potential approaches for HIV gene therapy

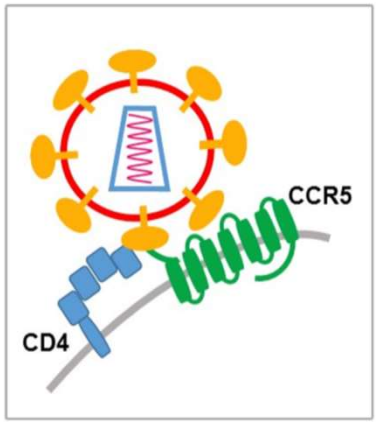
Protect: Make the target CD4 T cells resistant to HIV

Attack: Enhance immune responses against HIV

Purge: Remove the reservoir of latent HIV proviruses

Protecting cells by CCR5 knockout

CCR5 was the first target for gene editing



- CCR5 is the major entry co-receptor for HIV
- CCR5-negative individuals are highly resistant to HIV
  (Two copies of the defective CCR5Δ32 gene)
- HSC transplants from CCR5Δ32 donors are the only known HIV cures

CCR5Δ32 HSC transplants have cured HIV

	Berlin Patient[1]	London Patient[2]	Dusseldorf Patient[3]
Cancer	AML	HL	AML
ART post-HSCT	none	16 mths	66 mths
HIV remission	> 10 yrs	18 mths	3 mths

Timothy Ray Brown

Adam Castillejo

1. *Hutter NEJM 2009*
2. *Gupta Nature 2019*
3. *Jensen CROI 2019*

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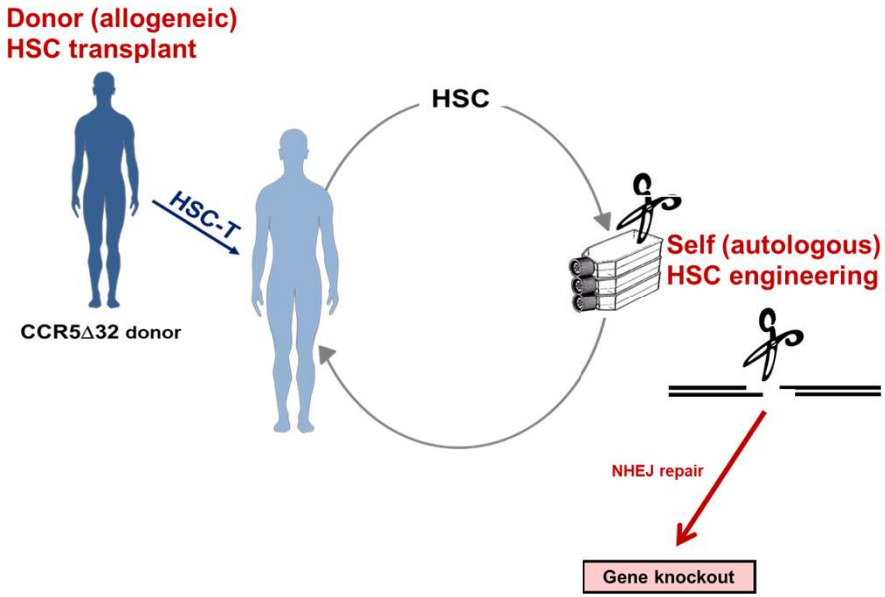
amfAR
MAKING AIDS HISTORY

Gene editing to knockout CCR5



Current clinical trials targeting CCR5

CCR5Δ32 donor HSC, for blood cancers
- IciStem consortium

Autologous T cells edited with ZFNs*
- U Penn, UCSF, UCLA, Sangamo Therapeutics

Autologous HSC edited with ZFNs*
- City of Hope, Sangamo Therapeutics

Edit donor HSC with CRISPR/Cas, for blood cancers
- Affiliated Hospital to the Academy of Military Medicine, Beijing University

* Zinc finger nucleases, an alternative to CRISPR/Cas9

CCR5-ZFN HSC trial

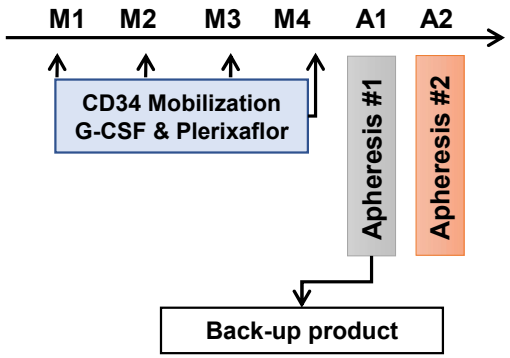
Pilot Study to Evaluate the Feasibility, Safety and Engraftment of ZFN CCR5-Modified CD34+ Hematopoietic Stem/Progenitor Cells in HIV-1 (R5) Infected Patients

- **Clinical PI:** Amrita Krishnan, MD
- **Lead City of Hope investigator**: John A Zaia, MD
- **Lead Sangamo Therapeutics investigator:** Adrian Woolfson, MD, PhD
- **Clinical Sites** (enrollment and follow-up)**:**
  - **Mills Clinical Research**: Anthony M Mills, MD (Los Angeles, CA)
  - **Quest Clinical Research**: Jay Lalezari, MD (San Francisco, CA)
  - **UCLA CARE Center**: Ronald Mitsuyasu, MD (Los Angeles, CA)

ClinicalTrials.gov Identifier NCT02500849

CCR5-ZFN HSC trial

HSC collection:
~ 1 Week

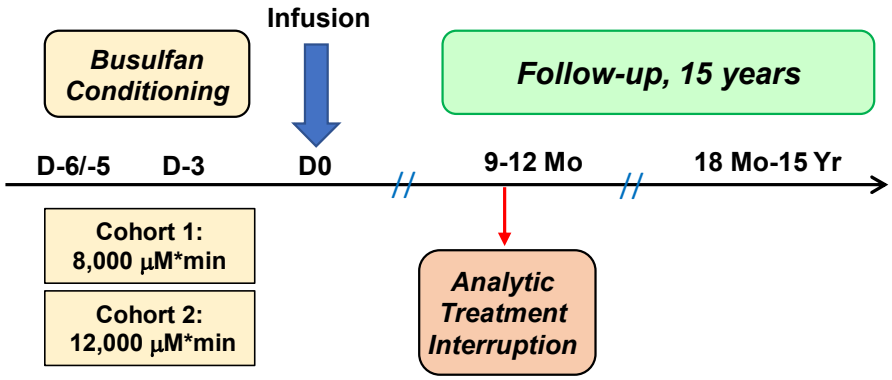


Cell editing, manufacture
~ 6 weeks

ZFN mRNA electroporation

SB-728mR

Treatment & follow-up
- ATI at 9 months



**** ATI eligibility criteria**
- Aviremia (<20gc/mL)
- CD4+ count ≥450 cells/μL OR CD4+ ≥350 cells/μL and CD4% ≥25%
- Detectable CCR5-modified CD4+ cells in blood

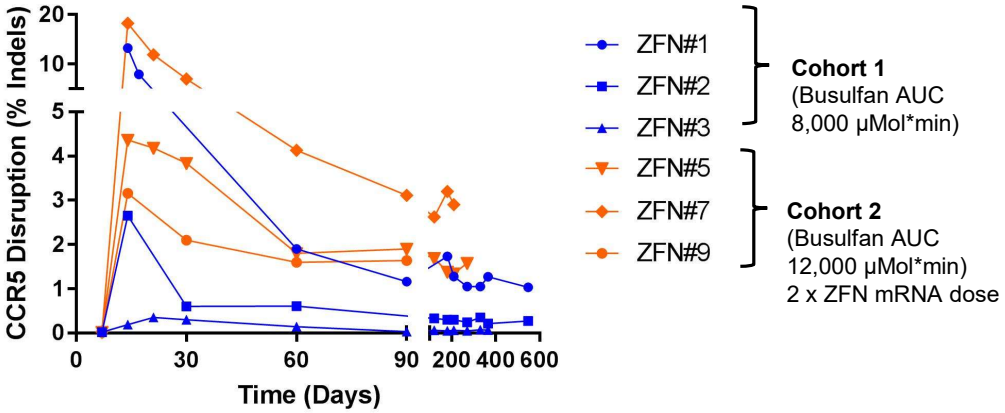
Sangamo THERAPEUTICS City of Hope CIRM CALIFORNIA'S STEM CELL AGENCY

Study status and summary

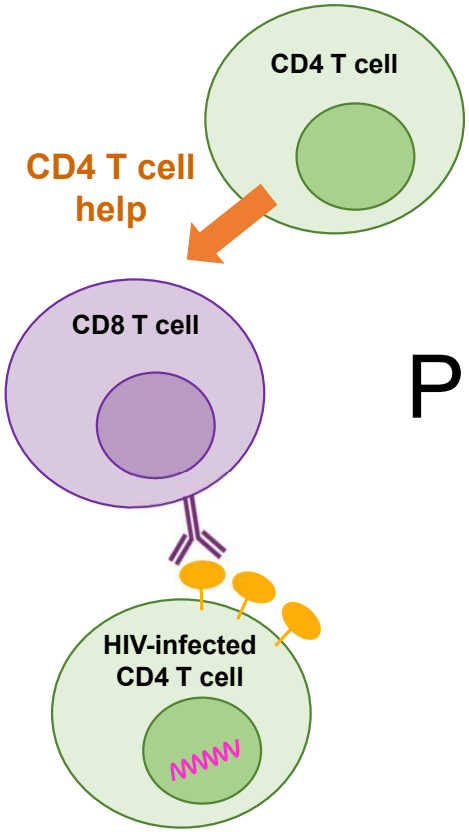
- 14 subjects enrolled, 8 treated
- Two subjects on ATI

Subject	ZFN#1	ZFN#2	ZFN#3	ZFN#5	ZFN#7	ZFN#9
% indel IP	43.25	20.04	8.00	24.80	34.82	21.75

CCR5 Disruption in Peripheral Blood Post Infusion



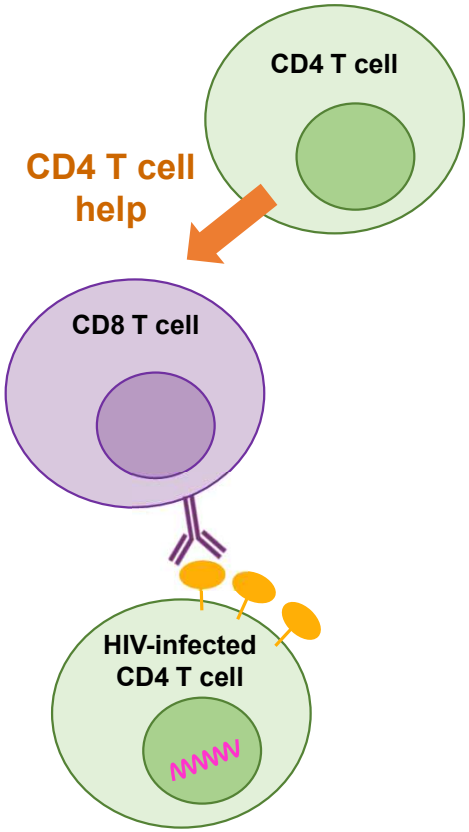
Day 0, infusion of investigational product (IP)



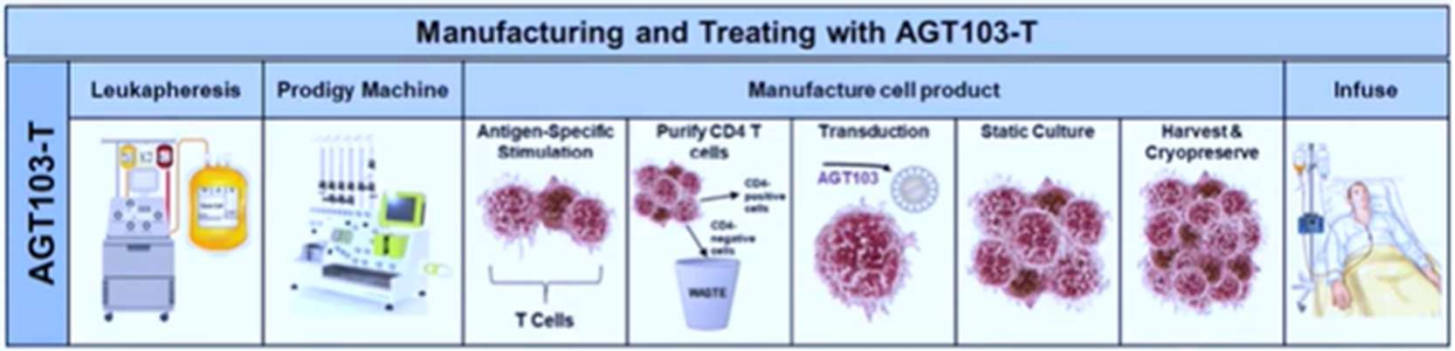
Preserving HIV-specific T cell help

Preserving CD4 T cell help – American Gene Technologies

NCT04561258
Oct 2020, DC



- HIV Gag-specific CD4 T cells isolated from an HIV+ individual's blood
- Massively expanded *ex vivo* by stimulating with HIV Gag peptides
- Made resistant to HV with a lentiviral vector (shRNAs targeting CCR5 and HIV Tat and Vif)



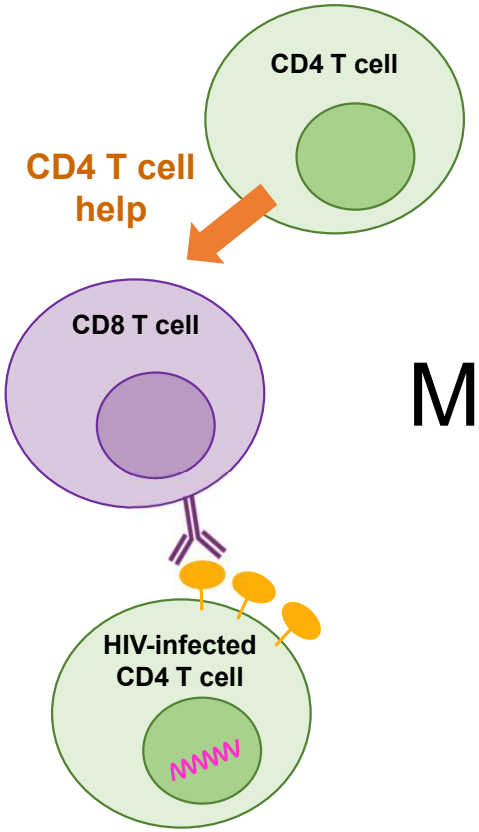
Isolate HIV Gag-specific CD4+ T cells from blood, **<1 million**

HIV Gag peptide stimulation to **expand**

Add LV vector to **protect**

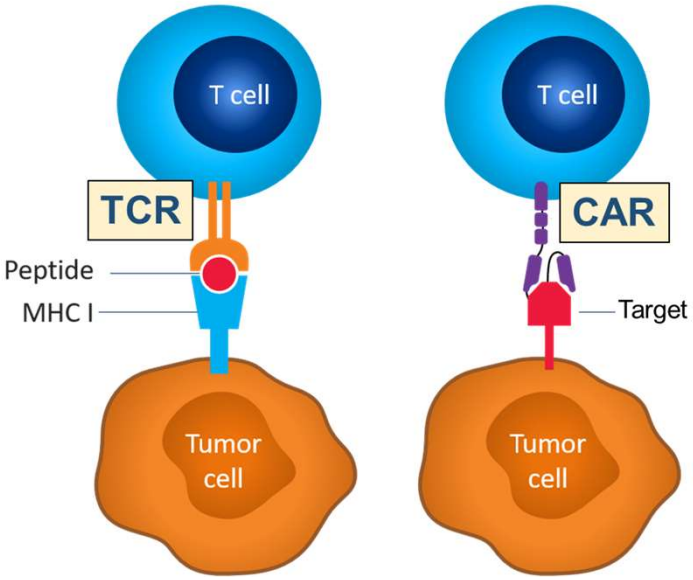
1 billion

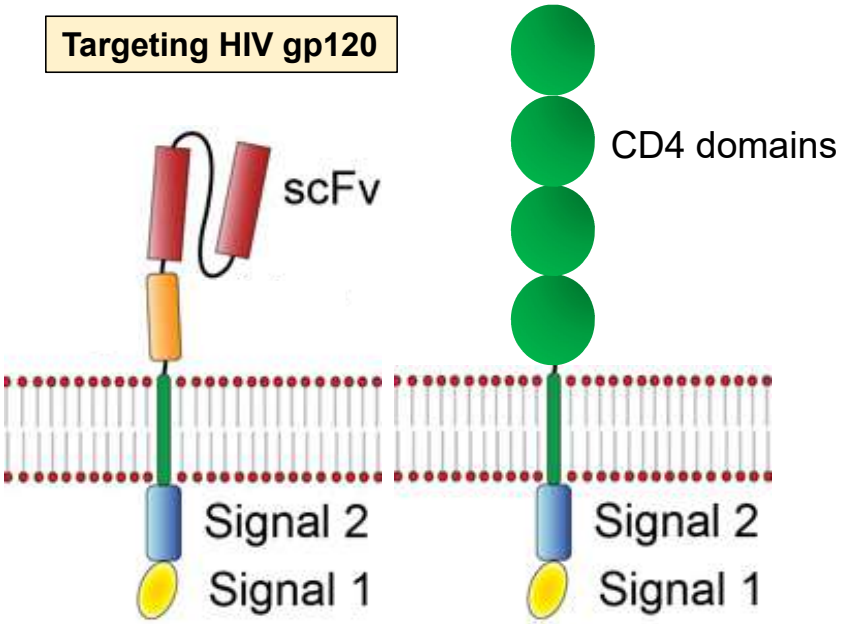
Reinfuse



Making HIV-specific CD8 T cells

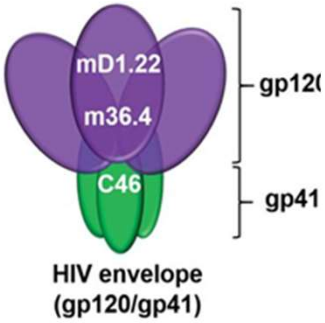
Making better CD8 T cells – CAR T cells

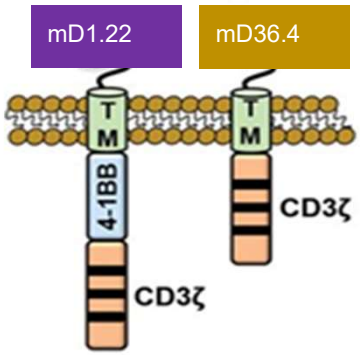




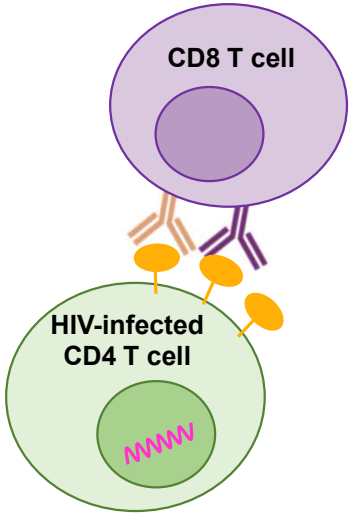
Making better CD8 T cells – Lentigen

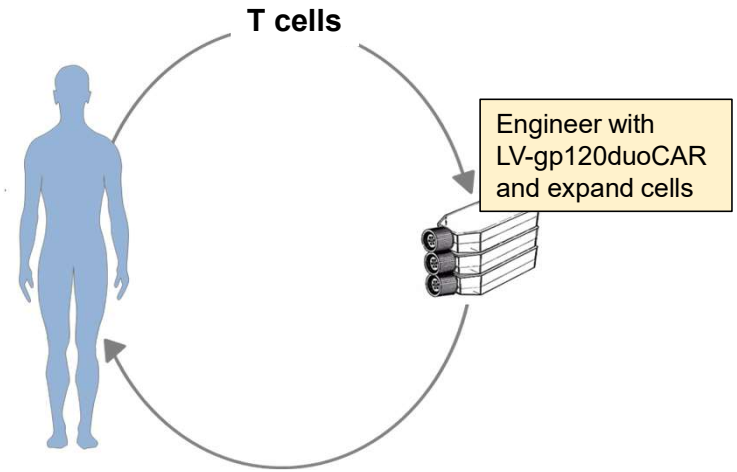
NCT04648046,
Dec 2020, UCSF





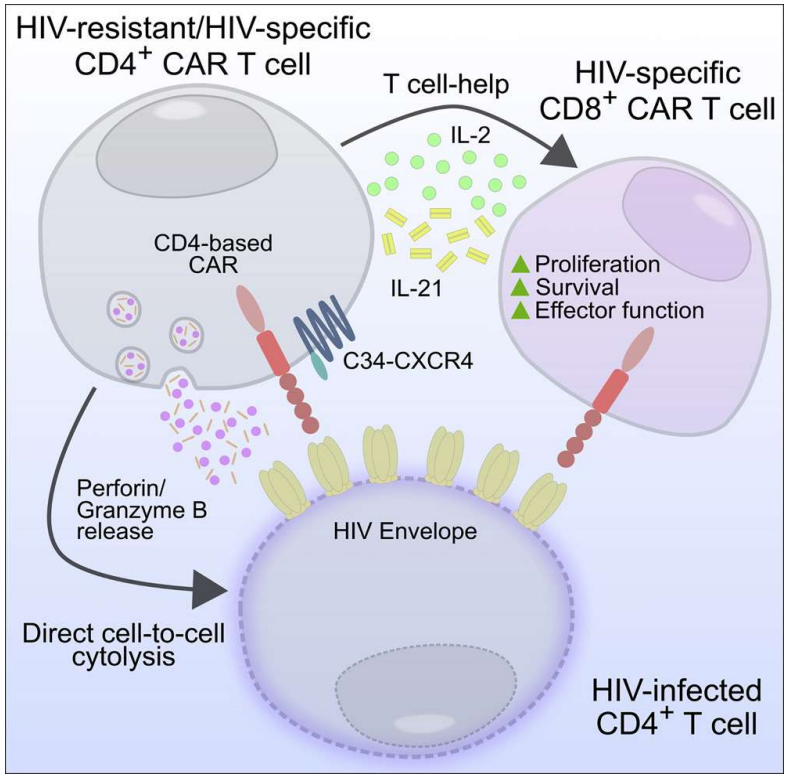
gp120duoCAR





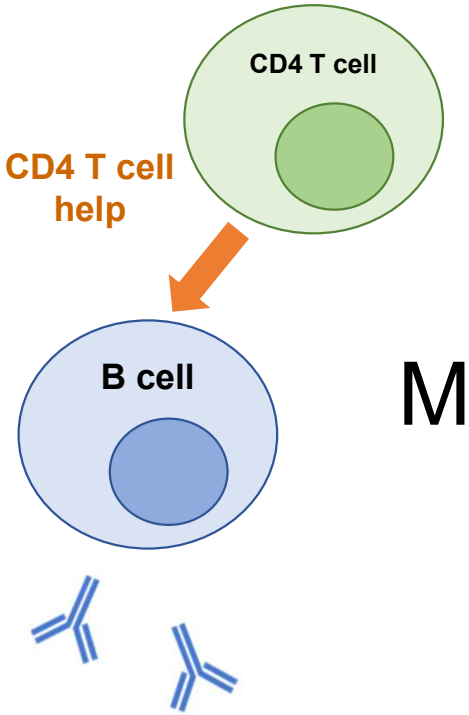
Kim Anthony-Gonda et al., Sci Transl Med, 019;11:eaav5685

HIV-resistant CD4+ CAR plus CD8+ CAR T cells



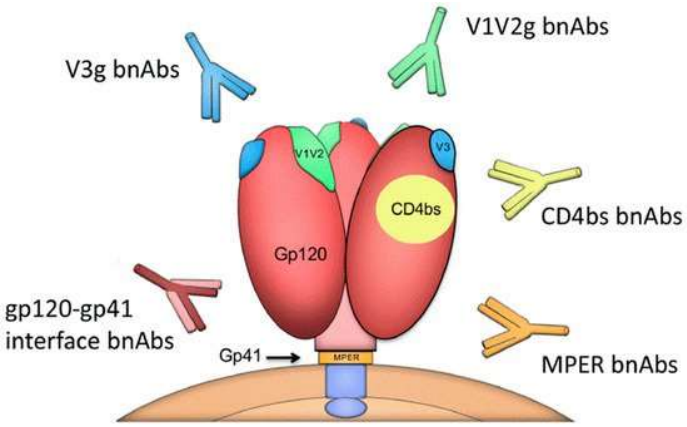
- CAR is CD4 extracellular domains
- T cells made HIV-resistant by expressing an entry inhibitor
- Engineering CD4 plus CD8 cells improves activity

HIV-Resistant and HIV-Specific CAR-Modified CD4+ T Cells Mitigate HIV Disease Progression and Confer CD4+ T Cell Help In Vivo
Maldini et al., Mol.Ther. 2020



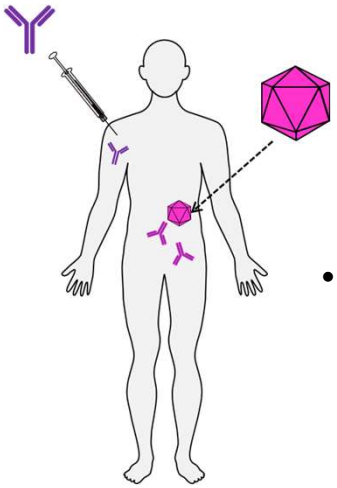
Making better antibodies

Broadly neutralizing antibodies as HIV therapeutics



Classes of anti-HIV bnAbs

- Rare, highly evolved antibodies that develop in some HIV+ individuals and can suppress multiple strains of HIV
- **<u>However, bnAbs can't be induced by vaccination</u>**



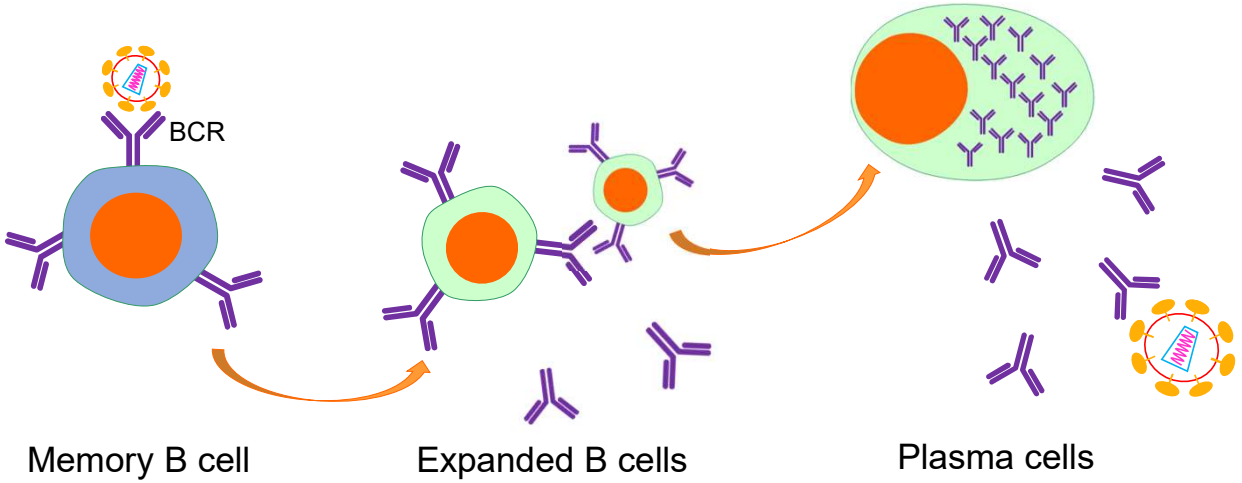
- bnAbs injected as monoclonal antibodies, or expressed from AAV gene therapy vectors injected into muscle

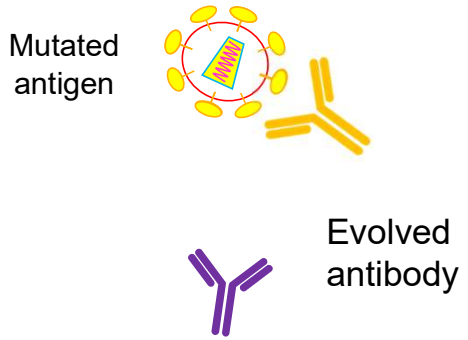
Naturally-produced antibodies are a perfect drug

Antibody production responds to antigen load and is boosted by re-infection or vaccination

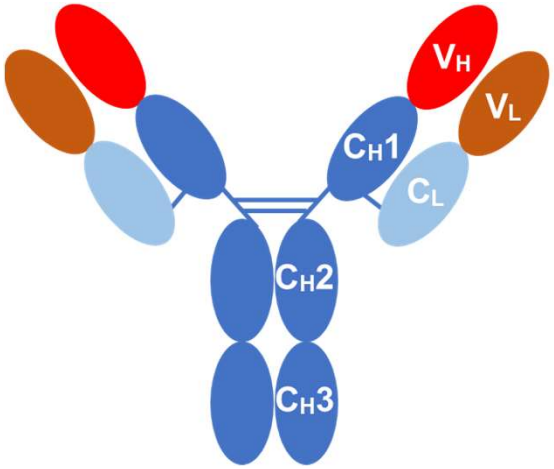
Plasma cells are factories for long-term antibody secretion

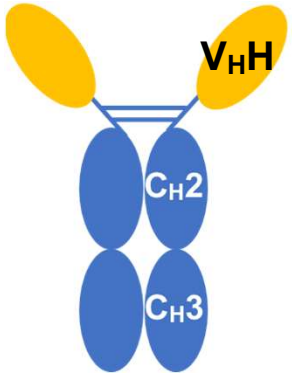
Antibodies can be improved by mutation and affinity maturation





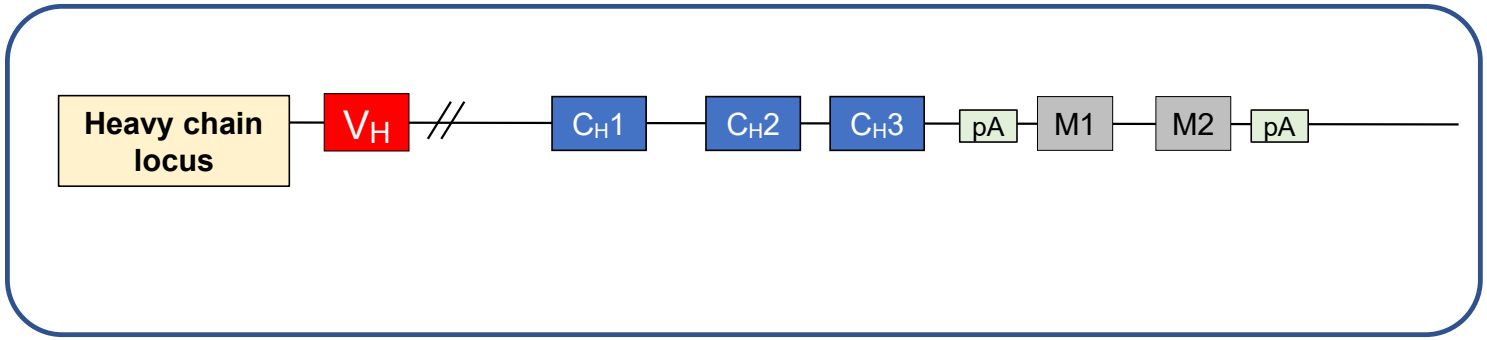
Our strategy mimics heavy chain only antibodies

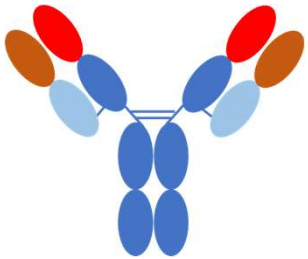




- **H chain only** antibodies from camelids
- **V_HH** is a compact single domain (“nanobodies”)
- **Lack of C_H1** removes the need for an L chain partner

Editing strategy for human heavy chain only Abs





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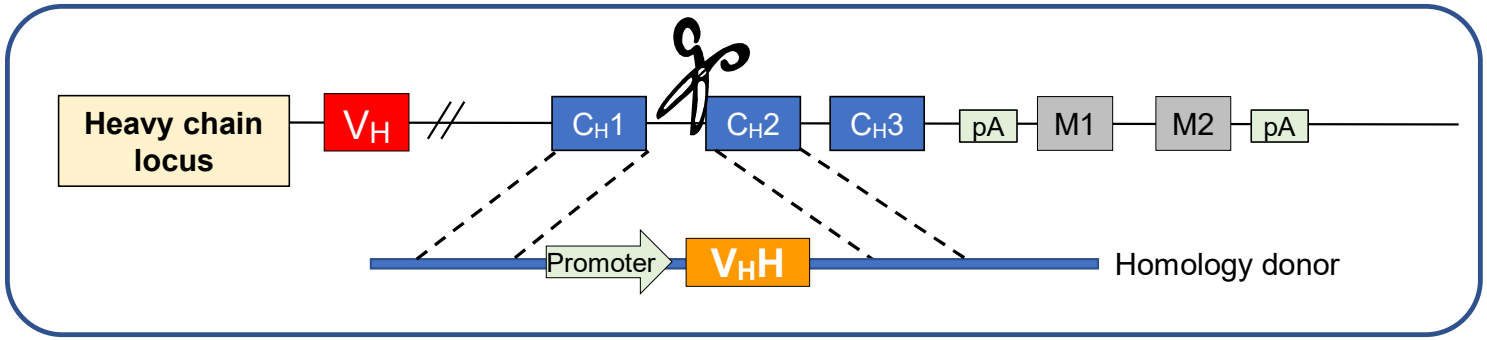
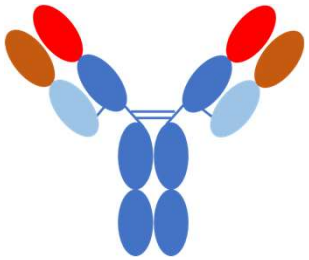
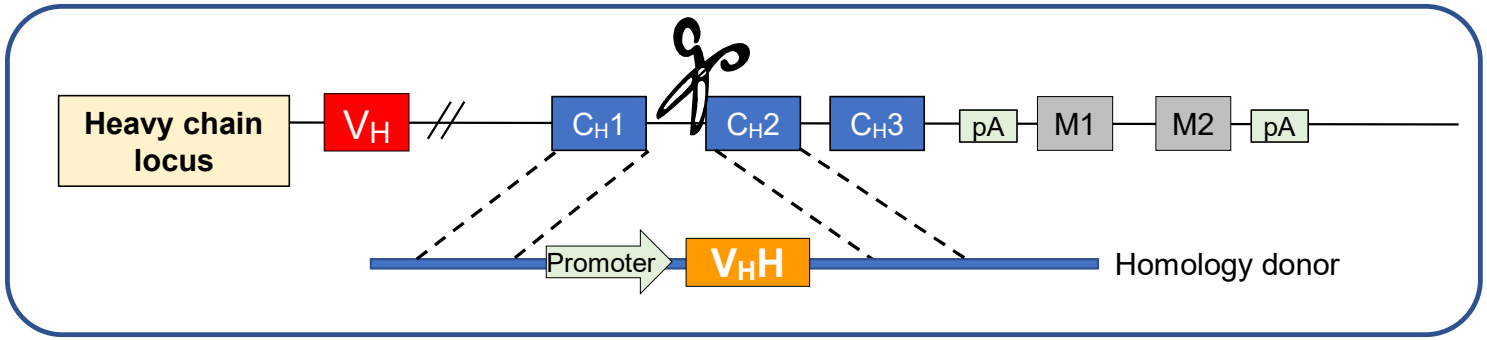
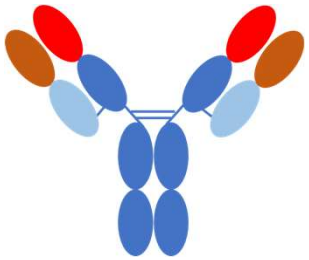
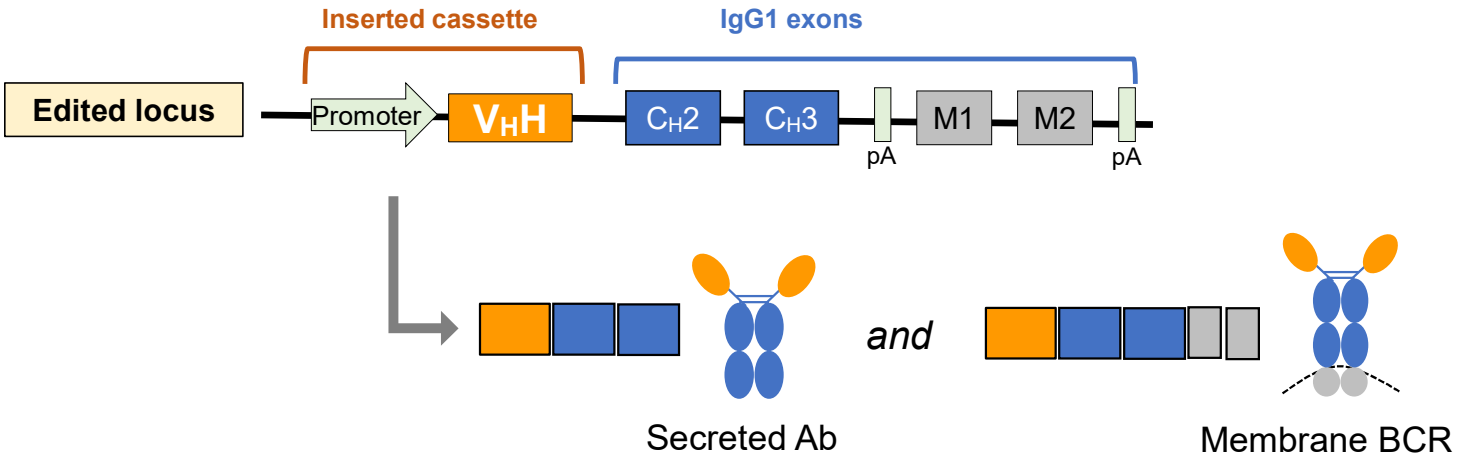




Editing strategy for human heavy chain only Abs

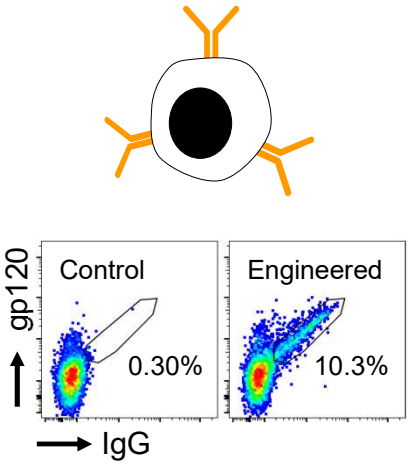




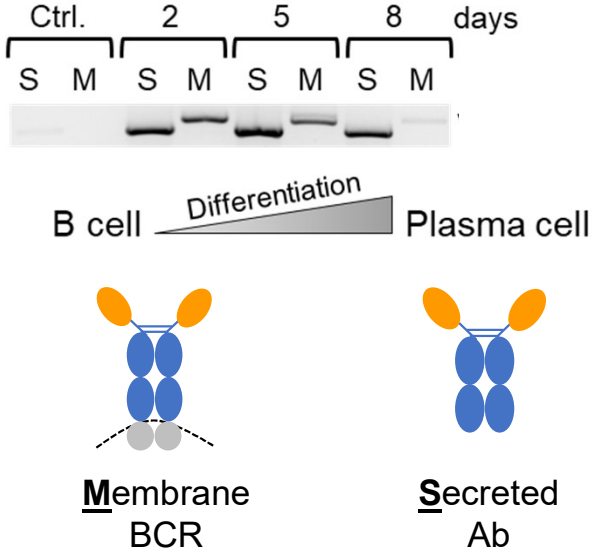


Engineered human B cells make functional heavy chain bnAbs

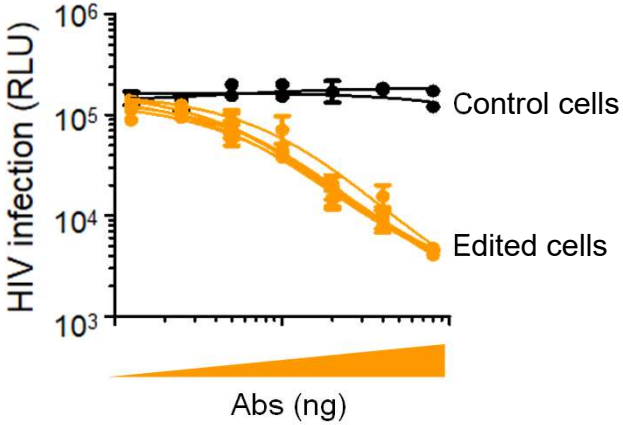
BCR on cell surface

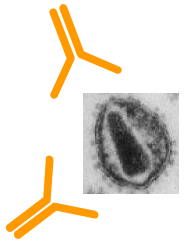


mRNA isoform shifts from BCR to Ab



HIV neutralization assay





Summary

- Gene therapy is increasingly being used for diseases with few/no other options
- FDA has approved 4 gene therapies and >900 in development
- Advances in CRISPR/Cas9 gene editing is expanding the options
- Public awareness and acceptance is growing, helped by the success of current trials for SCIDs, SCD, cancer and blindness

    - and maybe even the COVID RNA and adenoviral vector vaccines?

HIV gene therapy approaches

Protect: Make the target CD4 T cells resistant to HIV

- **CCR5 resistance**

Attack: Enhance immune responses against HIV

- **Expand and protect CD4 T cell help**
- **CAR T cells to improve CD8 T cells**
- **Engineer B cells to express bnAbs**

Purge: Remove the reservoir of latent HIV proviruses

- ***In vivo* delivery of CRISPR/Cas to destroy HIV**

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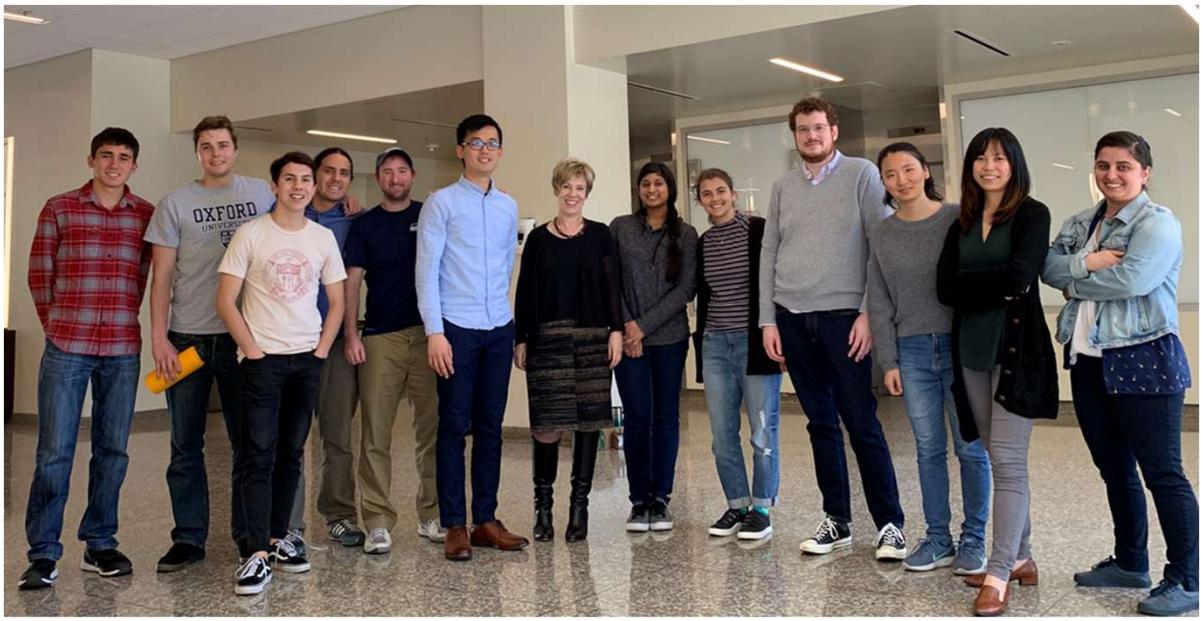

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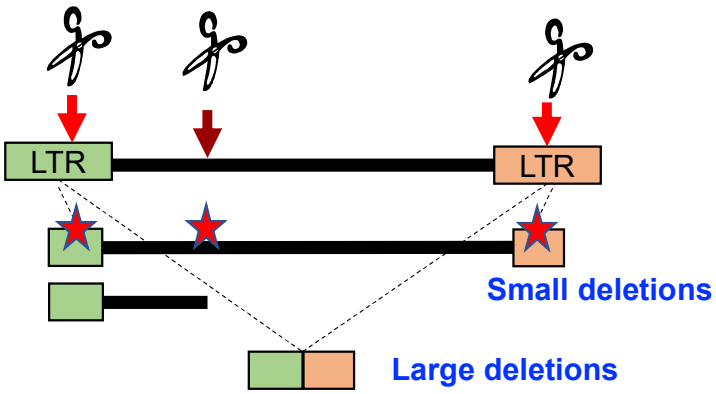
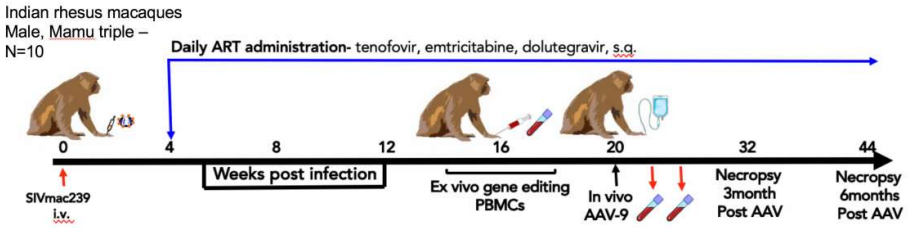
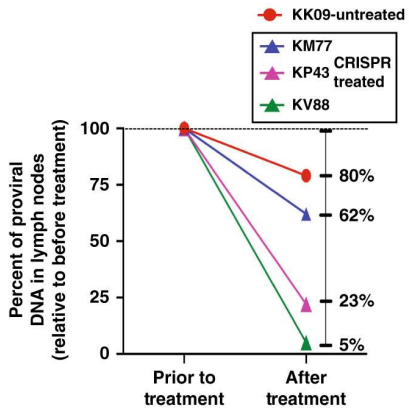
Excising proviral DNA – Excision Biotherapeutics



EBT-101 – inject 10^{13} AAV9 vectors/kg carrying saCas9 and 2 gRNAs





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Mountain West