Towards an HIV Cure: Industry Collaboration Group

Cell and gene therapy for HIV cure: Platforms, progress and practical considerations

Session 1: Engineering for success – Gene editing from lab to clinic

Stay muted and with **camera off**

Webinar is being recorded and will be posted on the IAS+ platform

Share your thoughts please use the chat/Q&A

IAS

International AIDS Society iasociety.org

Learn more:

https://bit.ly/3ZJPktS

Co-chairs

Devi SenGupta

Gilead Sciences

Roger Tatoud

Origena Consulting / IAS Consultant

Presenters

Jan Claas-von Jachmann

PROVIREX Genome Editing Therapies GmbH

Maria Salgado Bernal

IrsiCaixa AIDS Research Institute

James L. Riley

University of Pennsylvania

Learn more:

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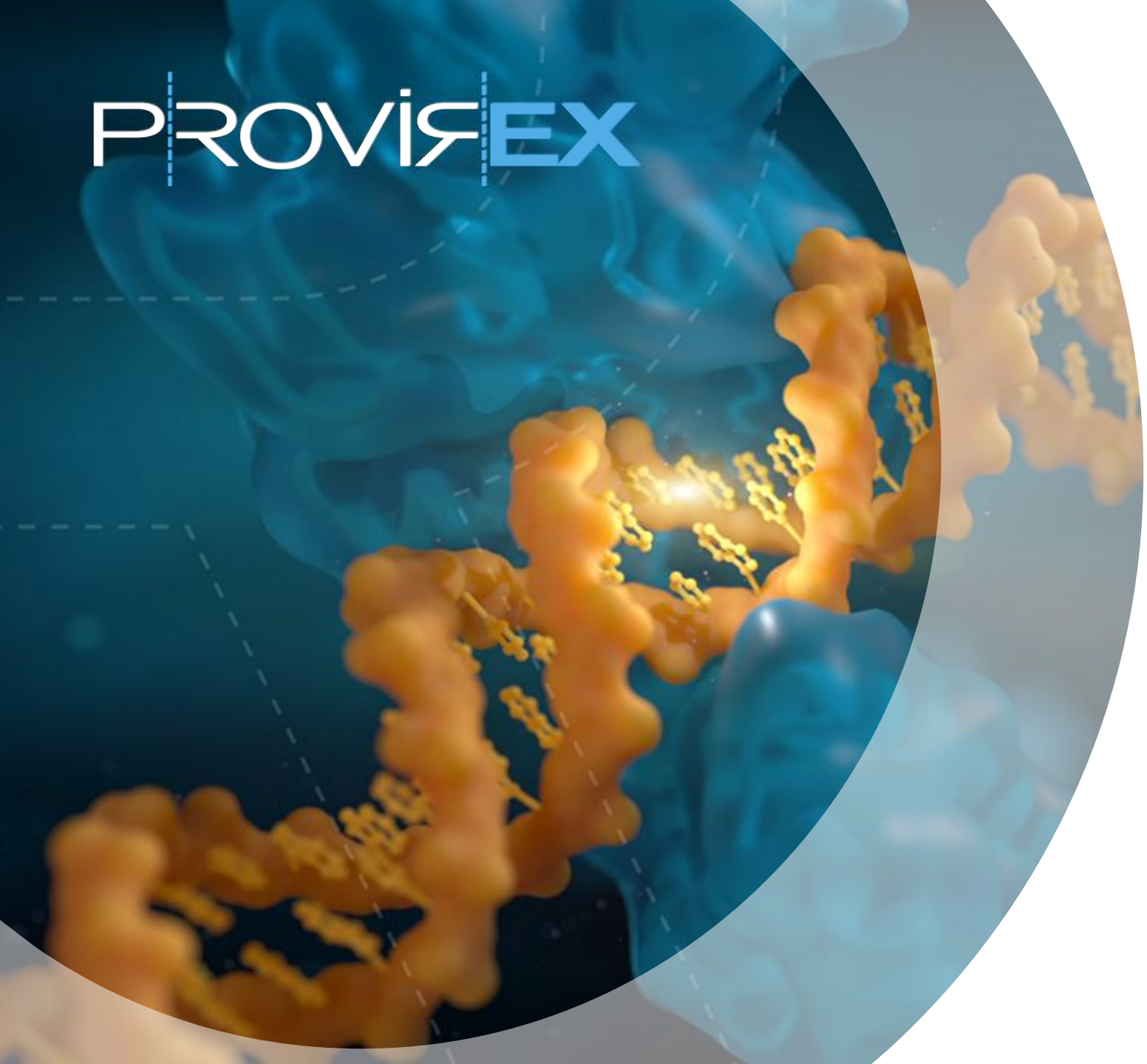


The future of HIV therapy

PROVIREX-Technology: Error-free Genome Editing for Eradication of life-threatening Persistent Virus Infections

bioventure®
Lab to Life Investments.

IFB | innovations starter

Who we Are

PROVIREX Spin Off from Leibniz Institute of Virology (LIV) Founded in 2019

Dr. Jan-Uwe Claas-von Jachmann
Partner CEO
25+ years in Biotech & Pharma, inc. Pre-clinical Research to Head of International Marketing and General Management.

PD Dr. Jan Chemnitz MBA
Co-Founder CSO
20+ years of experience in HIV cure research. Expert in recombinase-based genome editing. Leads scientific strategy and clinical integration.

Oliver Ahnfeld
Co-Founder CFO
20+ years of experience as Managing Partner in financial planning, corporate strategy, and business transformation.

Dr. Niklas Beschorner
Co-Founder Head of R&D / Manufacturing
15+ years of experience in HIV cure research with deep expertise in gene therapy delivery system. Specialist in recombinase technology.

Dr. Maike Voges
Co-Founder Head of QM
15+ years of research experience in virology, immunology, and infectious disease, including HIV and HPV. Expert in ATMPs & lentiviral vectors.

Dr. Michael Hammerschmid
Quality assurance
Biotechnology executive with 25+ years of experience spanning biotech production, startup leadership and entrepreneurship.

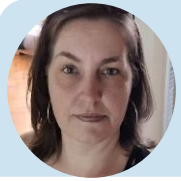
Dr. Melanie Wittner
Scientist
10+ years of research experience, including postdoctoral work at University Medical Centre Hamburg-Eppendorf and industry R&D at Evotec.

Dr. Volker Fehring
Formulation Development Expert
20+ years of experience in pharmaceutical formulation development. Expert in LNP for targeted drug delivery.

Dr. Ann-Kathrin Bernert
Scientist
8+ years of experience in immuno-oncology. Experienced in ATMP-development and GMP-compliant manufacturing.

Dr. Laura Mosbacher
Scientist
8+ years of research experience, including applied virology at LIV and industrial R&D at BioNTech and Sirion (AAV development).

Dr. Charlotte Flory
Scientist
8+ years of research experience, including applied virology at the Leibniz Institute for Virology.



Britta Weseloh
Lab Manager
30+ years of experience in molecular biology, including 18 years on HIV, HSV, SARS-CoV-2 at the Leibniz-Institute for Virology.

Dr. Ulrike Martin
Internal Project Manager
17+ years of experience as a senior scientist for research and development.

Dr. Oliver Zimmer
Pharmacist
7+ years of experience in the development and characterization of pharmaceutical formulations.

M.Sc. Daniel Foth
Technician
7+ years of experience in virology at Leibniz Institute of Virology and in GMP-compliant manufacturing.

Dr. Oliver Lentz
Key Account Manager
20 years of experience as product manager, MSL and key account manager.

Prof. Dr. Joachim Hauber
Co-Founder
Professor (em.) of Virology

Dr. Ilona Hauber
Co-Founder

Prof. Dr. Frank Buchholz
Co-Founder
Dean of Research at TU Dresden

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Current HIV therapies don’t offer a cure and still have shortcomings

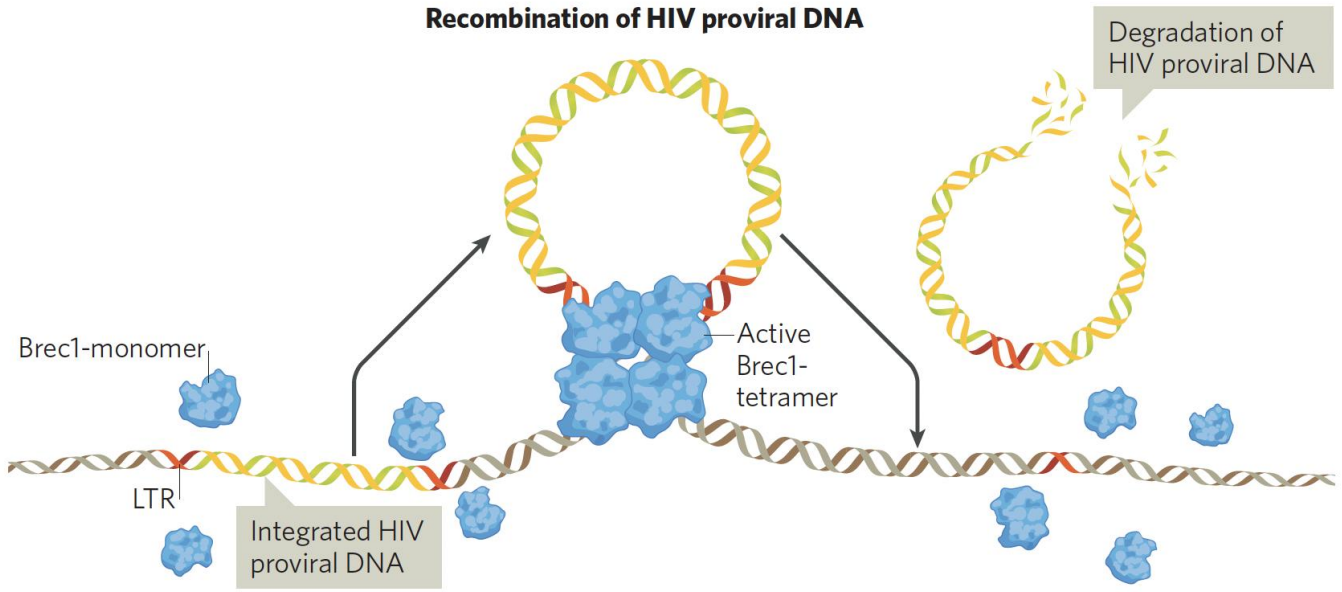
- Lifelong combined antiretroviral therapy (cART) is required:
- Despite advances in ART, high treatment burden leads to non-compliance in people living with HIV (PLWH):
  - **~5%** discontinue due to **drug resistance**[1]
  - The risk to develop a **malignancy with a high mortality** rate is substantially enhanced (up to100 fold) even under successful treatment with ART[8]
  - **~5%** stop therapy due to **psychological stress**[2]
    (e. g. stigmatization, depression, potential infection of partners)
- **29%** of PLWH experience **co-morbidities**[3], amplifying societal costs by €3k–€7k per patient year[4,5,6]
- **~1%** of people living with HIV **do not respond to any of the existing ART classes and develop AIDS symptoms**[7]

[1] WHO: HIV Drug Resistance
[2] Bloch, M., et al. (2020) HIV Me. Suppl. 3:2-16
[3] A. Lorenc et al. London J Prim Care. 2014, 6(4): 84-90
[4] Pourcher, V., et al., (2020) PLoSONE
[5] Christensen, S., et al., (2019) PLoSONE
[6] Guaraldi, G., et al., (2017) AIDS Research and Therapy
[7] Pantke A. et al., (2022), HIV Medicine: AIDS in the era of antiretroviral medicine
[8] Yuan et al., (2022), The Lancet

Eliminating HIV: Brec1 recombinase

- Site-specific recombinase ensures **error-free** gene editing
- **Ex-vivo gene transfer** through GMP-grade, self-inactivating lentiviral vector
- Tat-dependent conditional promoter ensures **specific gene expression** only in HIV-infected cells
- 3 Patents granted

Brec1-recombinase **precisely excises** the integrated HIV

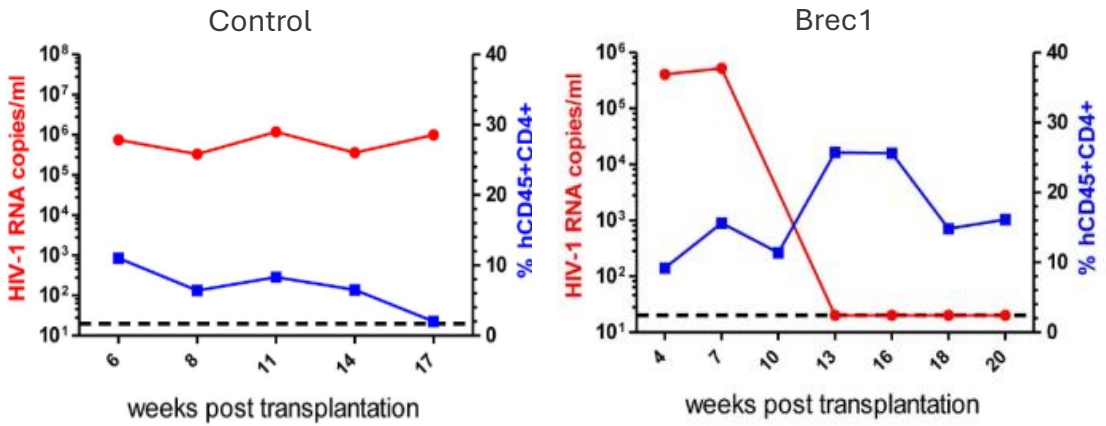


Reversal of HIV infection by Brec1-mediated genome editing reconstitutes host cell immune function and supplies a **functional cure to productive HIV infection**

HIV-Cure demonstrated in Animal Models

Efficacy

Brec1 removes HIV from CD4+ T cells of PLWH



Decrease in viral load (red graph) to below detection limit (< 20 HIV 1 RNA copies/ml) in Brec1 humanized mice.

Safety

- **No genotoxicity**
  - No off-target effects measured by full genome sequencing by NGS in primary T cells and capture sequencing in PBSC
  - No chromosomal rearrangements measured by SKY analysis and arrayCGH assay
  - No insertional mutagenesis by the transfer vector
- **No immunogenicity**
  - No adverse effect on T cell activation and cytokine release assay, no relevant T cell responses to Brec1 *in vivo*
- **No cytotoxicity**
  - No impact on HSC differentiation
  - No effect on cell cycle progression, growth and apoptosis
- **Healthy Brec1 transgenic mice**

Karpinski et al. 2016 Nat Biotech

Intellectual Property for the PROVIREX technology – Full Freedom to Operate

- PROVIREX holds **exclusive rights to a robust IP portfolio** covering the use of tailored recombinases for HIV treatment, including **three core patent families**
- Patents secure claims on **recombinase design**, **target site recognition**, and **therapeutic application** across multiple retroviral strains.
- Licences have been secured from Leibniz Institute of Virology, Max Planck Innovation & TU Dresden
- Comprehensive **Freedom-to-Operate (FTO) analysis**, conducted with leading EU patent law firms (Uexküll & Stolberg & df-mp), confirmed no infringement risks.

Patent	Description
WO2008083931A1	Use of a tailored recombinases for the treatment of retroviral infections
WO2011147590A2	Tailored recombinase for recombining asymmetric target sites in a plurality of retrovirus strains
WO2016034553A1	Well-tolerated and highly specific tailored recombinase for recombining asymmetric target sites in a plurality of retrovirus strains

Development strategy: Ex-vivo and In-vivo-Application (Direct Delivery)

2007 — HIV-1 proviral DNA excision first established

2016 — Brec1 removes HIV from CD4+ T cells of PLWH

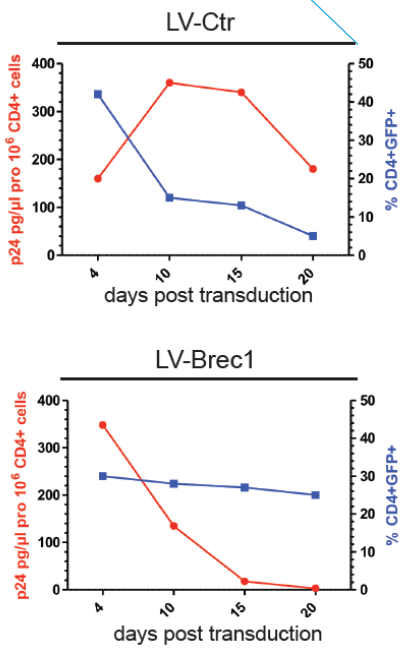
2024 — Brec1 activity confirmed in vivo

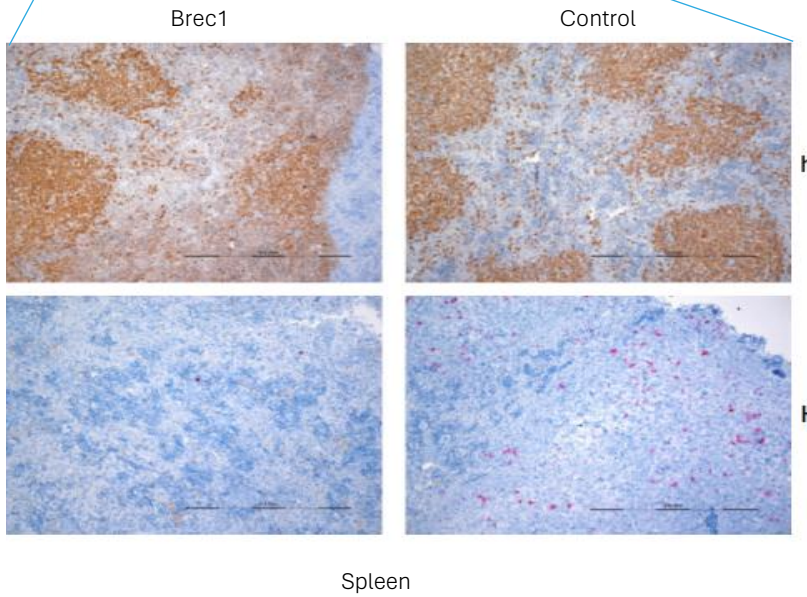
2026 — Pre-clinical safety evaluation

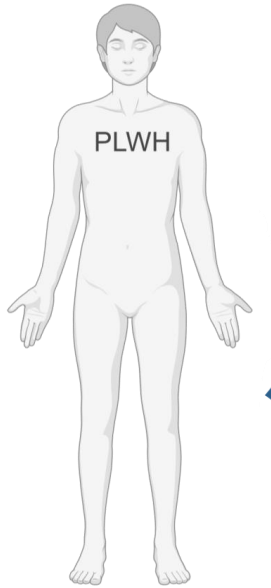
2027 — Phase I/II Brec1 clinical HSC trial

2028 — Develop regulatory strategy

2029 — Expand platform: Pre-clinical development of Direct Delivery Formulation (focus LNP)







Collection of CD34+ PBSC by leukapheresis

Ex vivo PBSC transduction with LV-Brec1

Autologous transfer of gene-modified PBSC

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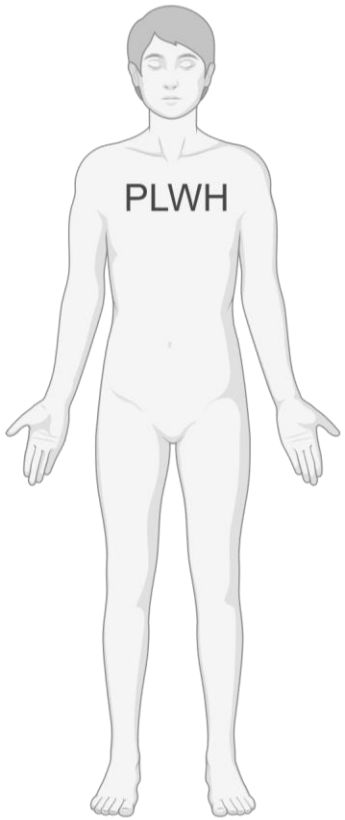
Phase Ib/IIa ATMP Trial – PRX001

- PLWH (8) on stable ART
- G-CSF mobilization
- Busulfan conditioning
- ART / ATI



Collection of
CD34⁺ PBSC
by leukapheresis

Ex vivo
PBSC transduction
with LV-Brec1

Autologous transfer
of gene-modified PBSC

Primary endpoints
Safety and tolerability of gene transfer into PBSC, Engraftment and detection of gene-modified cells

Secondary endpoints
Antiviral activity, i.e. control of viremia w/o medication (ATI)

Exploratory endpoints
Reduction of HIV reservoir (IPDA, TILDA), T cell activities (EliSpoT, ICS)

Phase Ib/IIa ATMP Trial – PRX001

Trial steered by Advisory Board with most renowned German HIV-experts

Trial sites

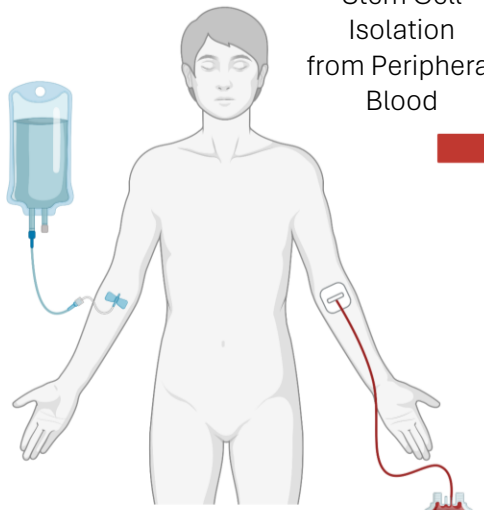
UNIVERSITÄTS KLINIKUM FRANKFURT GOETHE-UNIVERSITÄT

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

UKD Universitätsklinikum Düsseldorf

UNIKLINIK KÖLN

Stem Cell Isolation from Peripheral Blood



Inpatient Infusion of Brec1 Blood Stem Cells in Donor

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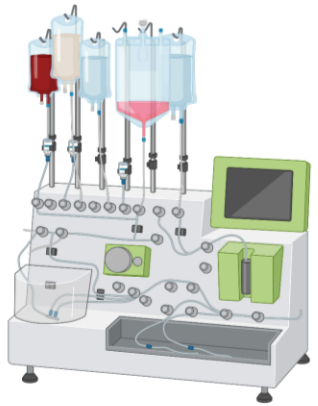
Automated Stem Cell Purification and ex vivo Brec1-Gene Transfer

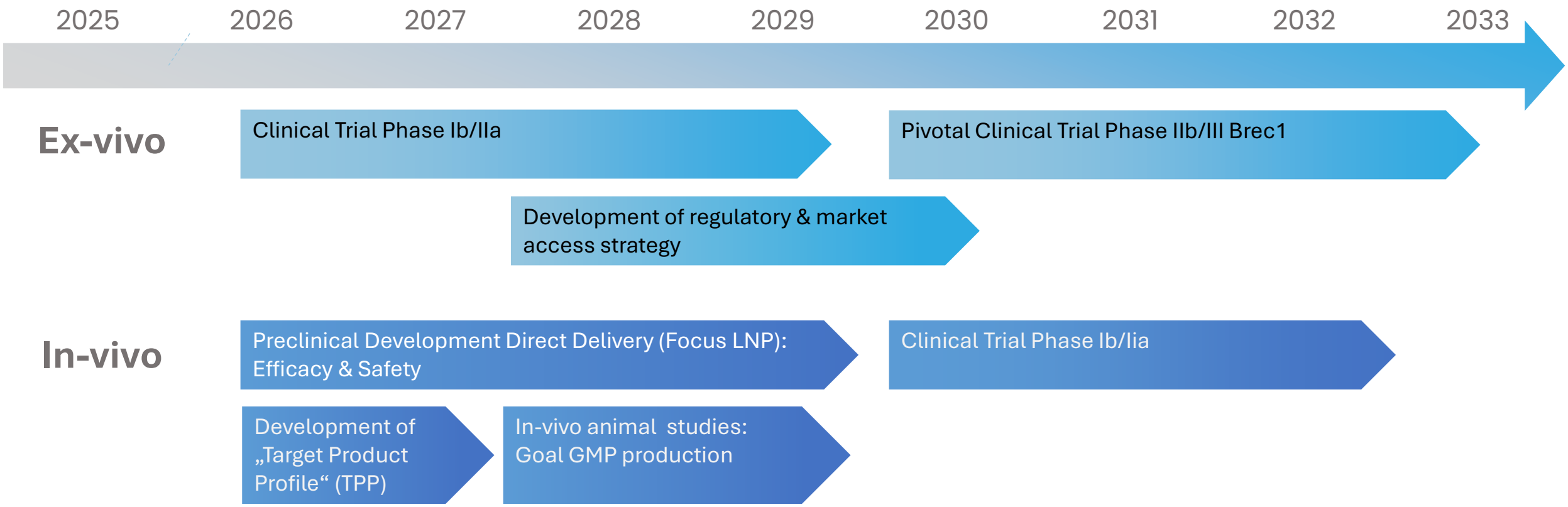


Quality Control

TecHHub | Bahrenfeld Hamburg

PROVIREX

Development Strategy: Ex-vivo and In-vivo-Application (Direct Delivery)

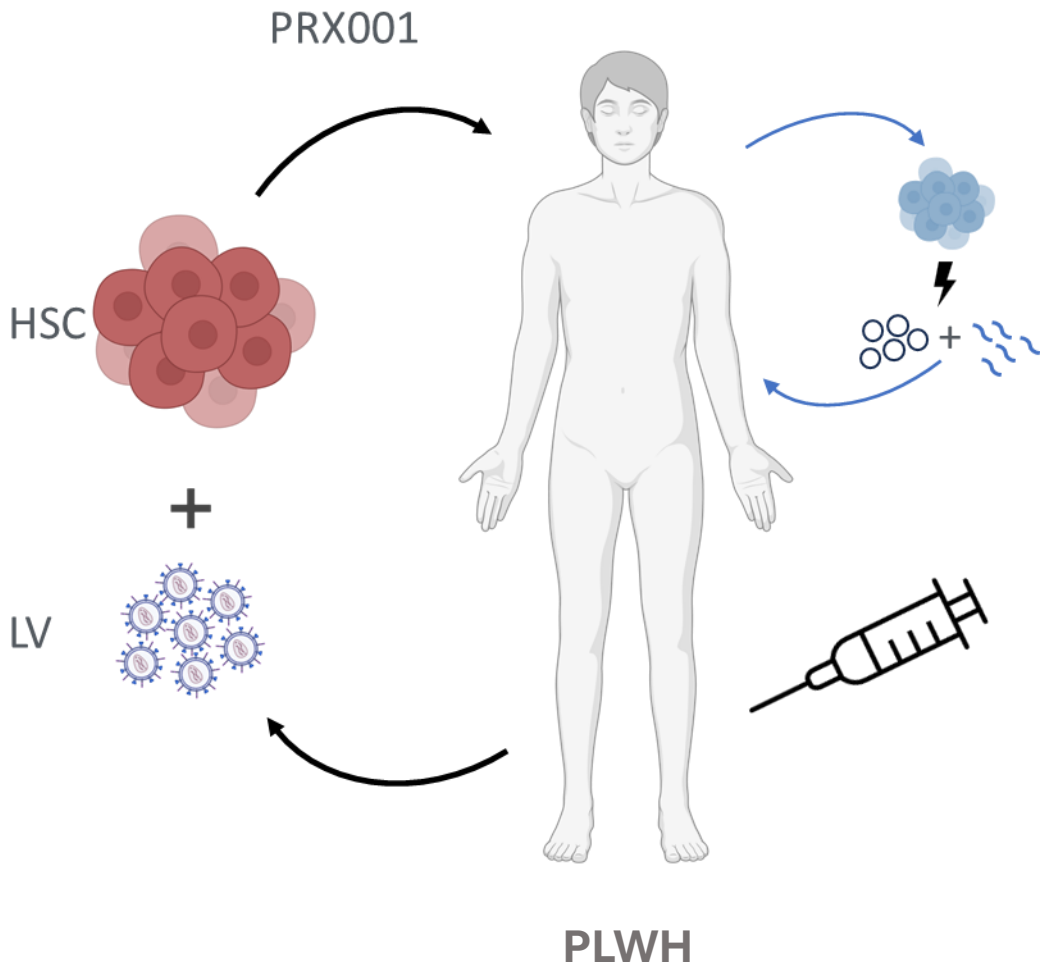


- **In vivo application will provide global access to a broader population of PLWH**

Direct Delivery development strategy
Direct administration of Brec1 into peripheral CD4⁺-T Cells

1) Ex vivo
Stem cells
(PRX001 study)

- Permanent introduction of Brec1 into blood stem cells
- Permanent production of HIV resistant cells
- Complex treatment



2) Ex Vivo
CD4⁺-T Cells in the blood

- Introduction of Brec1 into infected immune cells (CD4⁺T cells) outside the body after apheresis
- Established process: Use of transposase technology

3) In Vivo
CD4⁺-T Cells in the blood

- Introduction of Brec1 into infected immune cells (CD4⁺T cells) in the body
- Injection of lipid nanoparticles (LNP) coupled with CD4-specific nanobodies


Thank you

References

- Beschorner et al. (2024). Preclinical toxicity analyses of lentiviral vectors expressing the HIV-1 LTR-specific designer-recombinase Brec1. ***PLoS ONE*** 19(3): e0298542
- Karpinski et al. (2016). Directed evolution of a recombinase that excises the provirus of most HIV-1 primary isolates with high specificity. ***Nat Biotechnol***. 34:401-409.
- Hauber et al. (2013). Highly significant antiviral activity of HIV-1 LTR-specific Tre-recombinase in humanized mice. ***PLoS Pathog***. 9:e1003587.
- Buchholz & Hauber (2011). In vitro evolution and analysis of HIV-1 LTR-specific recombinases. ***Methods.*** 53:102-109.
- Sarkar et al. (2007). HIV-1 proviral DNA excision using an evolved recombinase. ***Science***. 316:1912-1915.

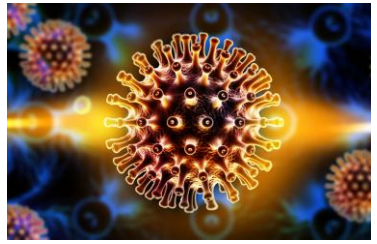
Host Factor Engineering: CAR-T cells therapy for HIV cure

María Salgado

(IrsiCaixa, IGTP)

February 19th, 2026

The HIV-1 Reservoir



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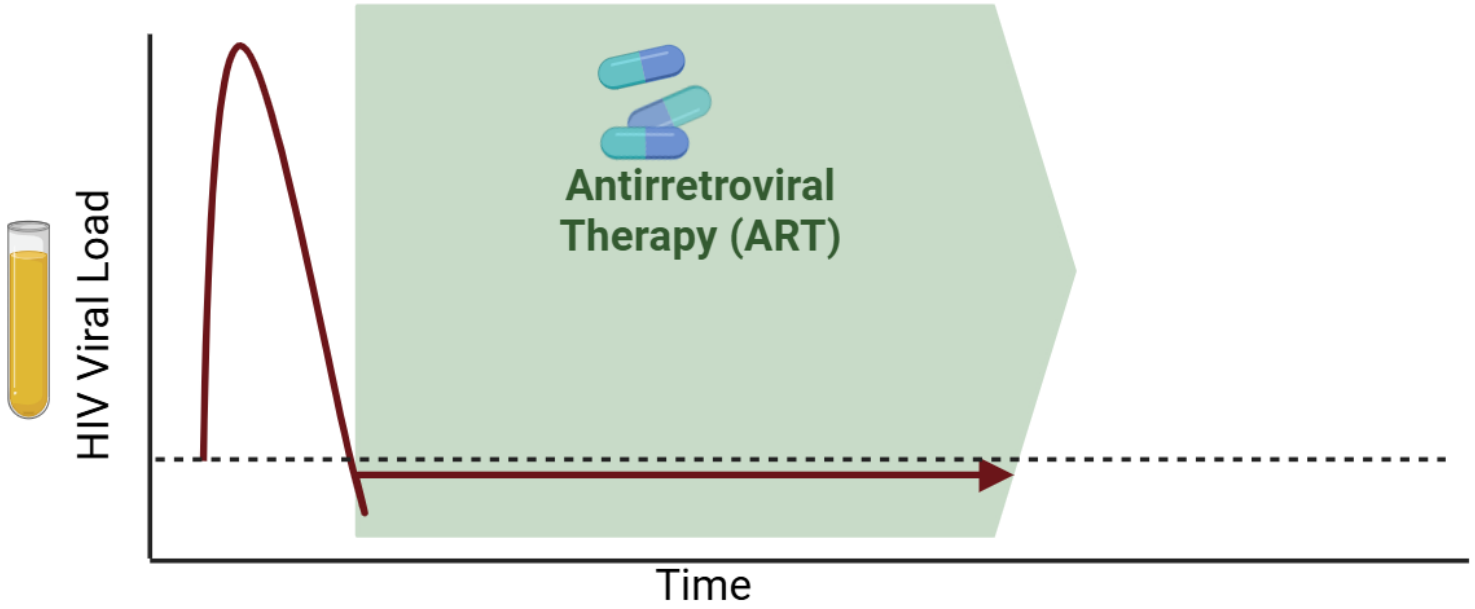

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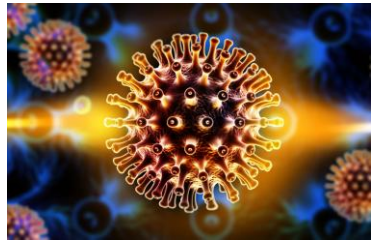

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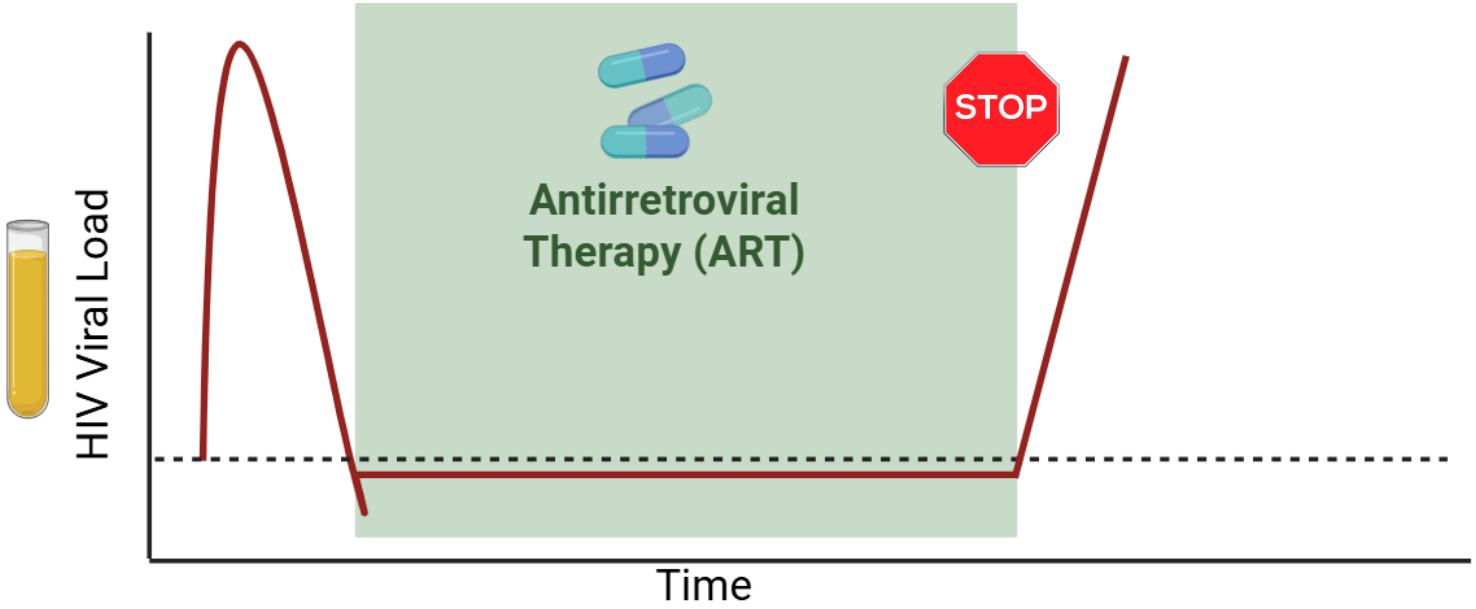

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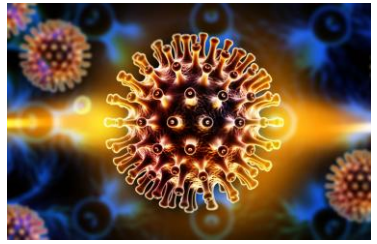

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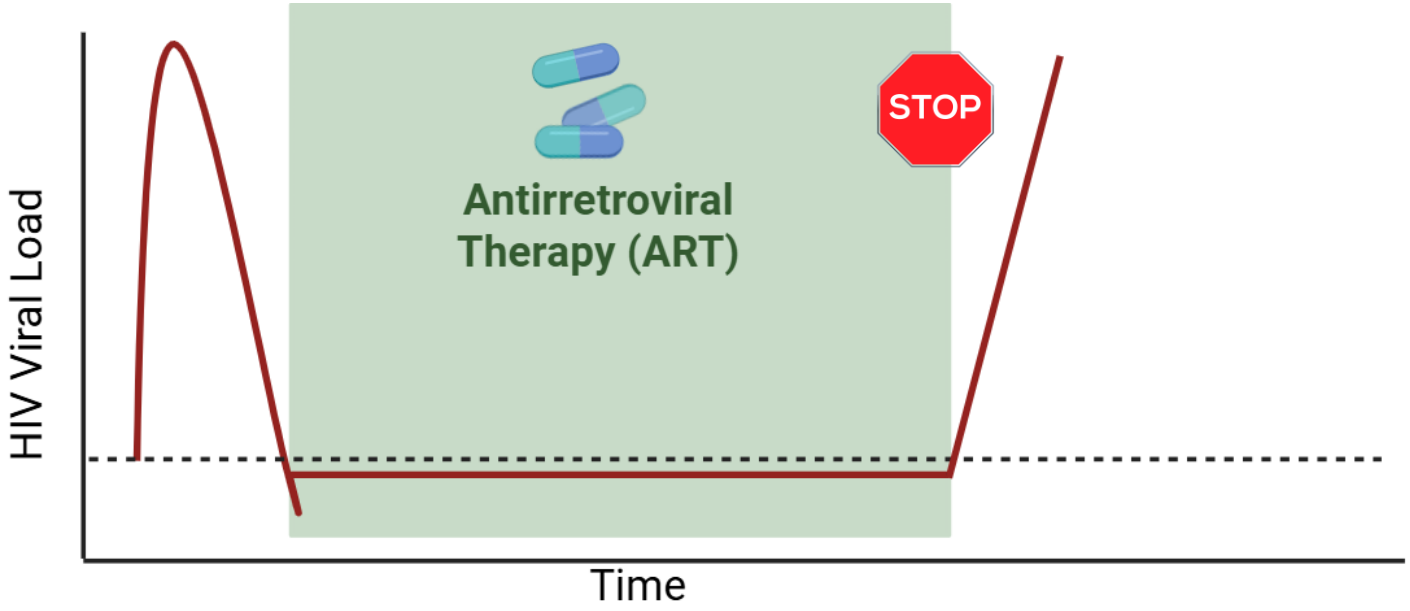
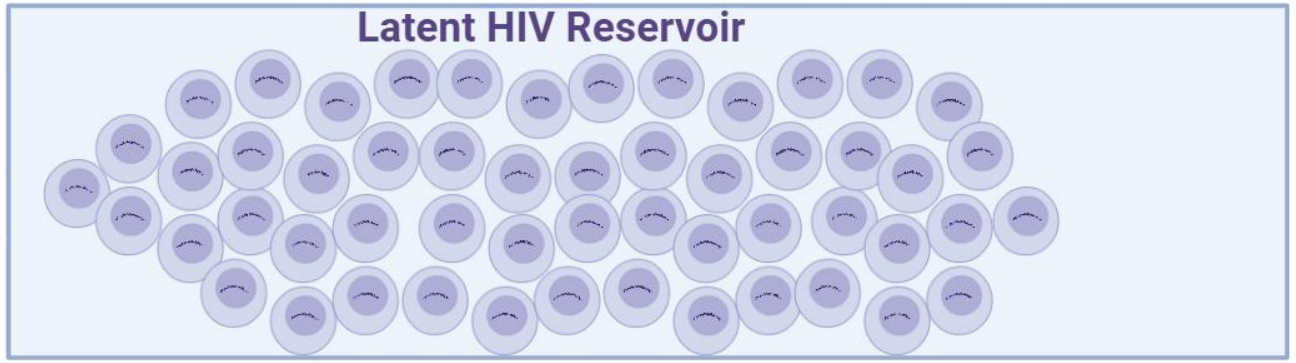


CD4⁺ T cells



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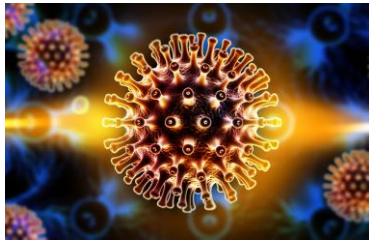

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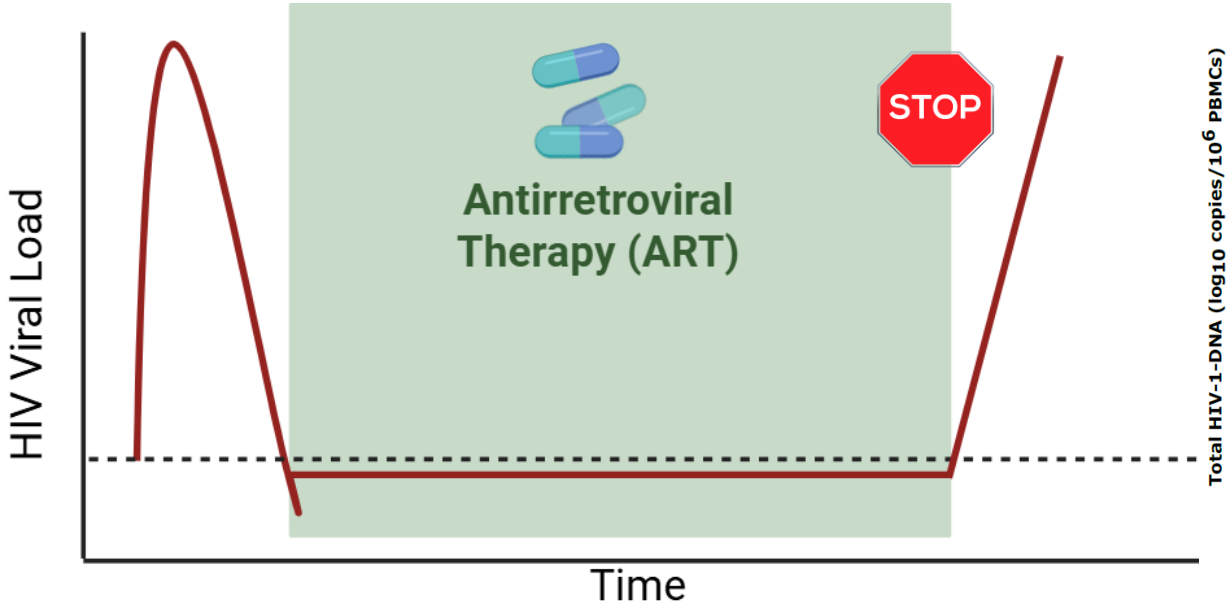
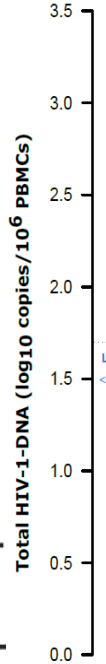
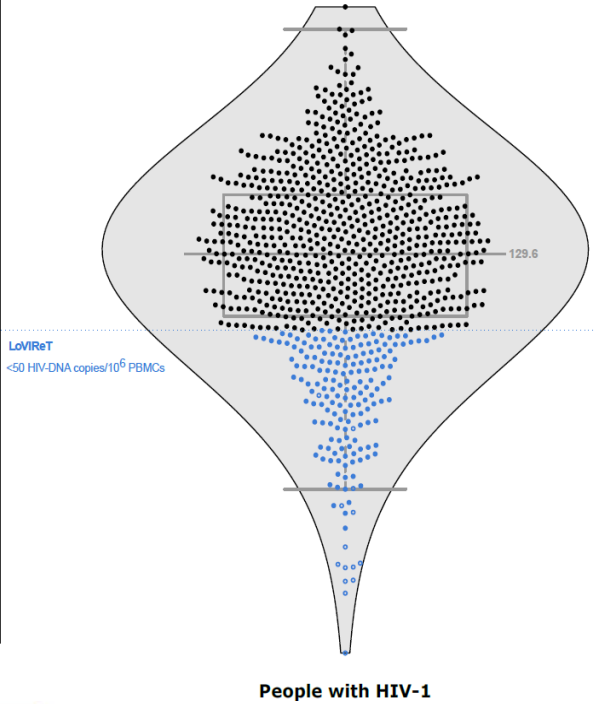
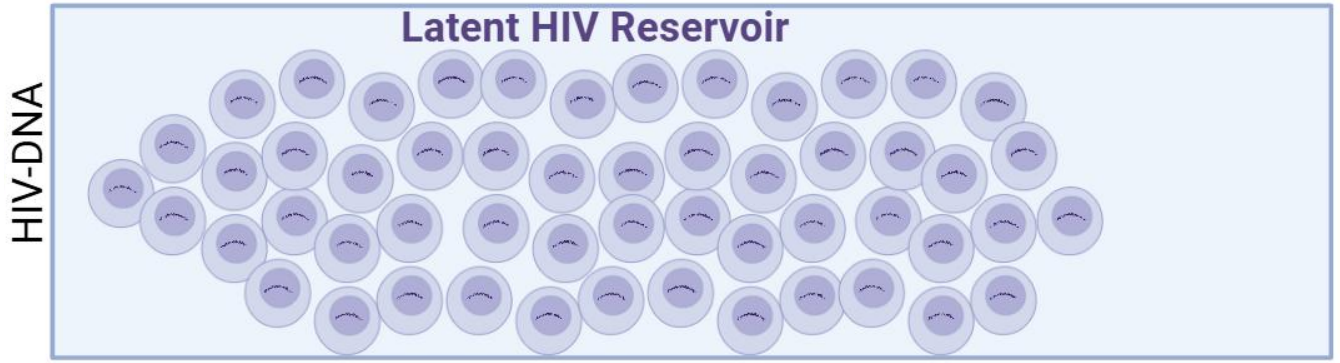






González-Navarro et al, 2024, JCI

CD4⁺ T cells



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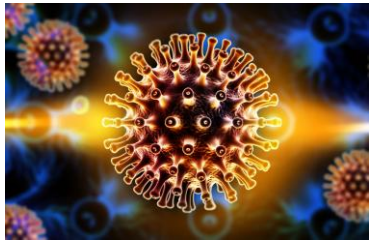

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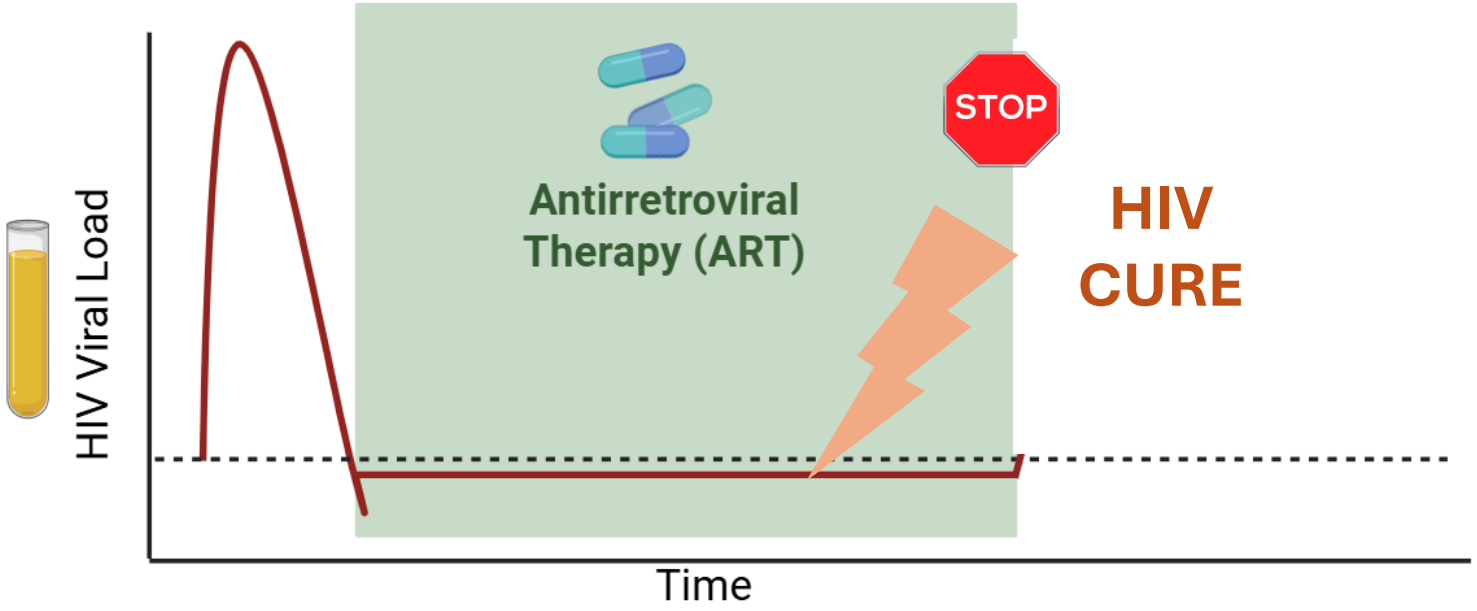
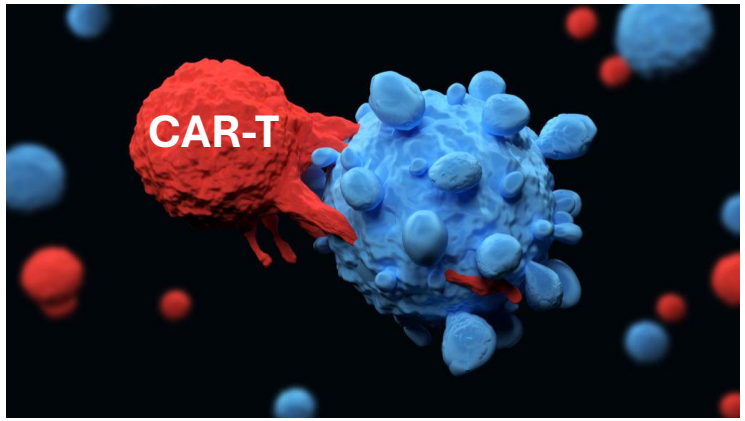
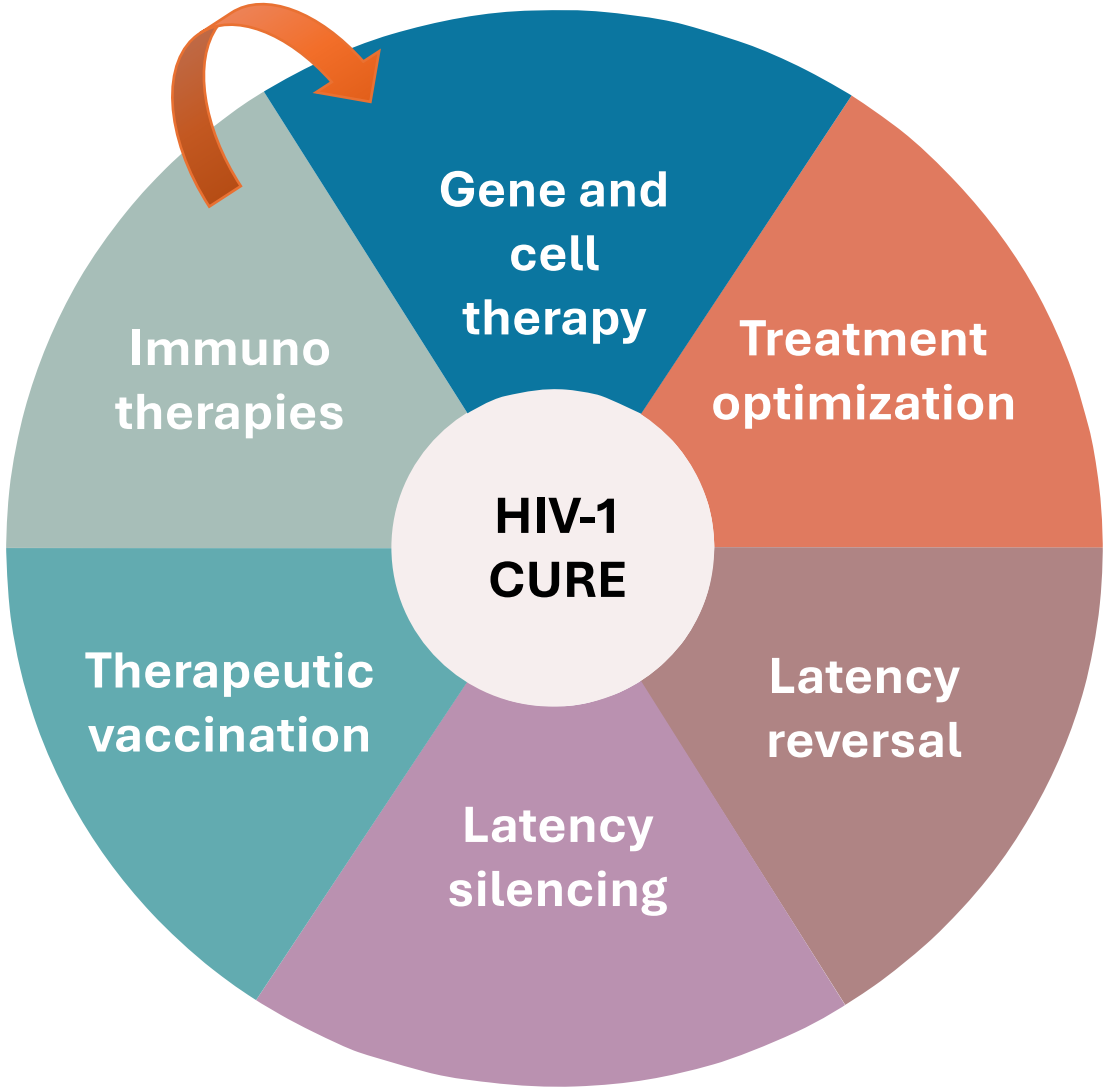


CD4⁺ T cells

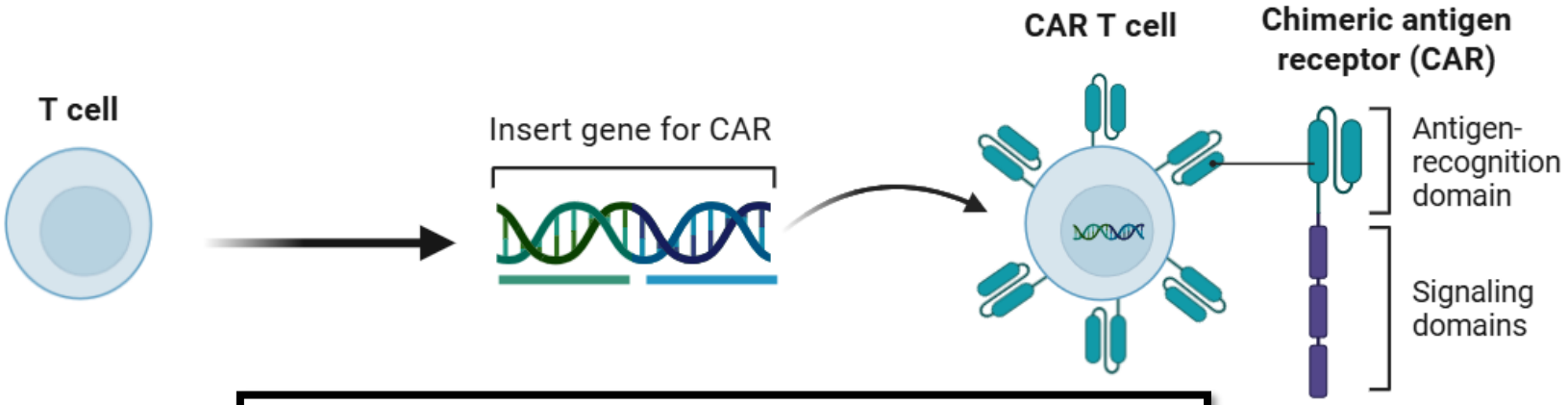
HIV-1 cure strategies

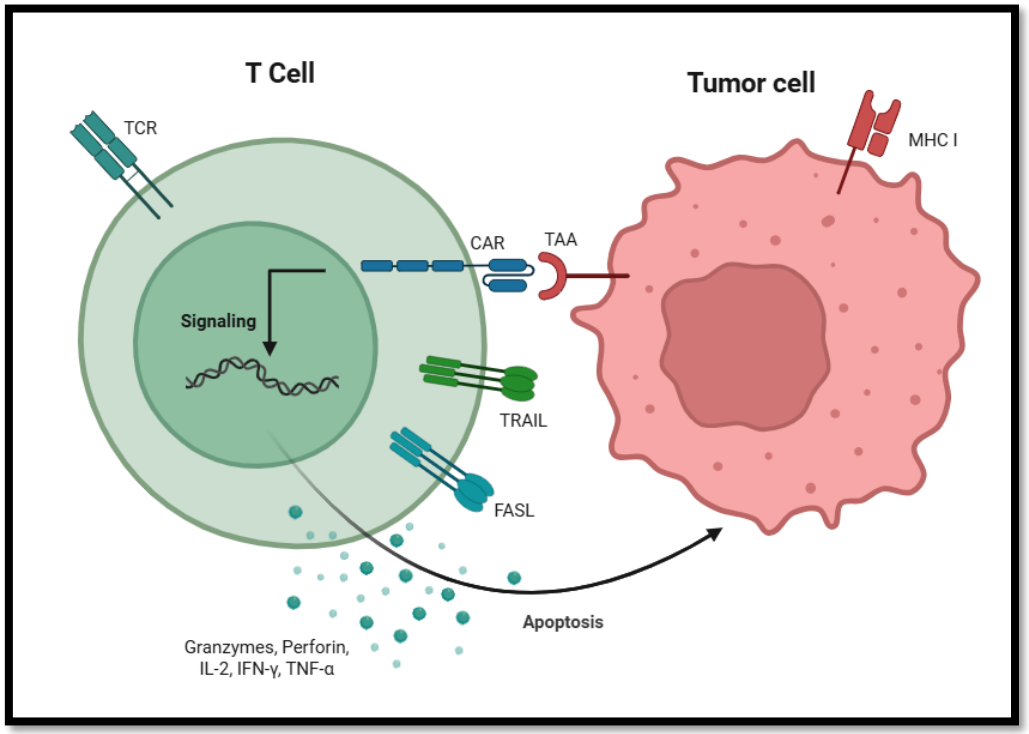


Science News, Jan 17th 2020



What is the Chimeric antigen receptor (CAR)-T cell therapy?





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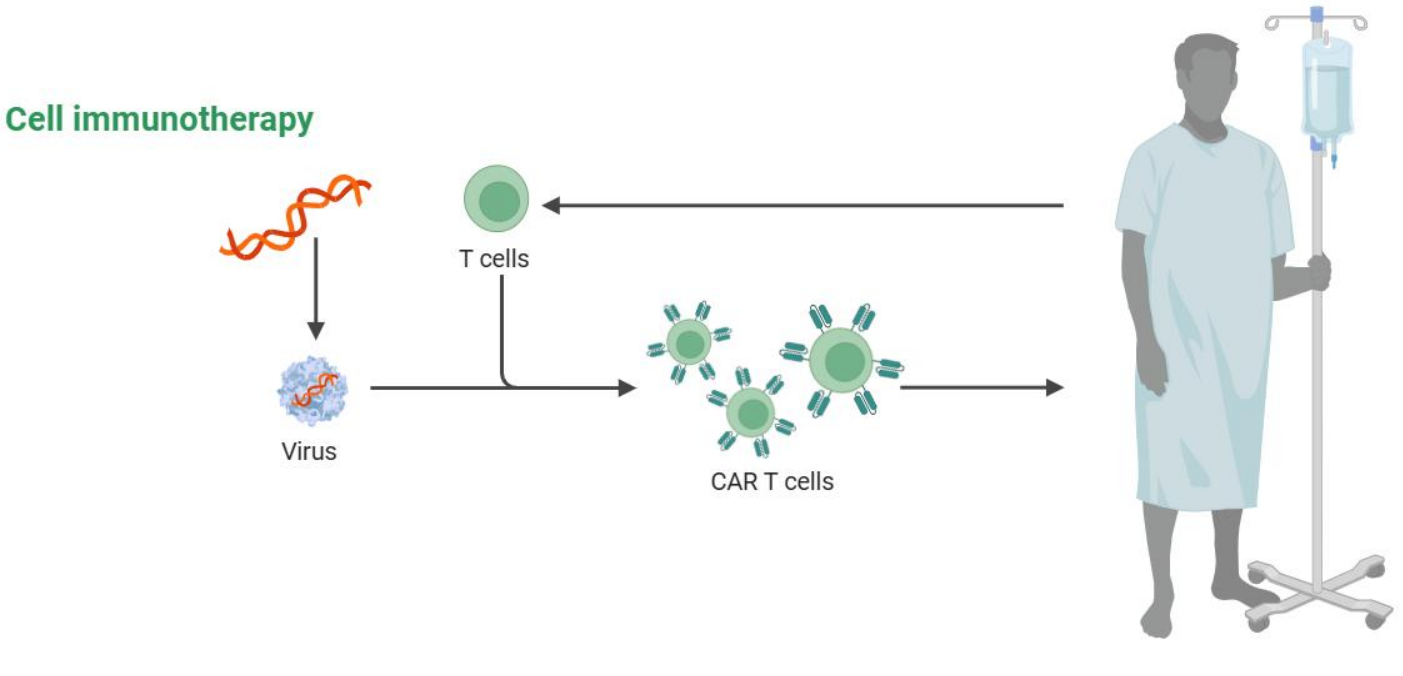
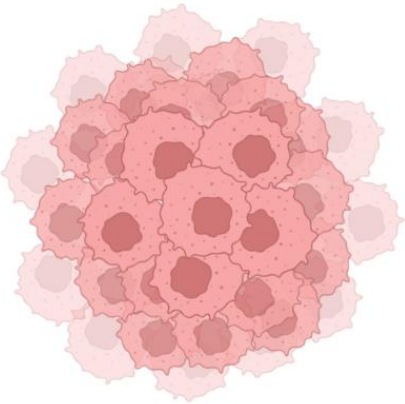
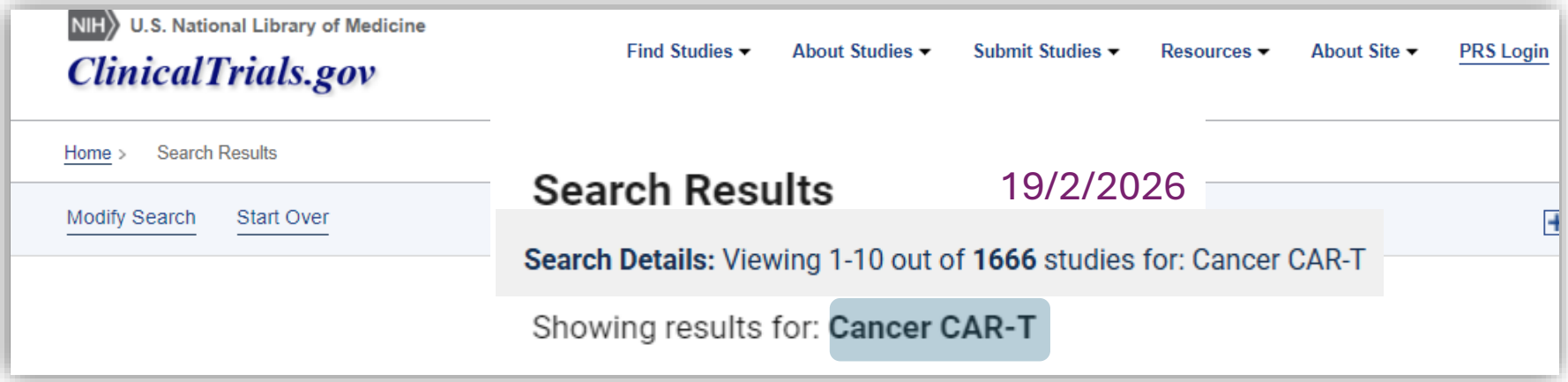
How is the use of CAR-T cells in the clinic?







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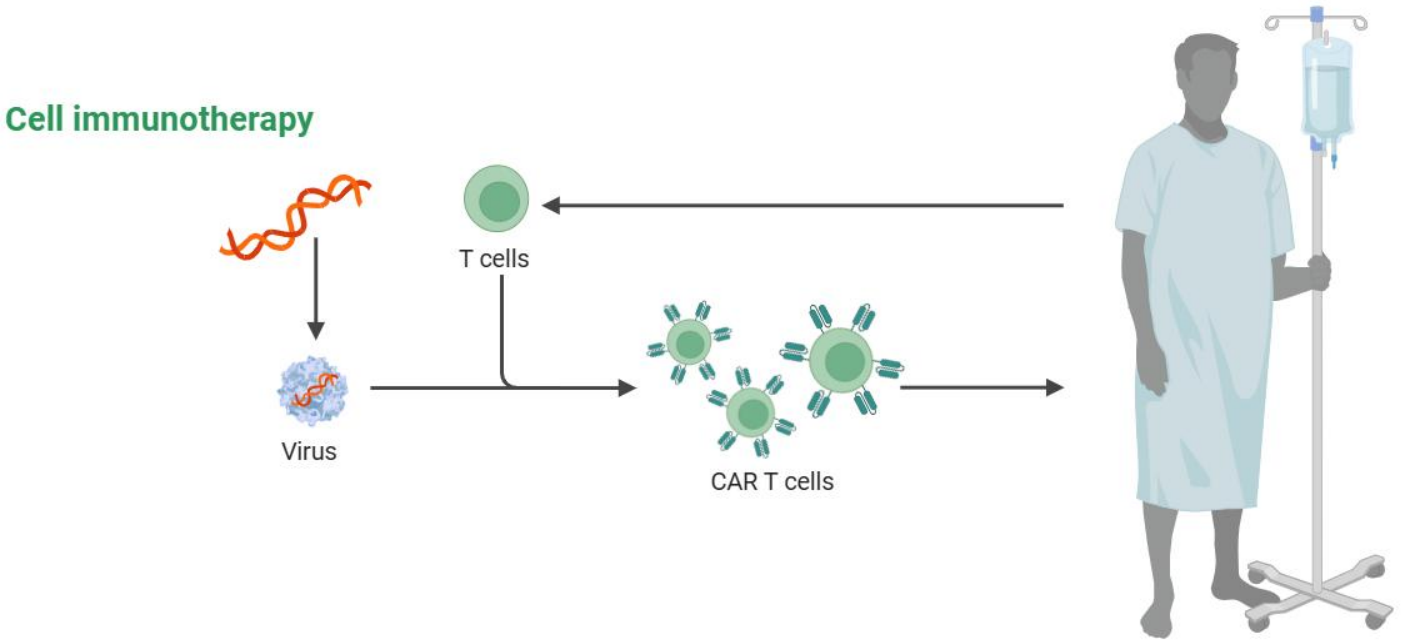

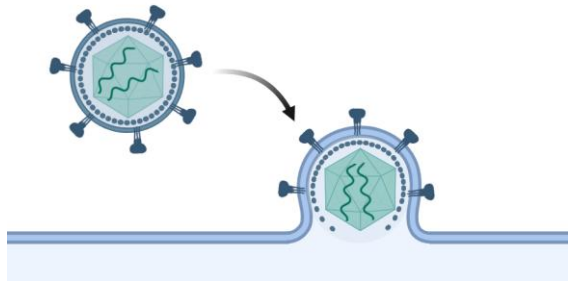

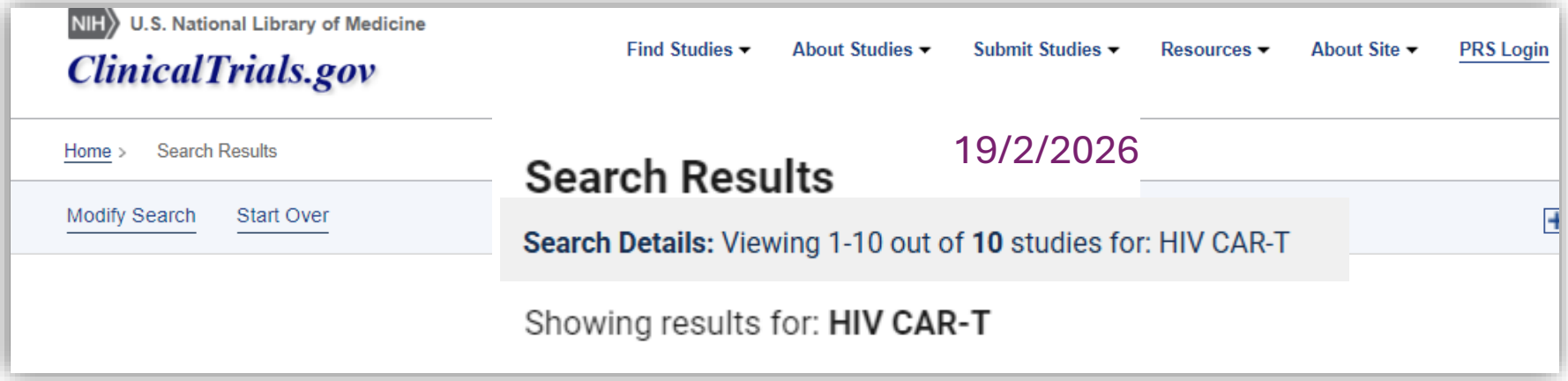

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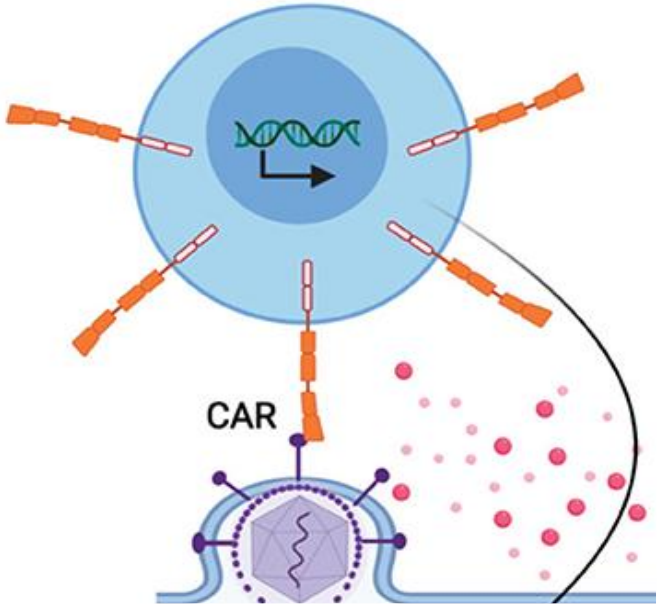
CAR-T cell strategies in HIV started in 90's

anti-HIV CAR-modified T Cells

CD4-receptor

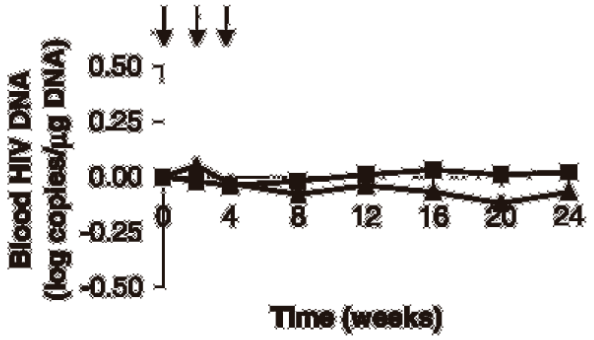


Mu *et al*, Front. Cell. Infect. Microbiol., 2020

MOLECULAR THERAPY Vol. 5, No. 6, June 2002

A Phase II Randomized Study of HIV-Specific T-Cell Gene Therapy in Subjects with Undetectable Plasma Viremia on Combination Antiretroviral Therapy

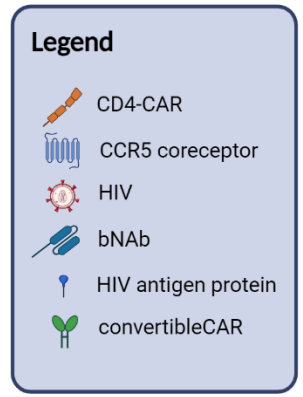
Steven G. Deeks,[1] Bridget Wagner,[2] Peter A. Anton,[3] Ronald T. Mitsuyasu,[3] David T. Scadden,[4] Christine Huang,[5] Catherine Macken,[6] Douglas D. Richman,[7] Cindy Christopherson,[8] Carl H. June,[9] Richard Lazar,[10] David F. Broad,[10] Sayeh Jalali,[10] and Kristen M. Hege[10,*]



No significant between-group differences were noted in viral reservoirs following therapy

Evolution in the use of CAR-T cells in HIV

CAR-T cell limitations | **New Approaches**

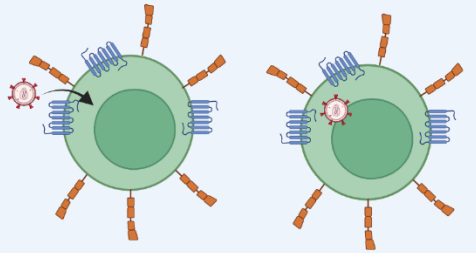


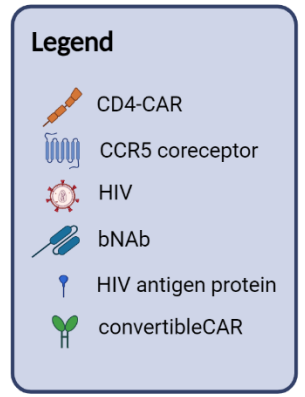
Campos-González et al, Viruses 2023

Evolution in the use of CAR-T cells in HIV

CAR-T cell limitations | **New Approaches**

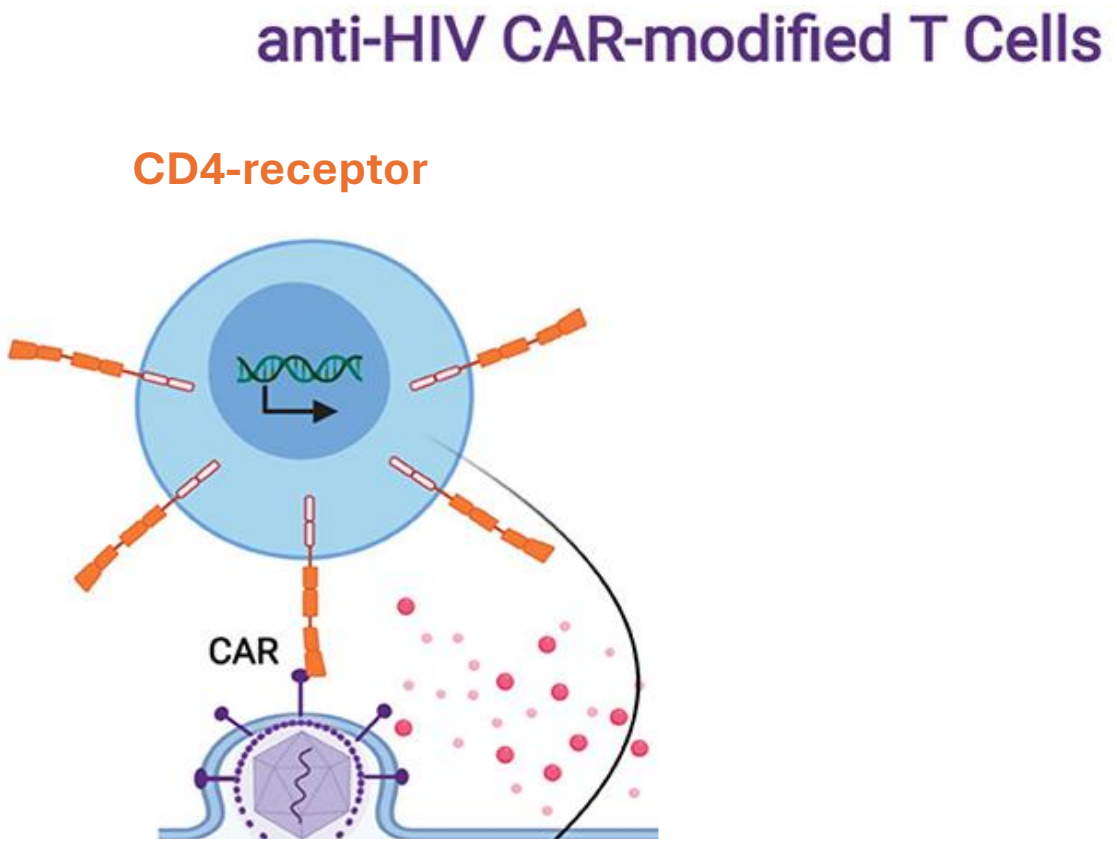
A. CAR-T cells infection





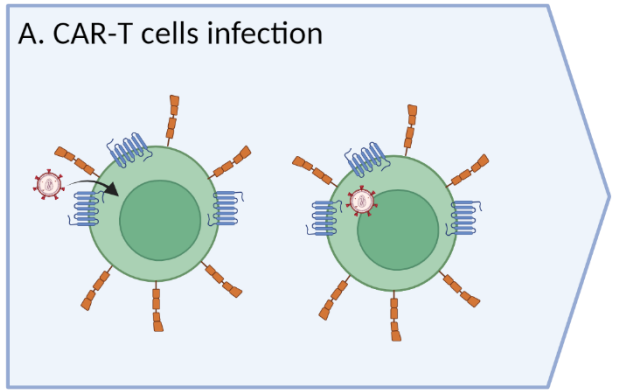
Campos-González et al, Viruses 2023

CD4 receptor can allow HIV infection



Mu *et al*, Front. Cell. Infect. Microbiol., 13 August 2020

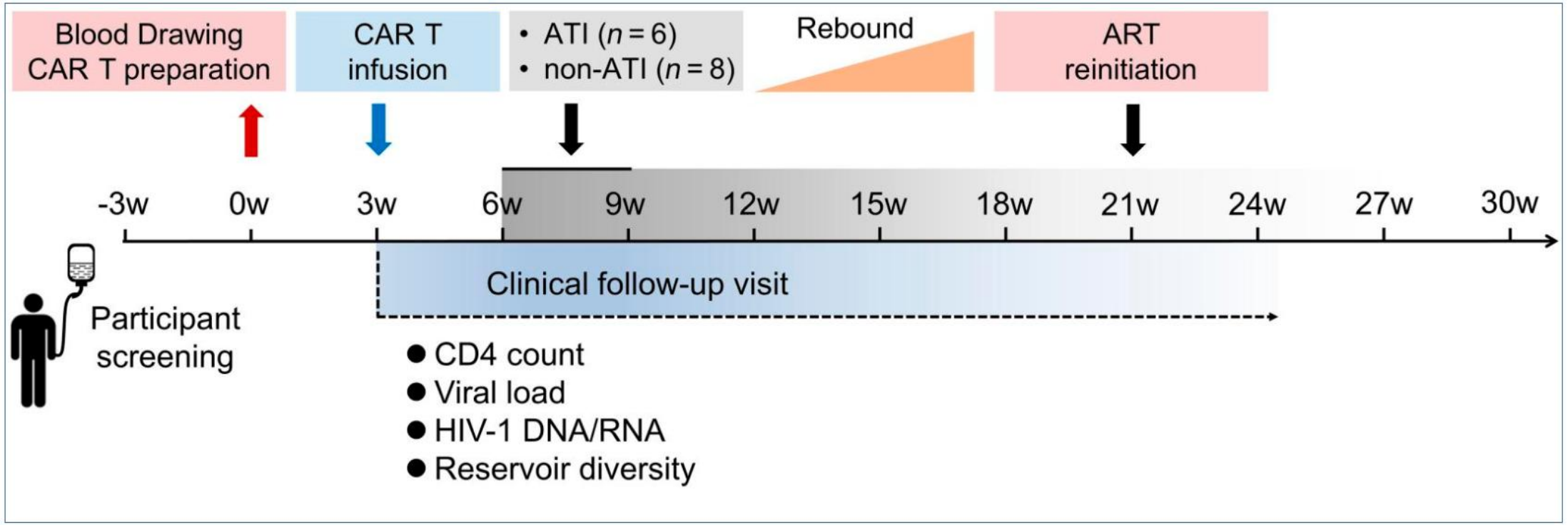
bnAbs as antibody to build the CAR-T

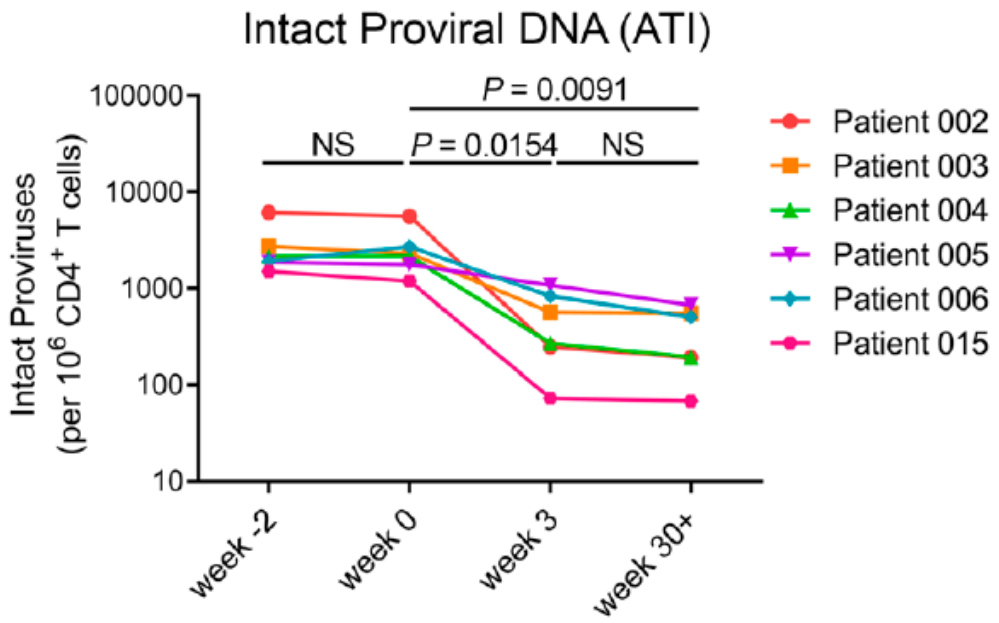


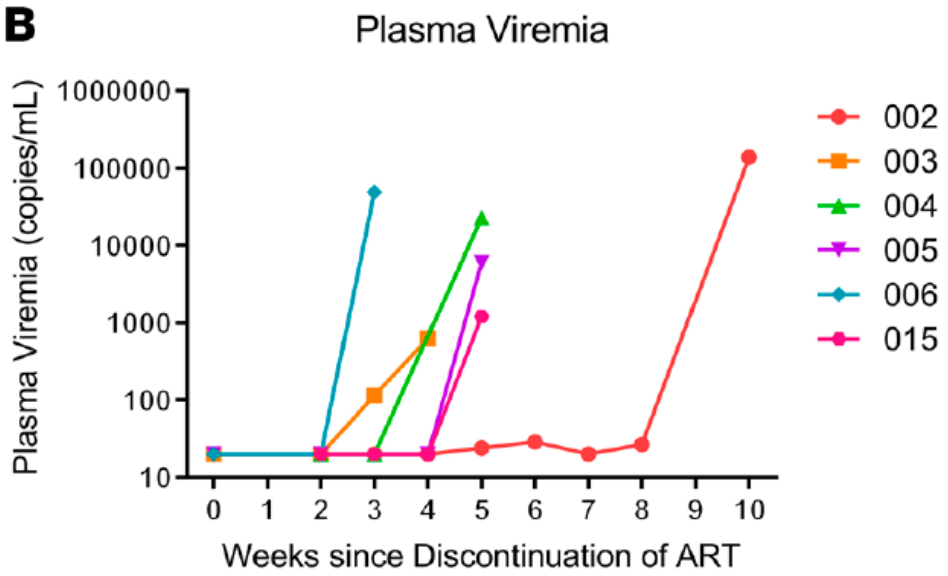
The Journal of Clinical Investigation Oct. 2021 CLINICAL MEDICINE

Broadly neutralizing antibody–derived CAR T cells reduce viral reservoir in individuals infected with HIV-1

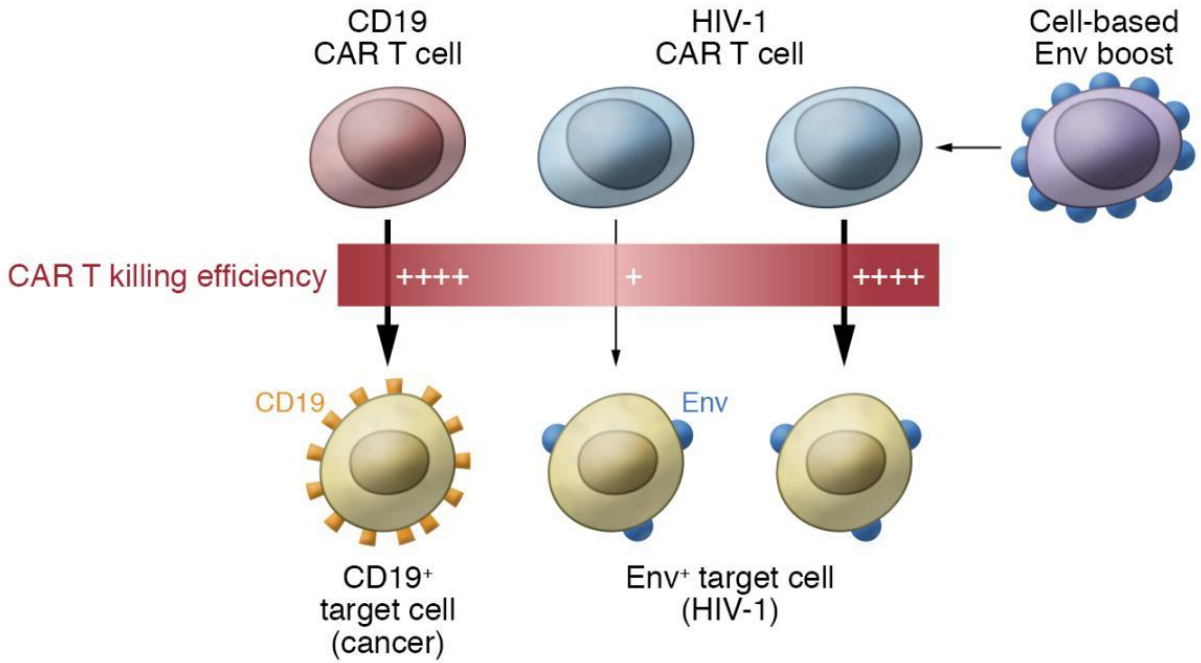
Bingfeng Liu,[1,2] Wanying Zhang,[1] Baijin Xia,[1] Shuliang Jing,[1] Yingying Du,[1] Fan Zou,[1,3] Rong Li,[1] Lijuan Lu,[1] Shaozhen Chen,[2] Yonghong Li,[2] Qifei Hu,[3] Yingtong Lin,[1] Yiwen Zhang,[1] Zhangping He,[1] Xu Zhang,[1] Xiejie Chen,[2] Tao Peng,[4] Xiaoping Tang,[2] Weiping Cai,[2] Ting Pan,[1] Linghua Li,[2] and Hui Zhang[1]







- bNAbs CD8+ CAR-T cells were safe and last for more than 30 weeks
- bNAbs CD8+ CAR-T cells were able to reduce caRNA and intact proviruses
- bNAbs CD8+ CAR-T cells did not prevent of viral rebound or bNAbs resistance



Lui et al, JCI, 2021

Integration of the CAR construct in the CCR5 gene

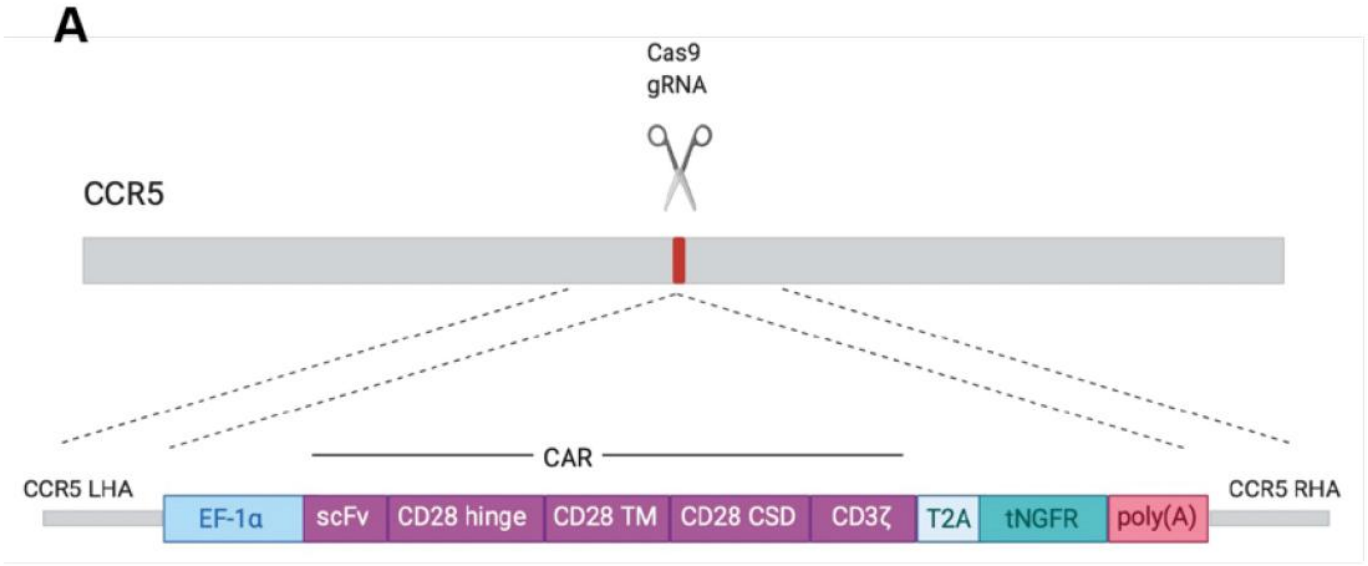
viruses

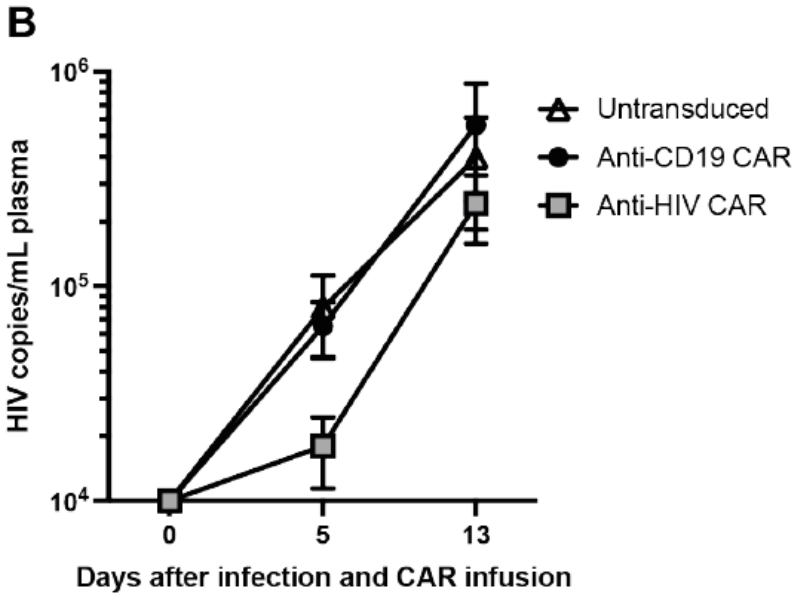
MDPI

Article

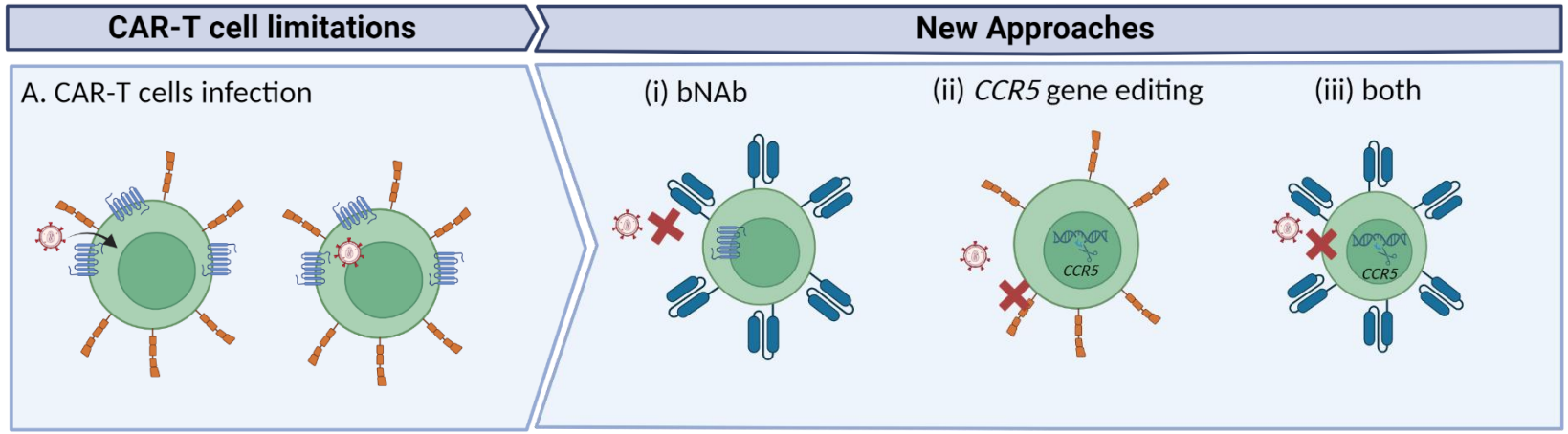
Development of HIV-Resistant CAR T Cells by CRISPR/Cas-Mediated CAR Integration into the *CCR5* Locus

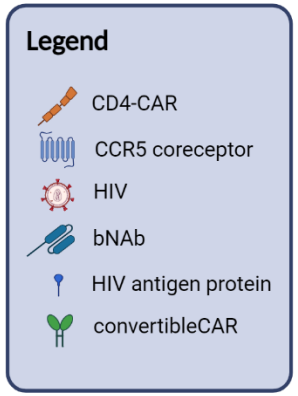
Frederik Holm Rothemejer [1,2], Nanna Pi Lauritsen [1,2], Anna Karina Juhl [1,2], Mariane Høgsbjerg Schleimann [1,2], Saskia König [3], Ole Schmeltz Søgaard [1,2], Rasmus O. Bak [3,4] and Martin Tolstrup [1,2,*]





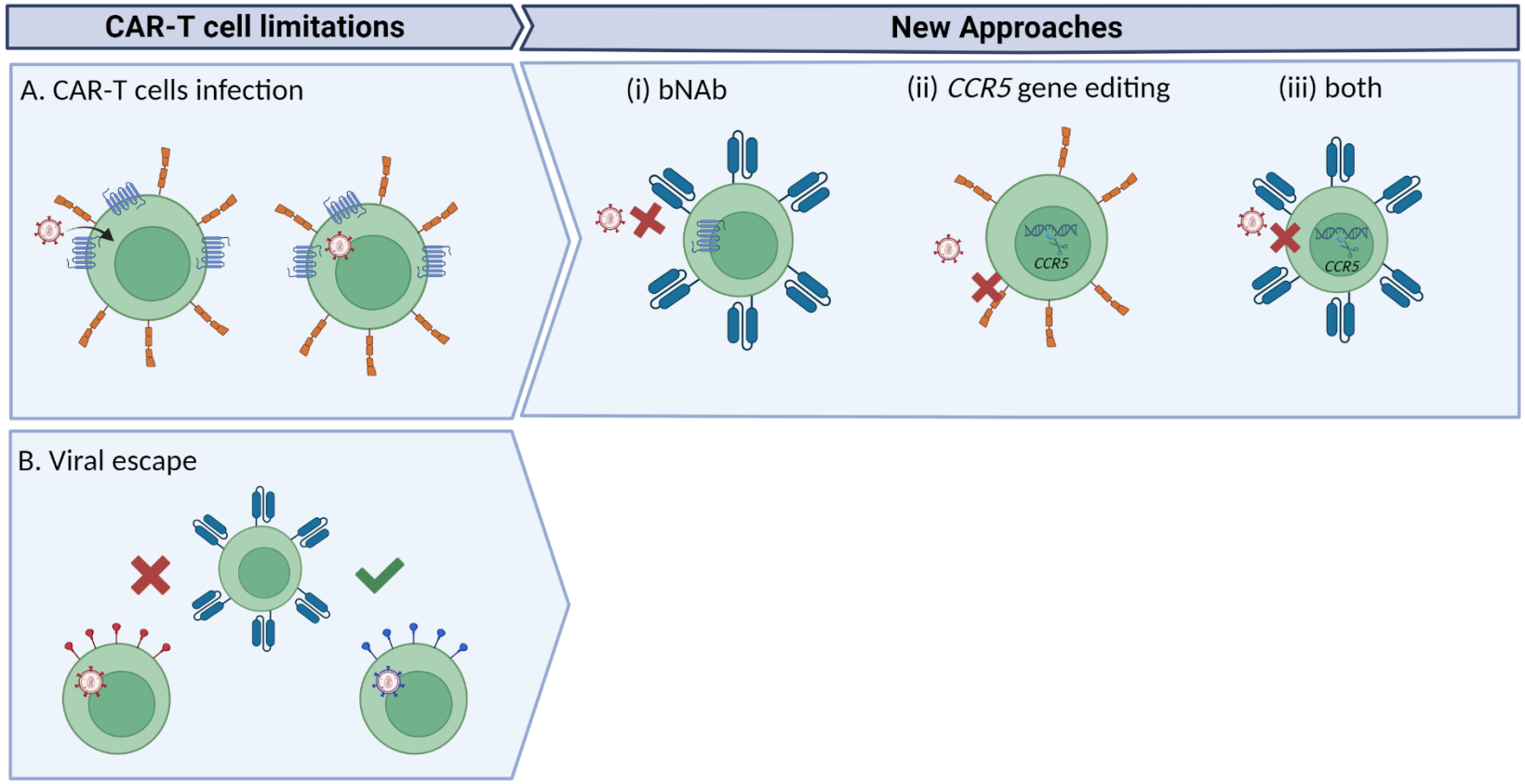
Evolution in the use of CAR-T cells in HIV



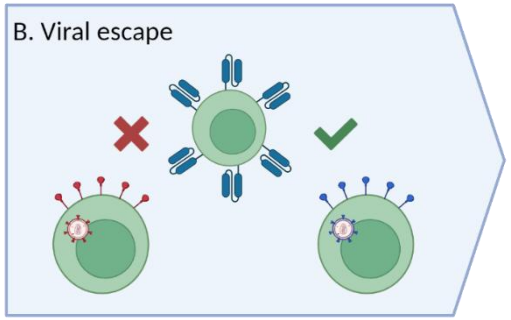


Campos-González et al, Viruses 2023

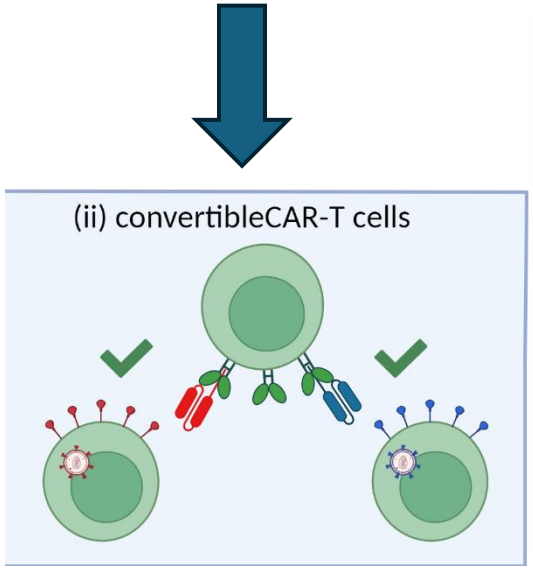
Evolution in the use of CAR-T cells in HIV



Campos-González et al, Viruses 2023



To avoid escape from bnAbs: ConvertibleCAR



Cell. 2019 October 31; 179(4): 880–894.e10. doi:10.1016/j.cell.2019.10.002.

Attacking Latent HIV with *convertible*CAR-T Cells, a Highly Adaptable Killing Platform

Eytan Herzig[1,2], Kaman Chan Kim[3], Thomas A. Packard[1,2], Noam Vardi[4,5], Roland Schwarzer[1,2], Andrea Gramatica[1,2], Steven G. Deeks[6], Steven R. Williams[3], Kyle Landgraf[3], Nigel Killeen[3], David W. Martin[3], Leor S. Weinberger[1,4,5], Warner C. Greene[1,2,7,*]

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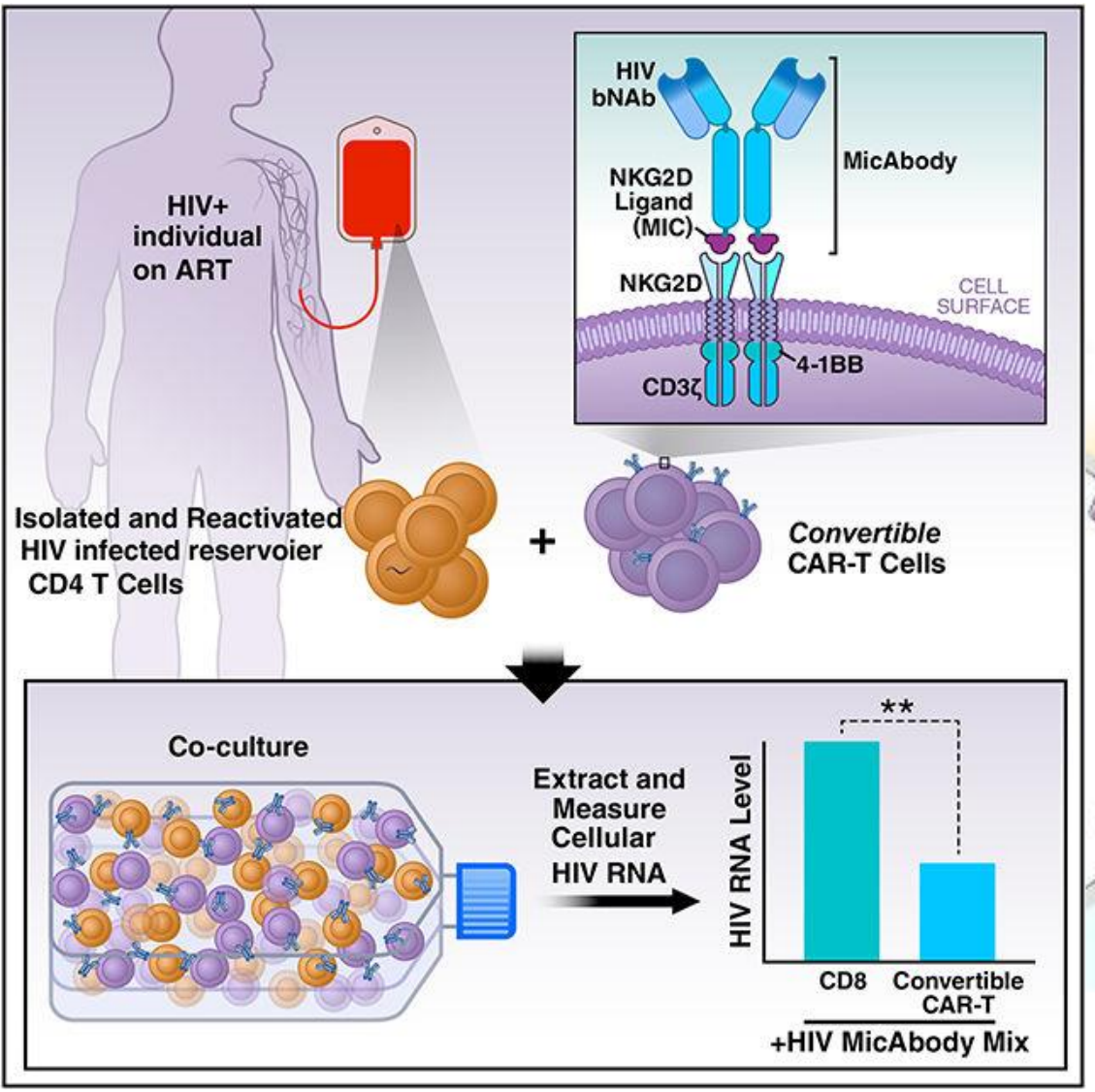
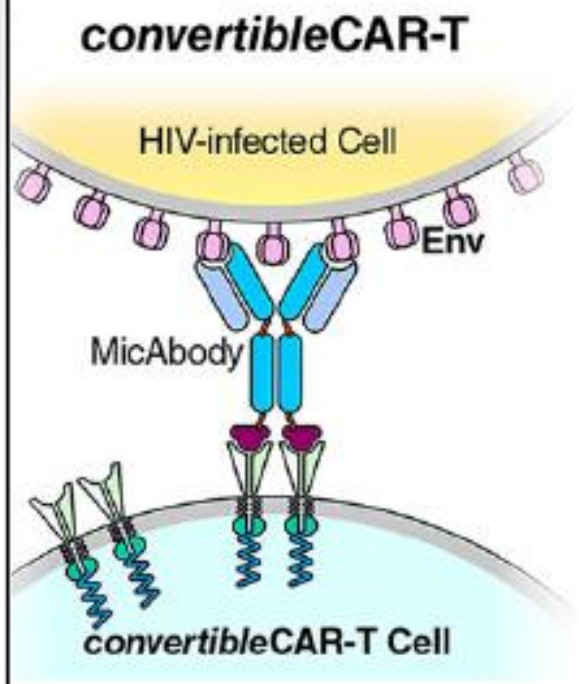
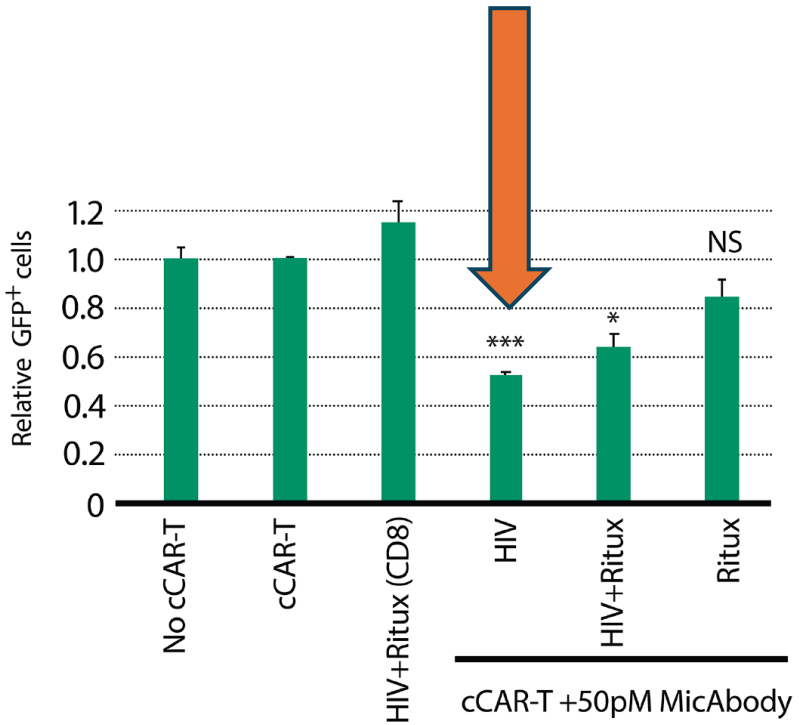
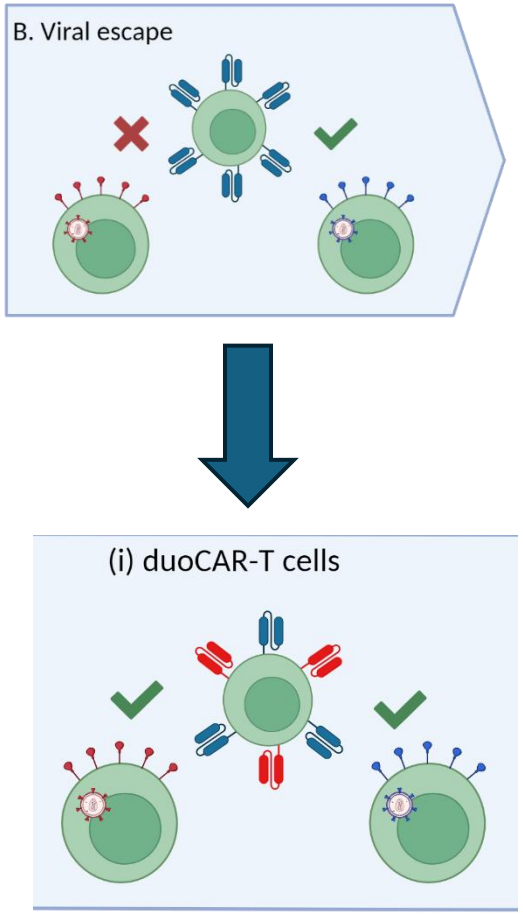








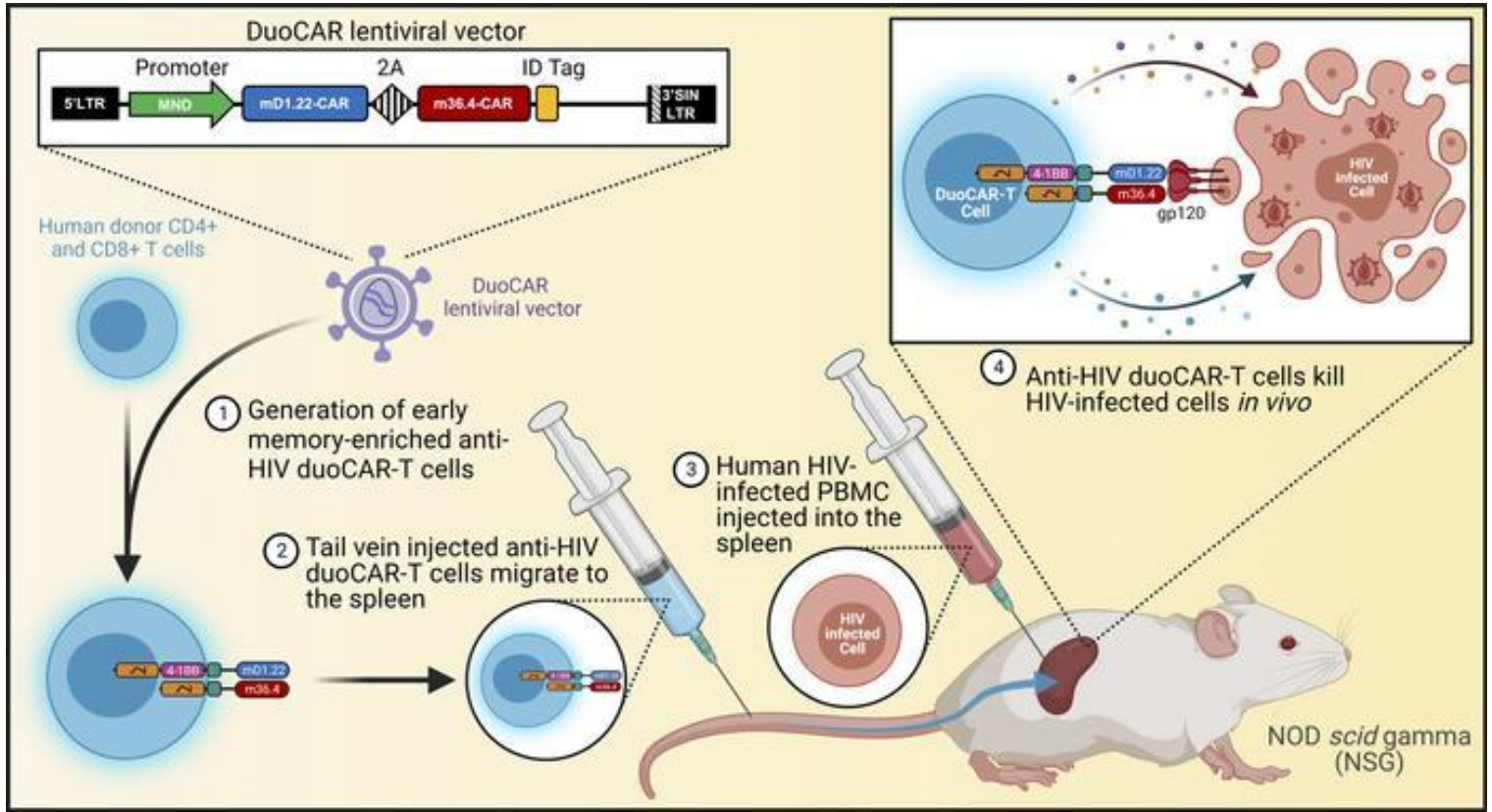
To avoid escape from bnAbs: DuoCAR

JCI insight

In vivo killing of primary HIV-infected cells by peripheral-injected early memory–enriched anti-HIV duoCAR T cells

Kim Anthony-Gonda, … , Harris Goldstein, Boro Dropulić

JCI Insight. 2022;7(21):e161698. https://doi.org/10.1172/jci.insight.161698.



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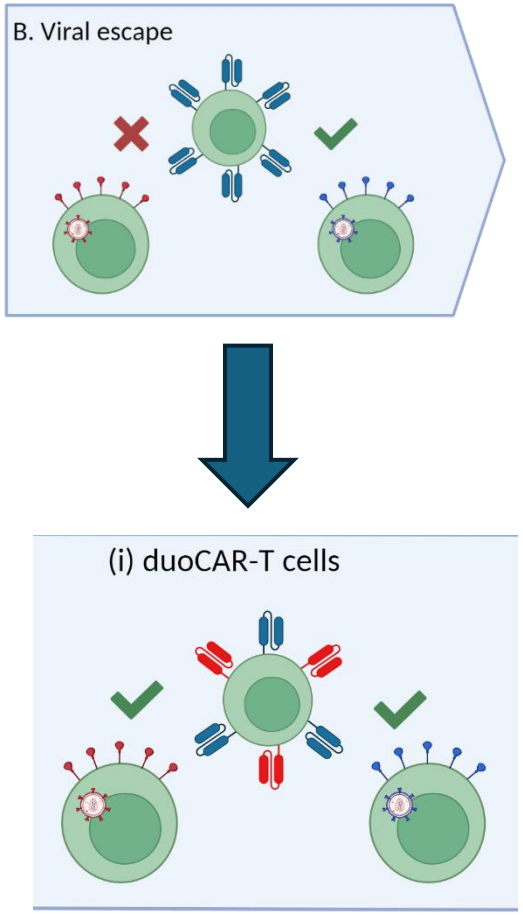

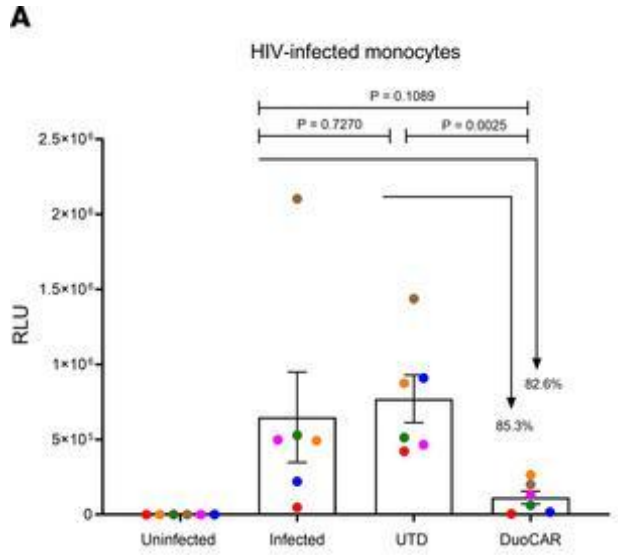

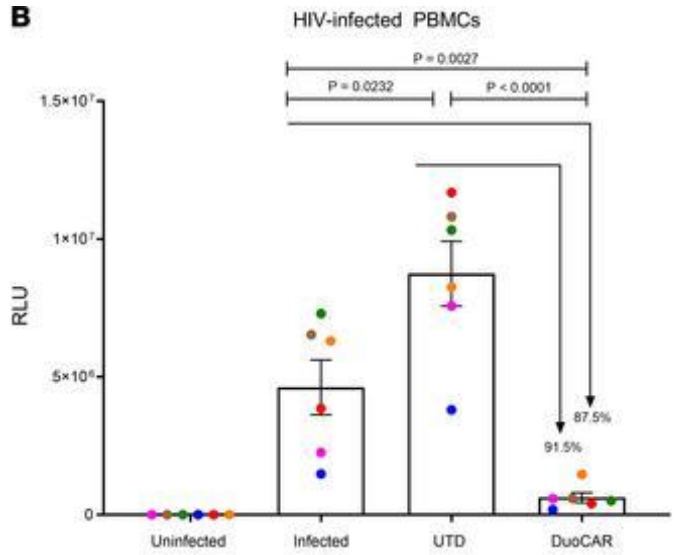

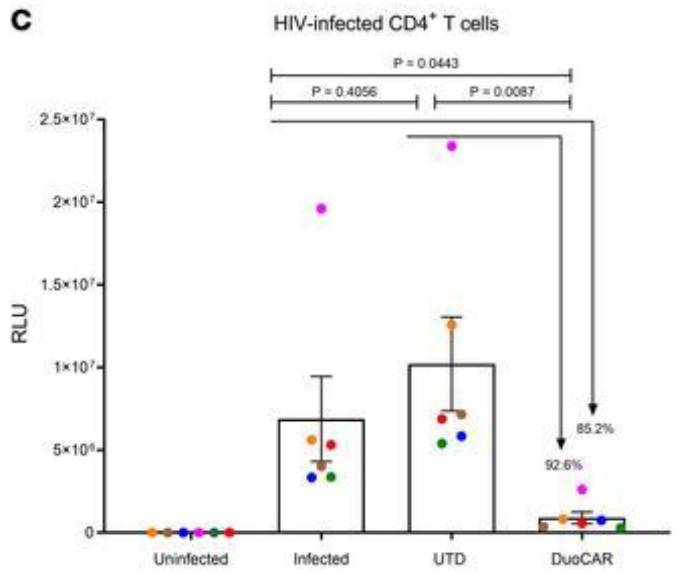

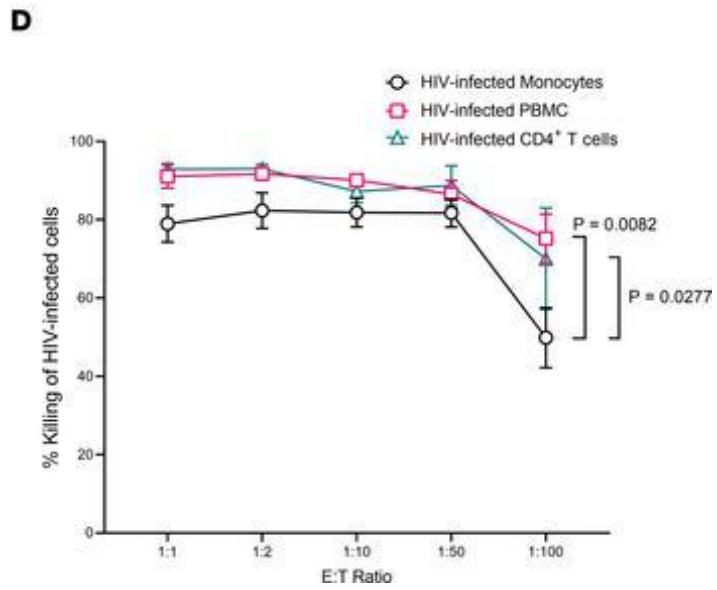

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RECRUITING

CAR-T Cells for HIV Infection

ClinicalTrials.gov ID NCT04648046

Sponsor Steven Deeks

Information provided by Steven Deeks, University of California, San Francisco (Responsible Party)

Last Update Posted 2023-05-30

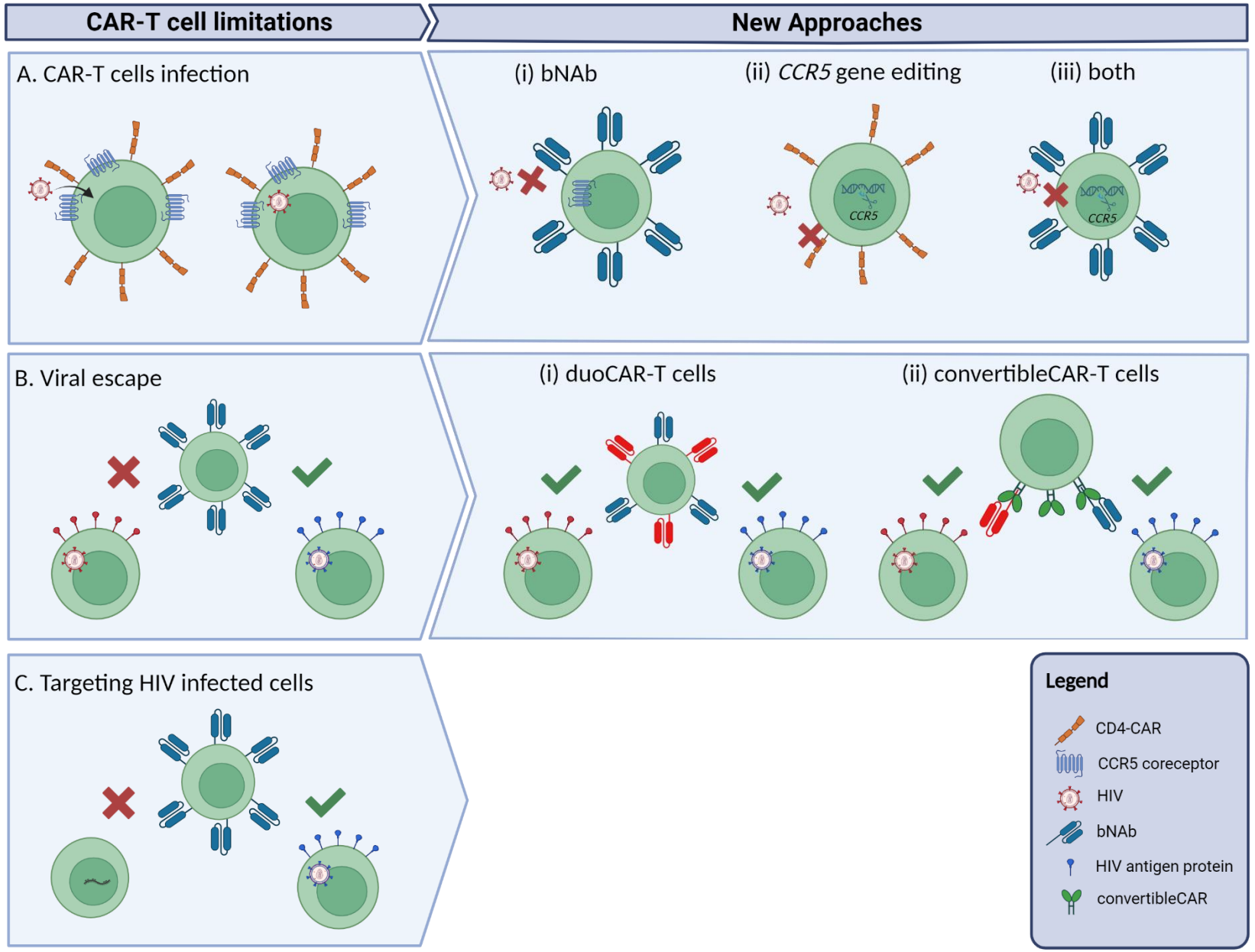
NEWS | 09 May 2023

First two patients receive CAR T cell therapy for HIV

Nature Medicine **explores the latest translational and clinical research news, with a phase 1/2 trial of a dual CAR T cell therapy that targets gp120.**

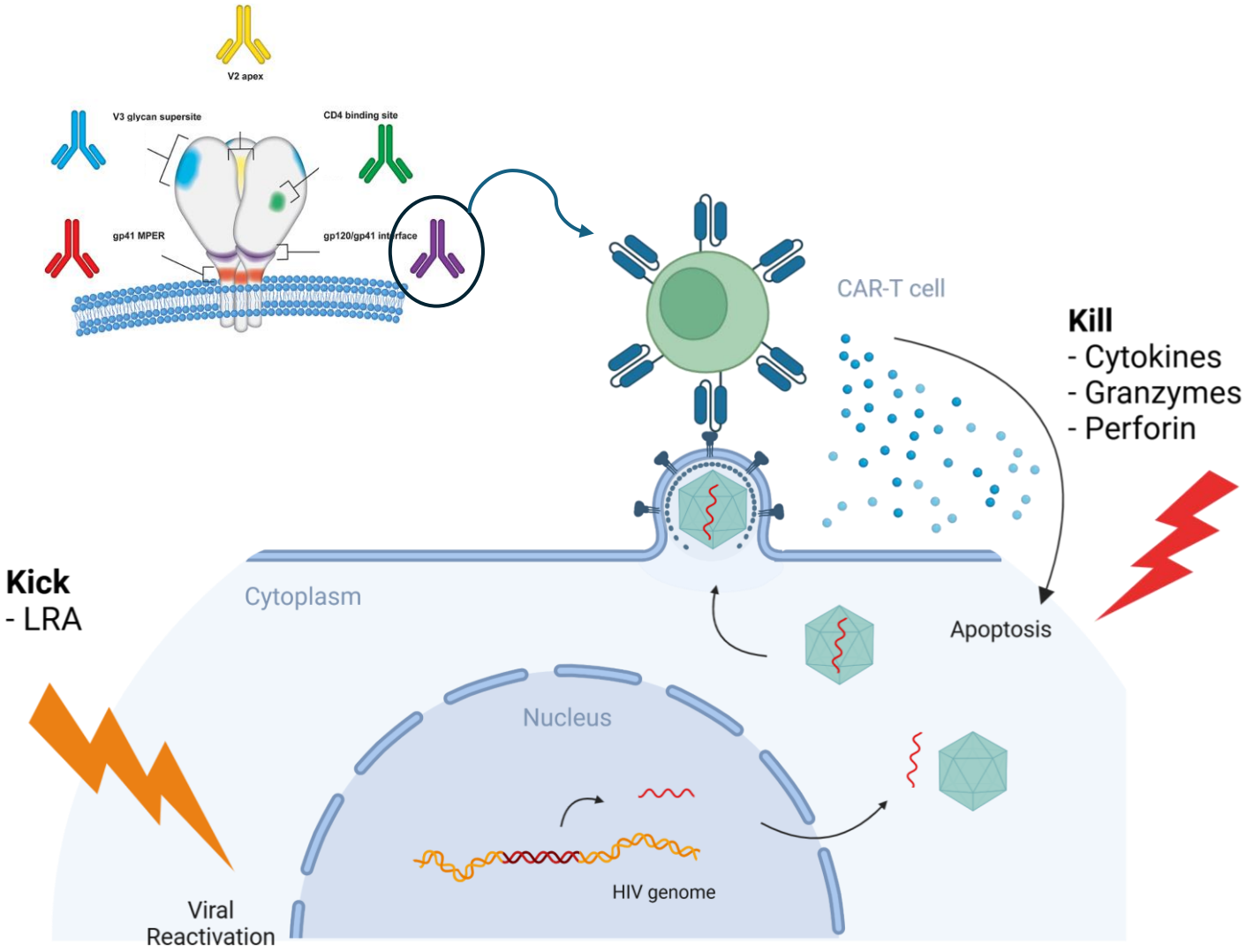
By Thiago Carvalho

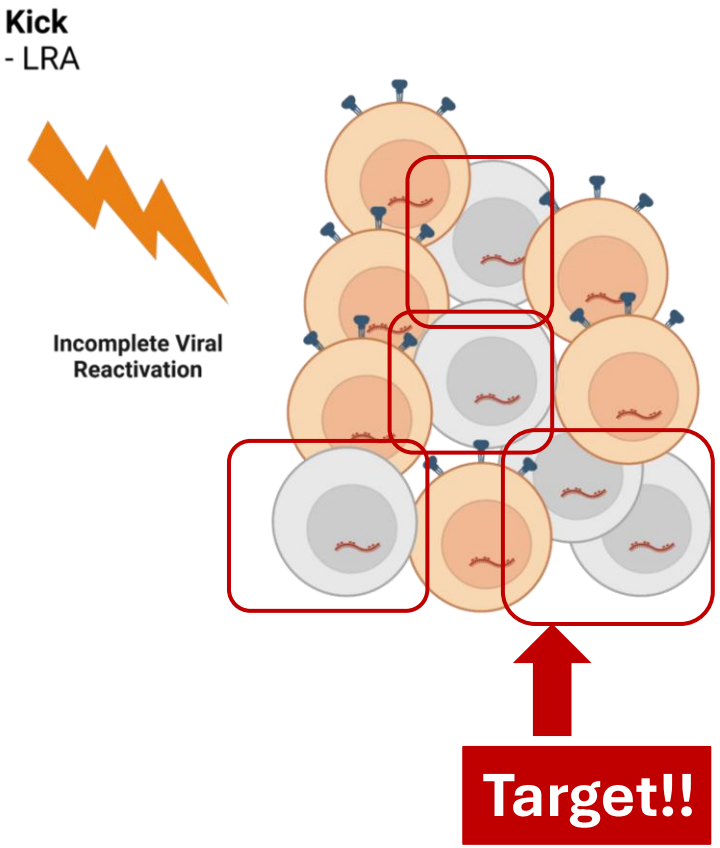
Evolution in the use of CAR-T cells in HIV



Campos-González et al, Viruses 2023

The challenge to target **ALL** latently infected cells





Adapted from: Mu et al. *Front Cell Infect Microbiol,* 2020
Adapted from: Spencer DA. et al. *Frontiers in Public Health*, 2021

The challenge to target **ALL** latently infected cells

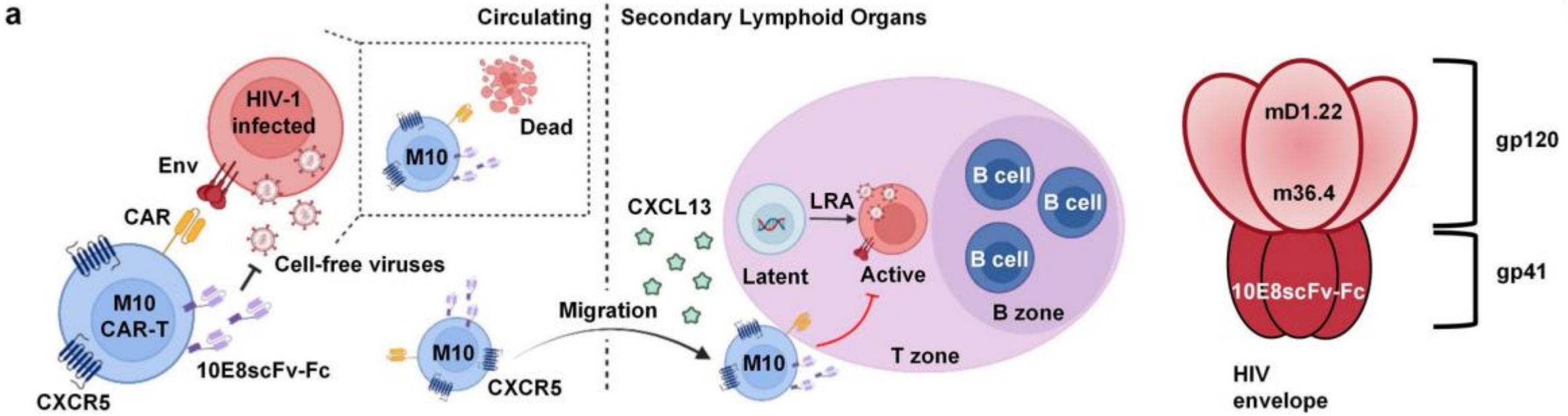
Mao et al. *Cell Discovery* (2024)10:49
https://doi.org/10.1038/s41421-024-00658-z

Cell Discovery
www.nature.com/celldisc

ARTICLE Open Access

Efficacy and safety of novel multifunctional M10 CAR-T cells in HIV-1-infected patients: a phase I, multicenter, single-arm, open-label study

Yunyu Mao[1], Qibin Liao[1], Youwei Zhu[1], Mingyuan Bi[2], Jun Zou[3], Nairong Zheng[1], Lingyan Zhu[1], Chen Zhao[1], Qing Liu[2], Li Liu[1], Jun Chen[1], Ling Gu[1], Zhuoqun Liu[1], Xinghao Pan[1], Ying Xue[1], Meiqi Feng[1], Tianlei Ying[1], Pingyu Zhou[4], Zhanshuai Wu[5], Jian Xiao[5], Renfang Zhang[1]✉, Jing Leng[5]✉, Yongtao Sun[2]✉, Xiaoyan Zhang[1]✉ and Jianqing Xu[1]✉



The challenge to target ALL latently infected cells

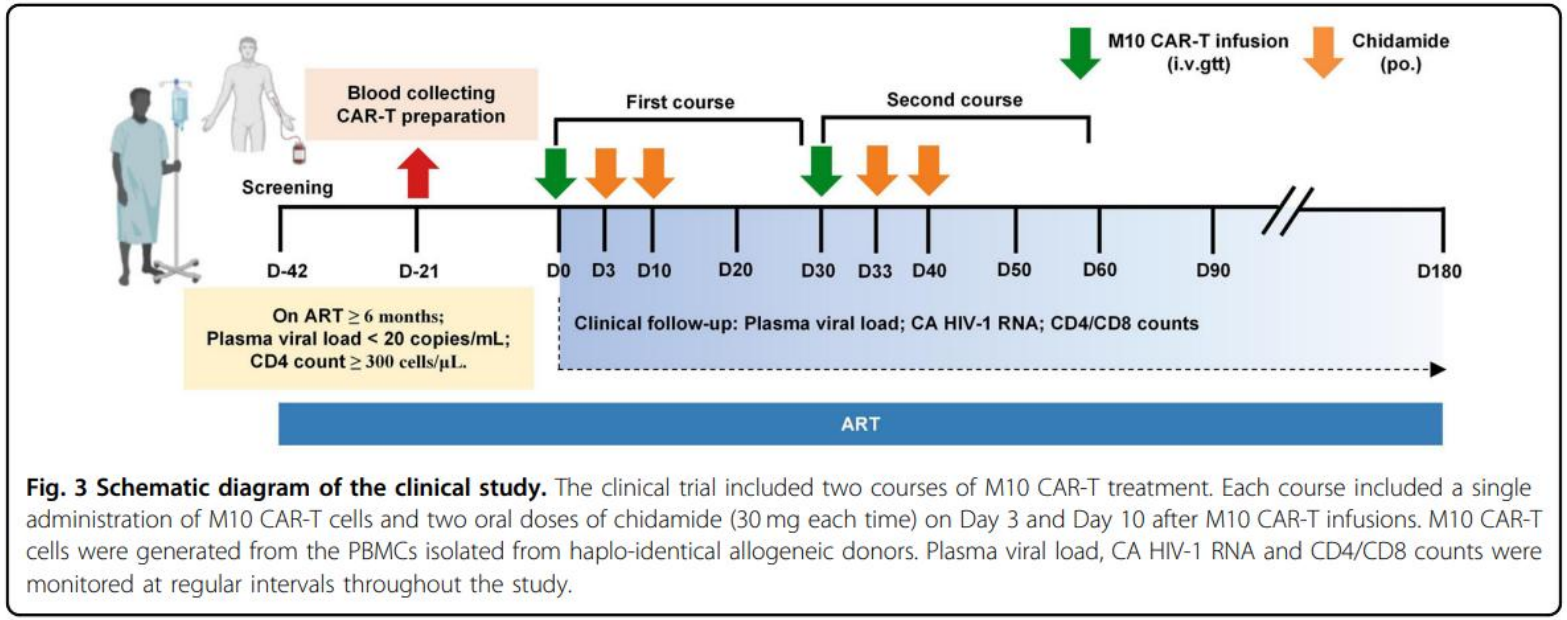
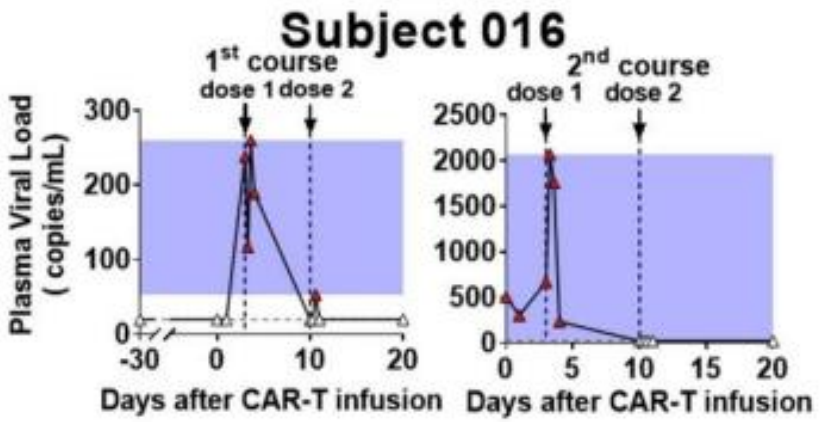
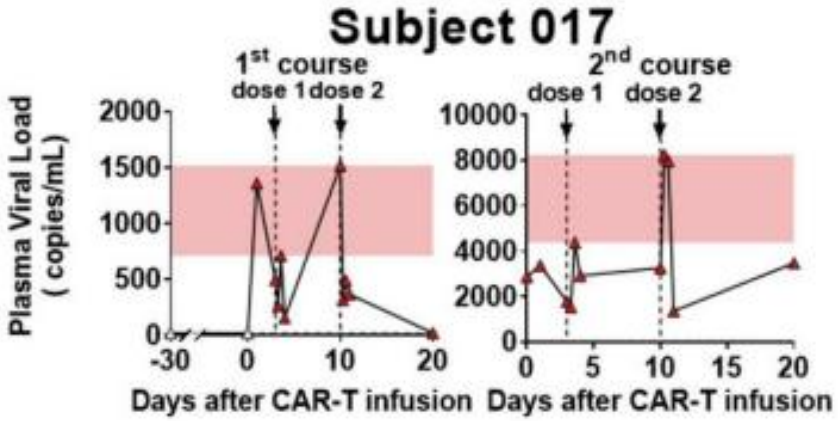


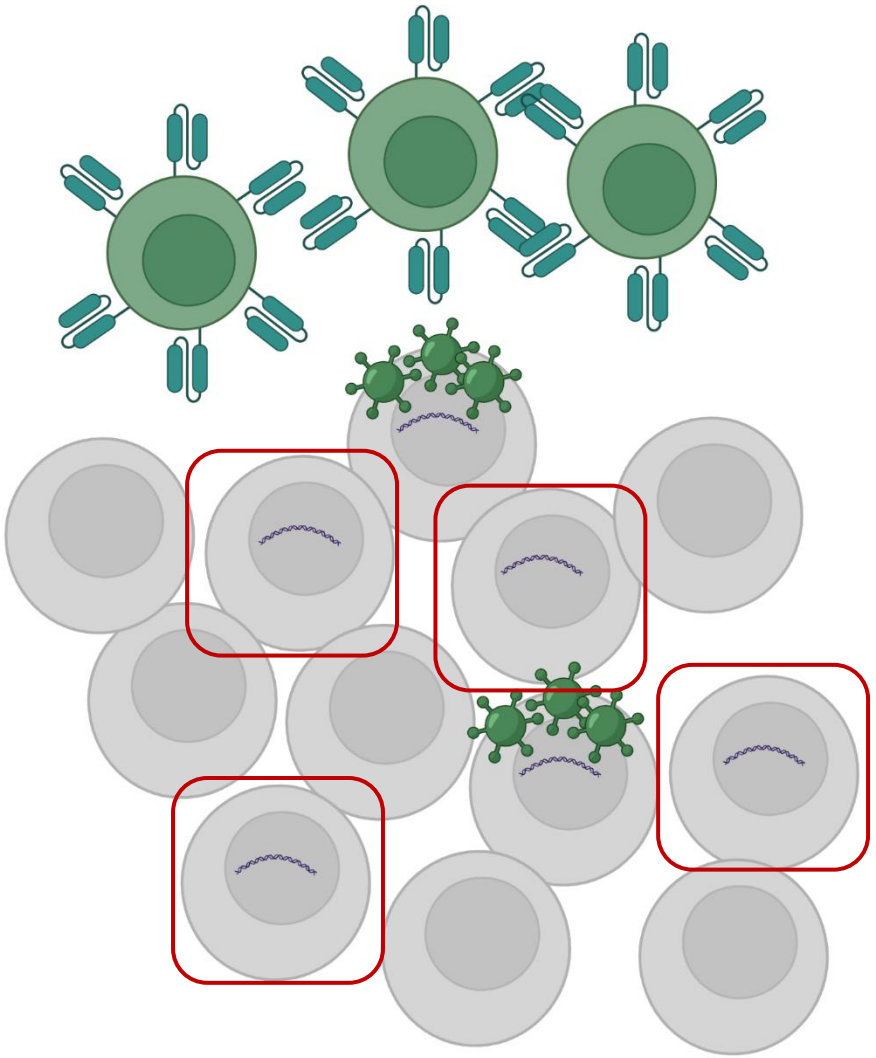
Fig. 3 Schematic diagram of the clinical study. The clinical trial included two courses of M10 CAR-T treatment. Each course included a single administration of M10 CAR-T cells and two oral doses of chidamide (30 mg each time) on Day 3 and Day 10 after M10 CAR-T infusions. M10 CAR-T cells were generated from the PBMCs isolated from haplo-identical allogeneic donors. Plasma viral load, CA HIV-1 RNA and CD4/CD8 counts were monitored at regular intervals throughout the study.





Mao et al. *Cell discovery,* 2024

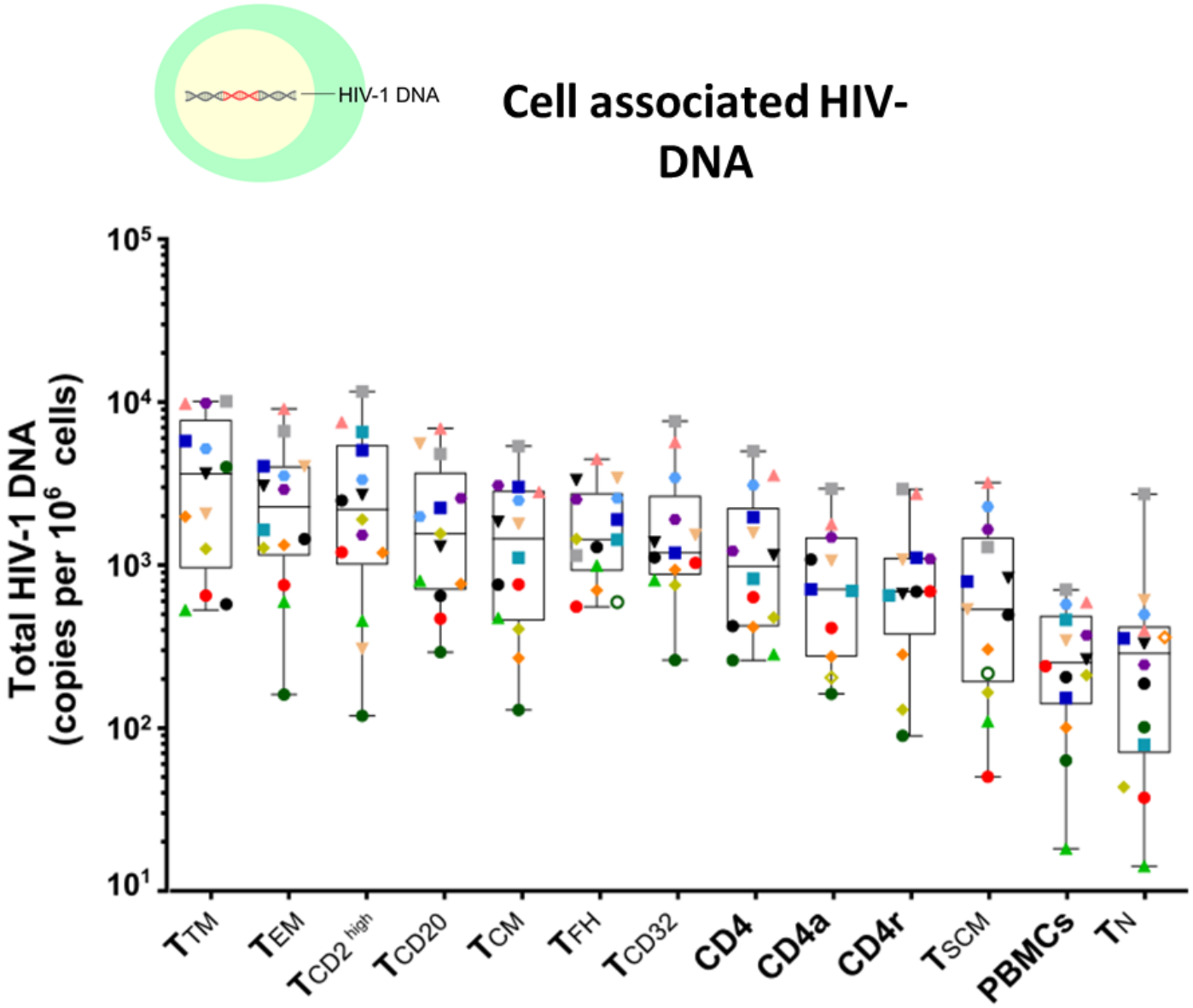
The challenge to target **ALL** latently infected cells



- Will be the LRAs able to reactivate the 100% of the latently infected cells?
- Will the CAR-T cells be able to kill all the gp120 expressing cells?
- Can we find a better marker of a latently infected cell?

Target!!

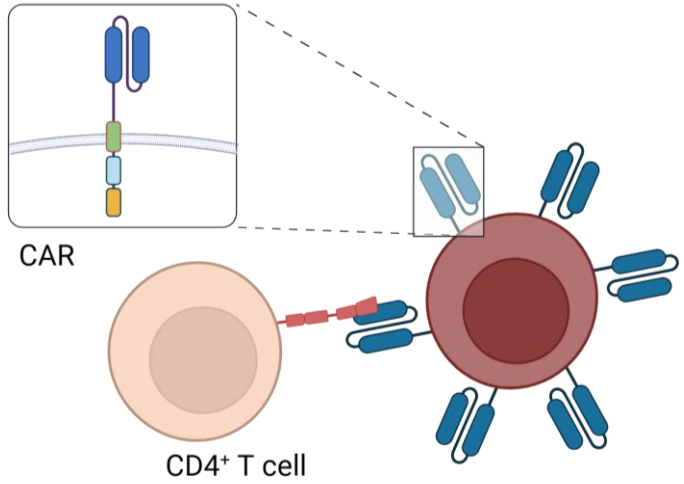
We miss a marker of latently infected cells



Galvez C. et al. *mBio*, 2021

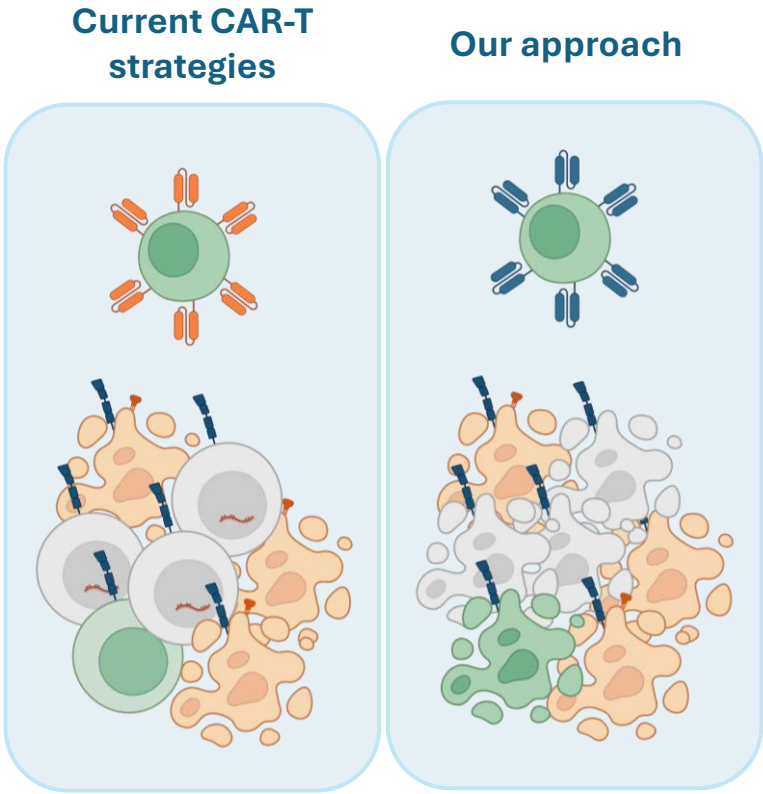
CAR-T cells alternative approach

CAR4



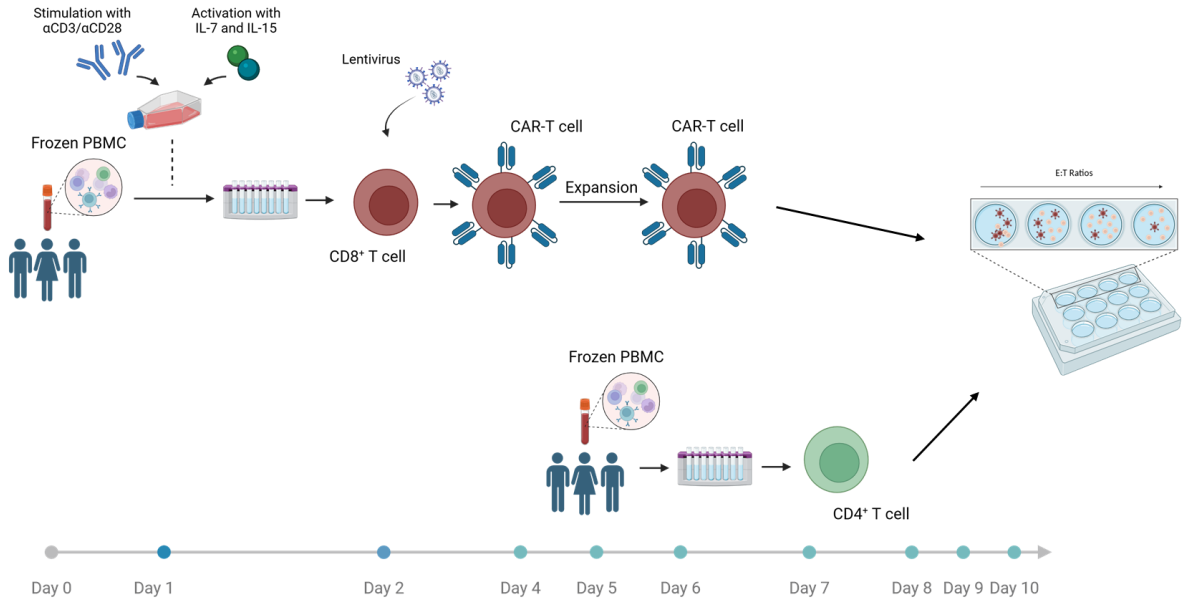
CAR Hu5a8

Mock



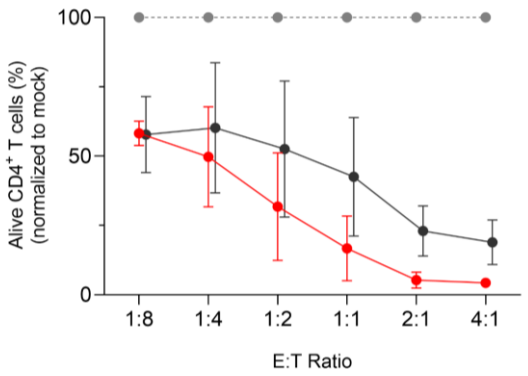
Can we eradicate the HIV-1 reservoir targeting CD4 as a PROVE OF CONCEPT?

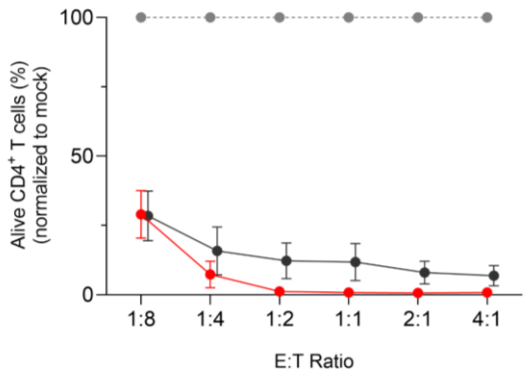
CAR-T cells from HD effectively eliminate healthy and HIV-1 infected autologous CD4 T cells

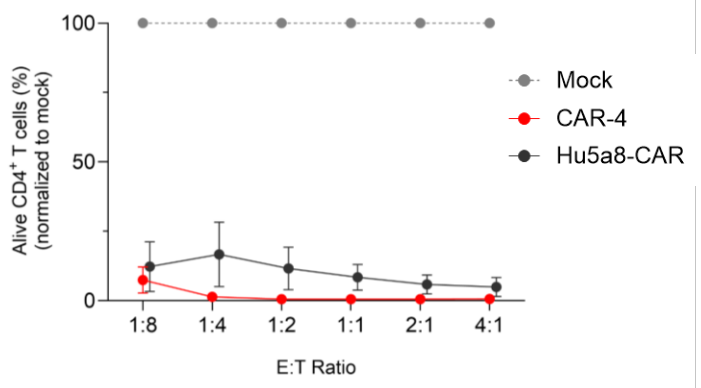


24h **48h** **72h**

Healthy Donors (HD)







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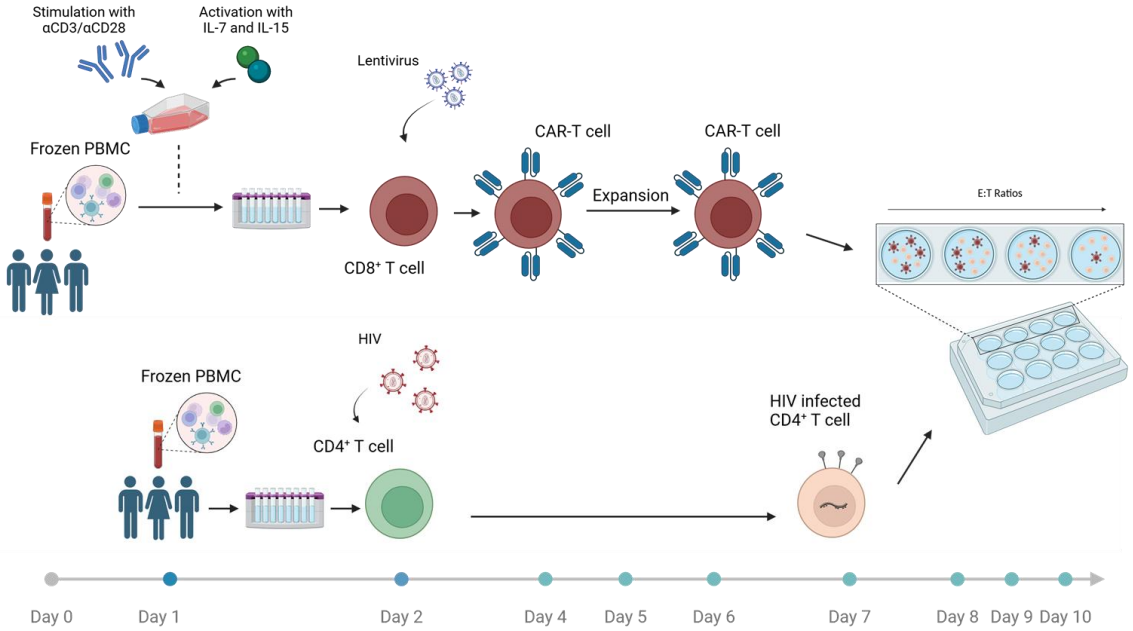
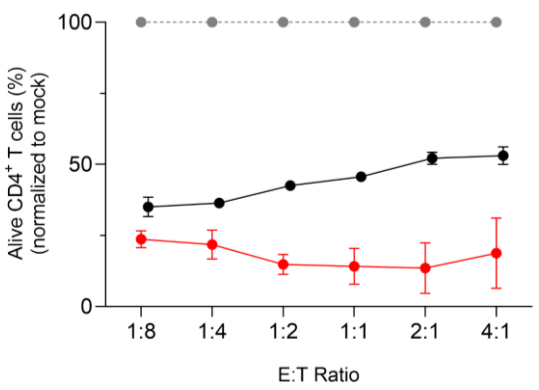
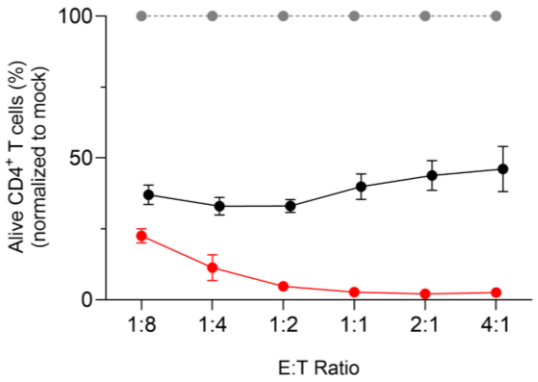
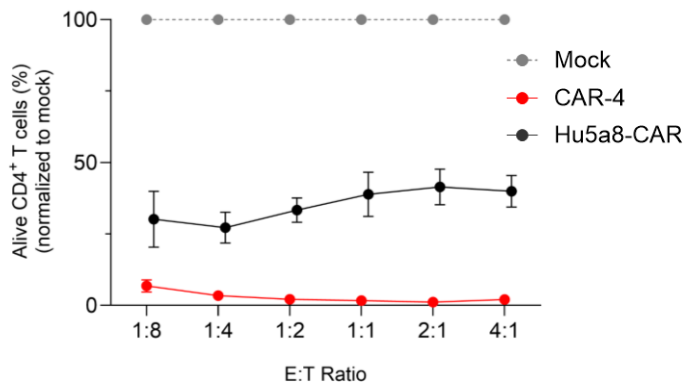


HIV-1 infected Donors



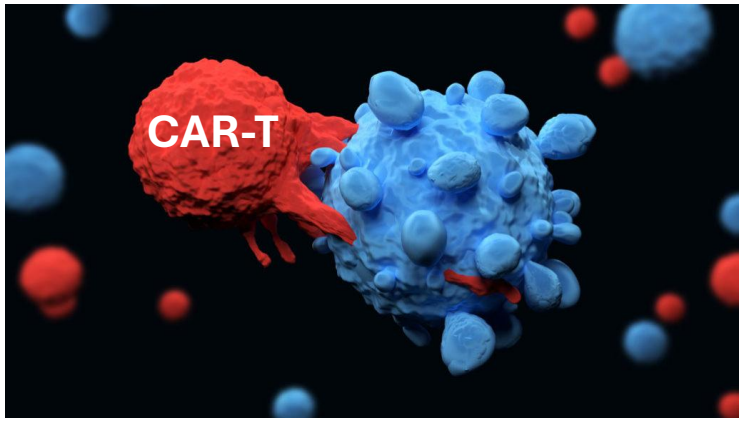




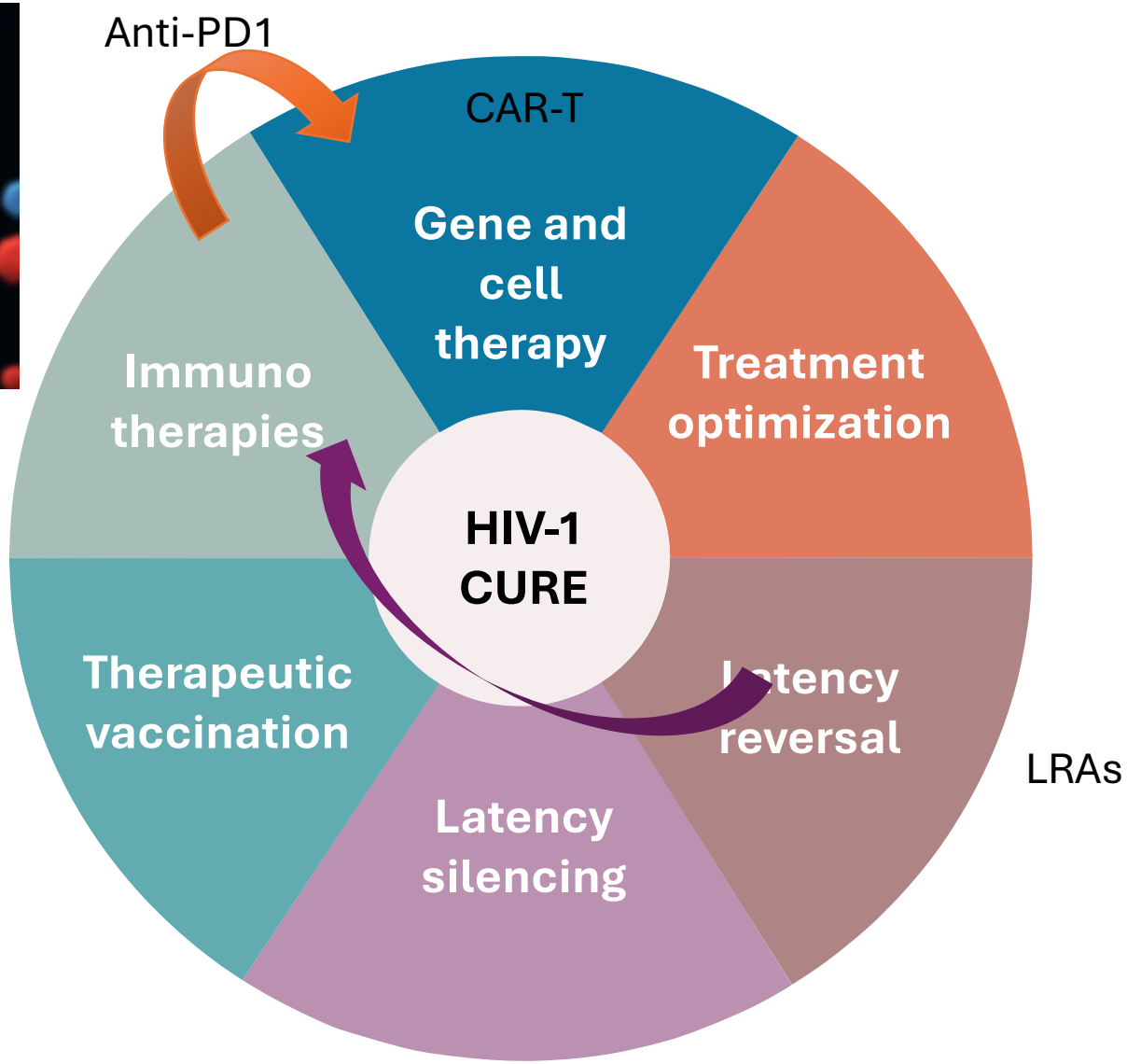
CAR-T cells approaches in HIV field: Pros and Cons

Cons	Pros
CD4 receptor confers susceptibility to the infection to the CARs	New generation and strategies of CARs are designed to avoid HIV infection
Viral escape to antibody-based CARs	They have demonstrated some HIV inhibition effect in active replication
Current CARs don’t target latently infected cells without the viral protein expression	They are safe in clinical trials

HIV-1 cure strategies



Science News, Jan 17th 2020



IrsiCaixa

IGTP Germans Trias i Pujol Research Institute

Josep Carreras LEUKAEMIA Research Institute

Thanks!

Gerard Campos
Àlex Pérez
Talía Velasco
Néstor Tirado
Javier Martínez Picado
Pablo Menéndez
Itziar Erkizia
Patricia Resa
Clara Téllez

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Lessons learned from Infusing Engineered T cells into People with HIV

Jim Riley

Penn Medicine

Center for Cellular Immunotherapies

The University of Pennsylvania has determined that Dr. Riley has a FCOI as he receives research support from two companies (Tmunity/Kite/Gilead) and BlueWhale Bio in which he has an equity interest. The University is actively managing this conflict.

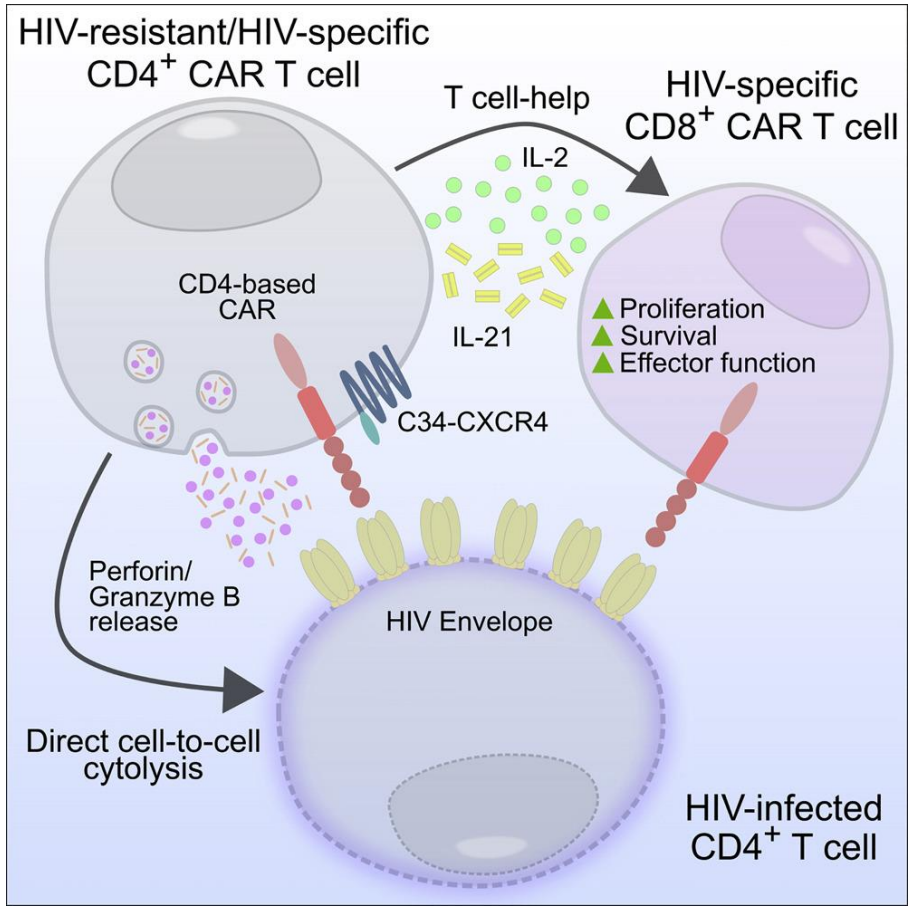
Why Engineered T cells?

- Elite Controlers are enriched for a few HLA alleles like HLA-B57

- Loss of CD4 T cell help is a major reason we do not clear HIV
- Long term ART reduces the number of HIV-specific T cells
- HIV escape renders many of these T cells ineffective

- Success of engineered T cells in cancer
- Change the rules of the game
- T cells are durable and could provide long term control

Key Issues for HIV T Cell Therapy



Identify: How can we safely identify latently infected cells? LRAs?

Redirect: How should we target HIV; CARs v TCRs

Protect: Restoration of CD4 T cell help will likely be key. How can we prevent these cells from becoming infected

Persist: Can we remove every single virus? If not, how can we maintain immune control for decades.

Disseminate: How can we get CAR T cells everywhere where HIV hides? Once they get there, can they kill?

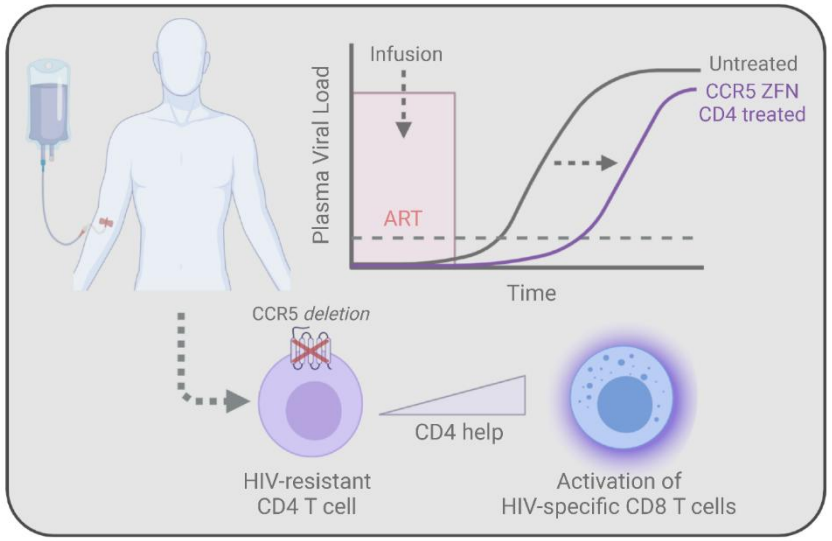
CCR5-edited CD4+ T cells augment HIV-specific immunity to enable post-rebound control of HIV replication 2021

Pablo Tebas,[1] Julie K. Jadlowsky,[2] Pamela A. Shaw,[3] Lifeng Tian,[4] Erin Esparza,[4] Andrea L. Brennan,[4] Sukyung Kim,[1] Soe Yu Naing,[1] Max W. Richardson,[2] Ashley N. Vogel,[4] Colby R. Maldini,[2] Hong Kong,[2] Xiaojun Liu,[4] Simon F. Lacey,[4] Anya M. Bauer,[1] Felicity Mampe,[1] Lee P. Richman,[1] Gary Lee,[5] Dale Ando,[5] Bruce L. Levine,[4] David L. Porter,[1] Yangbing Zhao,[4] Don L. Siegel,[4] Katharine J. Bar,[1] Carl H. June,[4] and James L. Riley[2]

Table 1. Patient demographics and cell manufacturing

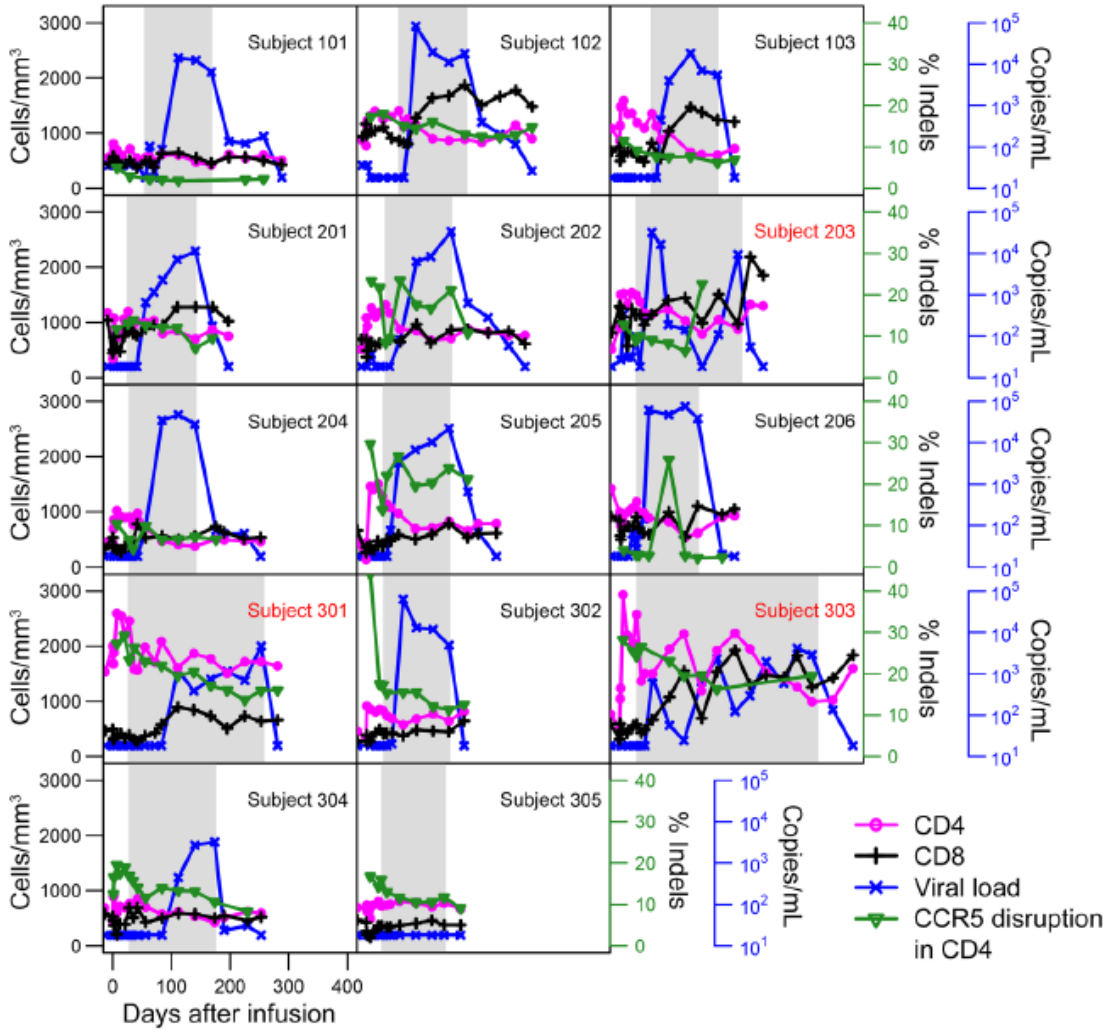
Cohort and patient no.	Age (yr)	Race or ethnicity	Sex	Duration of HIV infection (yr)	Baseline CD4+ T cell count per mm3	Baseline CD4+/CD8+ T cell ratio	SB-728mR-T dose	SB-728mR-T CD3+CD45+ (%)	SB-728mR-T cell modification (%)	ART regimen prior to ATI	Class
Cohort 1											
101	32	White	M	5.3	563	1.25	1.00×10^{10}	95.2	14.32	TDF/FTC/EFV	NNRTI
102	49	White	M	12.8	870	0.93	1.00×10^{10}	99.0	24.12	TDF/FTC/ATV/r	PI
103	41	Black	F	13.8	1081	1.62	1.00×10^{10}	98.6	28.63	TDF/FTC/DTG	INSTI
Cohort 2											
201	37	White	M	10.3	1179	1.14	1.00×10^{10}	98.2	26.81	TDF/FTC/ATV/r	PI
202	60	White	M	24.1	512	0.74	1.00×10^{10}	98.5	27.27	ABC/3TC/DTG	INSTI
203	54	Black	M	21.1	513	0.29	1.00×10^{10}	95.0	16.11	TDF/FTC/EVG/c	INSTI
204	49	Black	M	6.7	457	1.38	0.66×10^{10}	95.2	10.66	TDF/FTC/EVG/c	INSTI
205	45	Black	M	6.0	456	0.69	1.00×10^{10}	95.6	26.47	TDF/FTC/ATV/r	PI
206	43	Black, Hispanic	M	5.0	1421	1.55	1.00×10^{10}	99.2	21.51	ABC/3TC/DTG	INSTI
Cohort 3											
301	54	White	M	6.2	1535	3.27	1.00×10^{10}	95.0	33.87	TDF/FTC/RPV	NNRTI
302	50	White	M	7.5	456	1.62	1.00×10^{10}	98.6	23.04	TDF/FTC/EVG/c	INSTI
303	30	Black	M	1.7	763	1.28	1.00×10^{10}	96.6	24.20	TDF/FTC/RPV	NNRTI
304	19	Black	M	2.2	690	1.19	1.00×10^{10}	98.9	24.70	TDF/FTC/EVG/c	INSTI
305	39	Black	M	6.5	695	1.48	1.00×10^{10}	98.2	25.99	TDF/FTC/EVG/c	INSTI
Median	44			6.6	693	1.27	1.00×10^{10}	98.2	24.45		

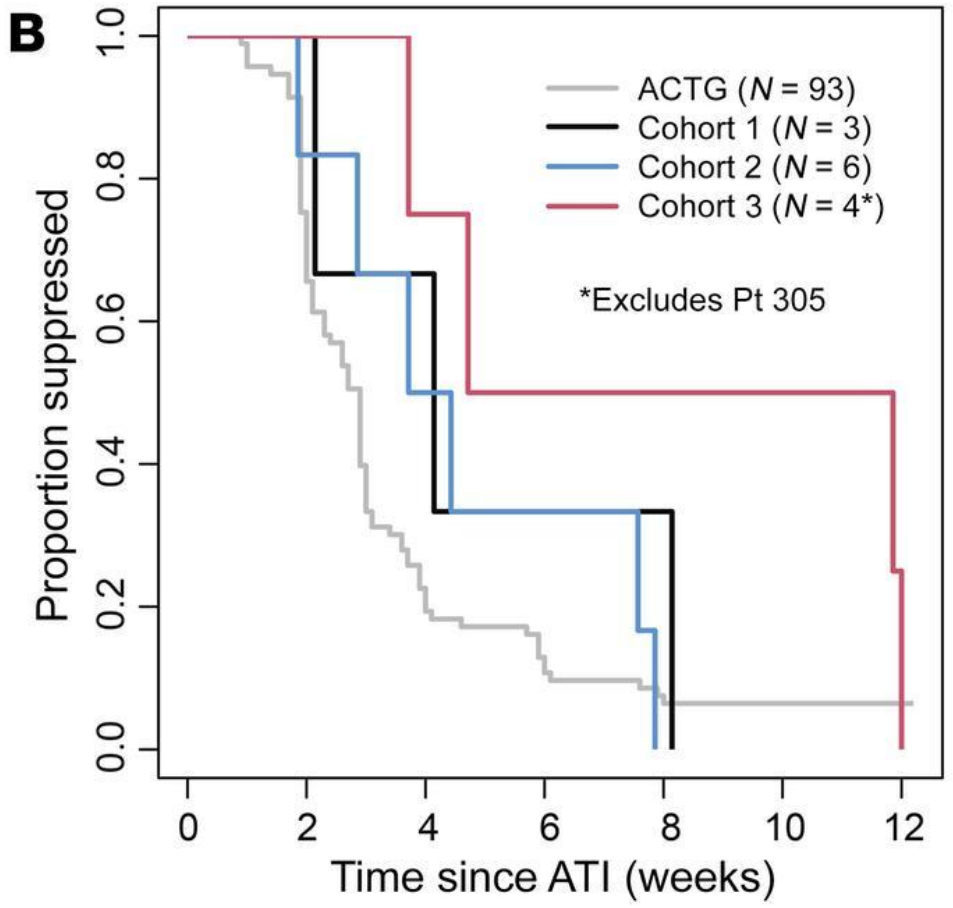
M, male; F, female; TDF, tenofovir; FTC, emtricitabine; EFV, efavirenz; ATVr, atazanavir/ritonavir; DTG,dolutegravir; INSTI, integrase strand transfer inhibitor; ABC, abacavir; 3TC, lamivudine; EVGc, elvitegravir/cobicistat; RPV, rilpivirine.



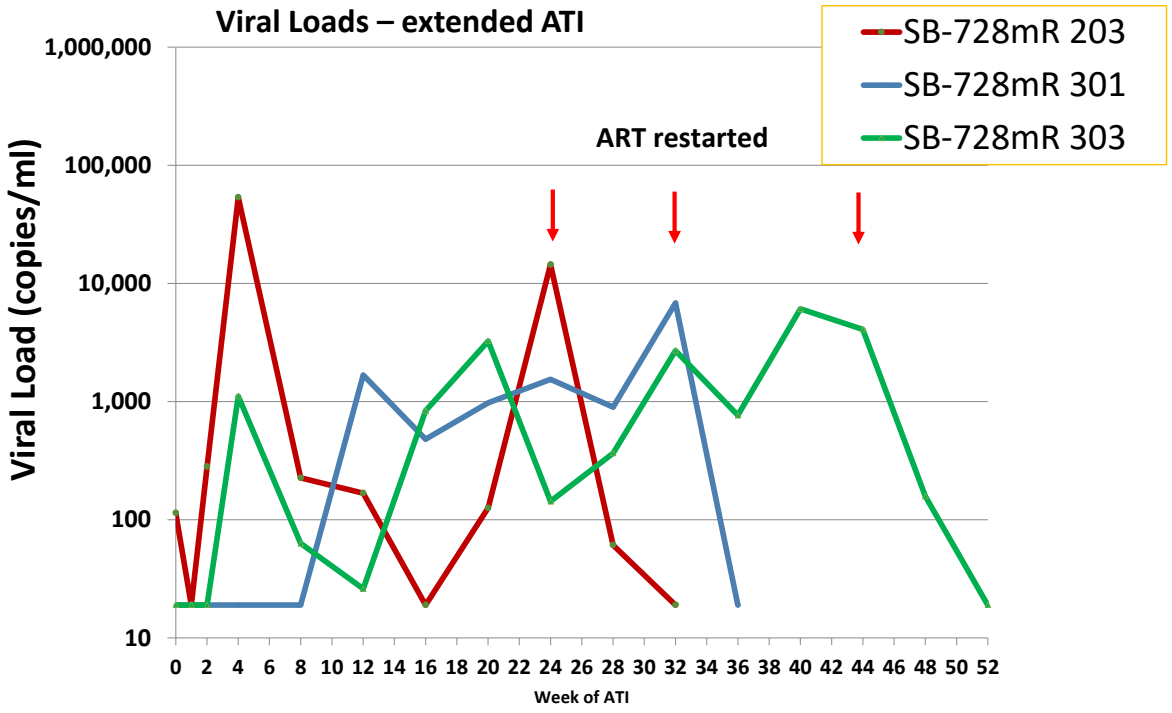
Pablo Tebas

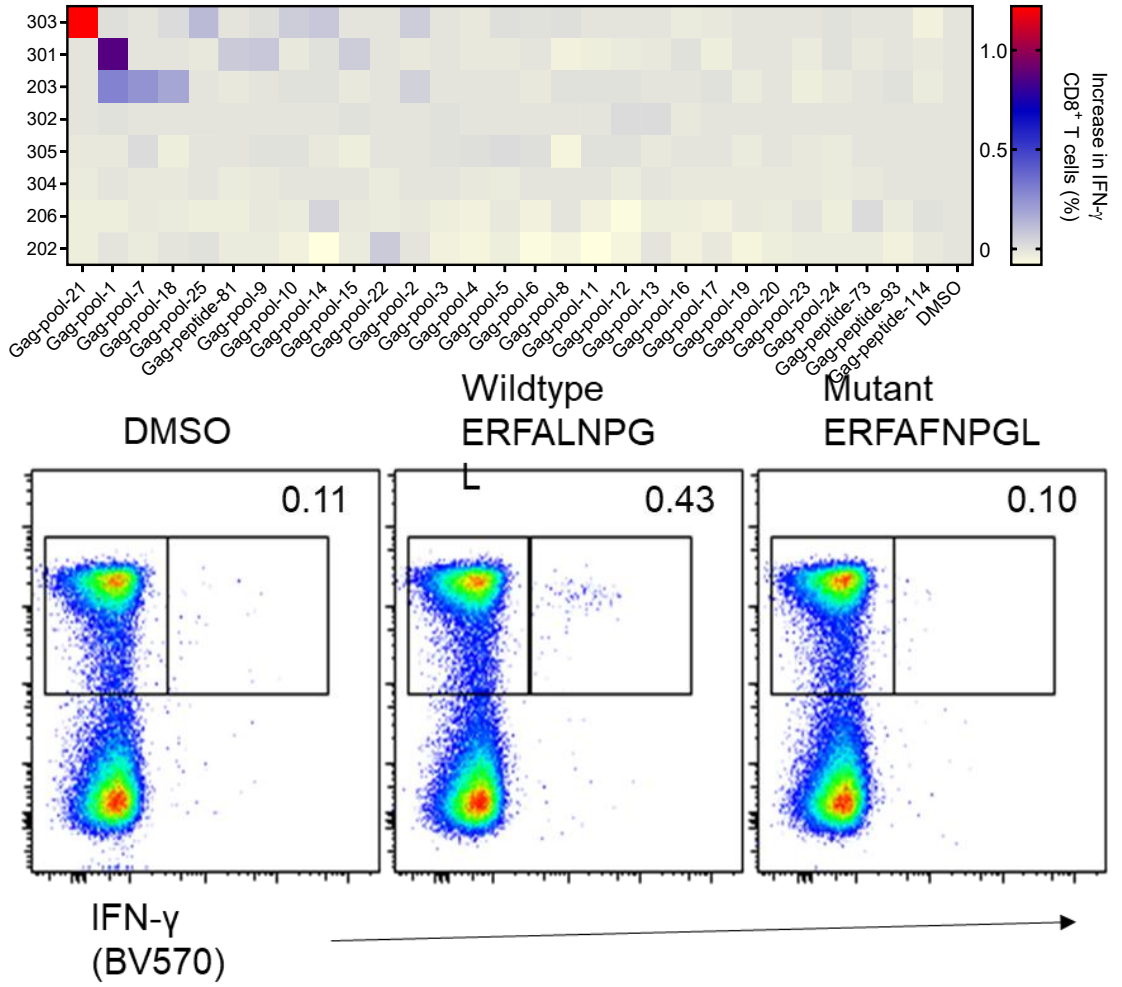
Large increase in peripheral CD4 counts after infusion of CCR5 edited CD4 T cells





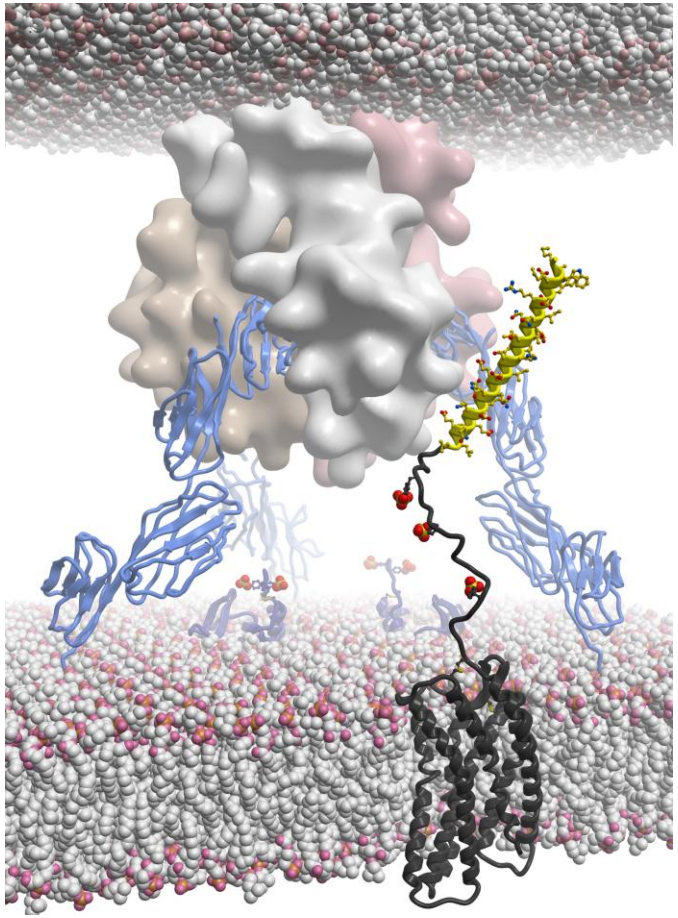
Post rebound control correlated with improved HIV specific T cell responses





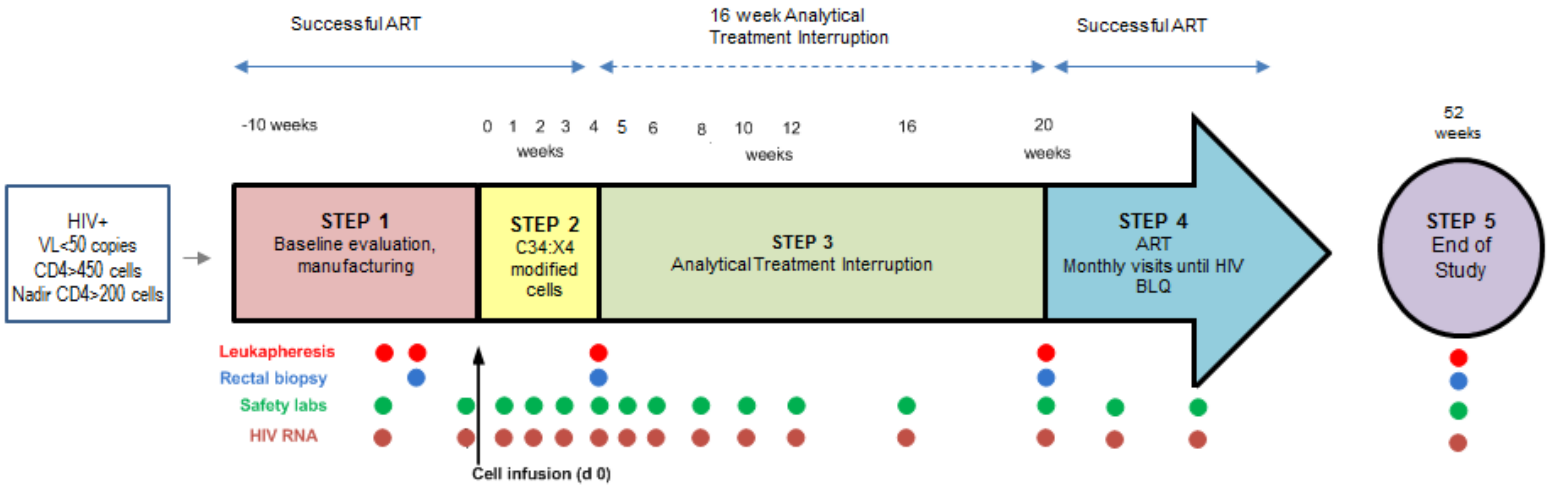
Drawbacks: Due to low editing on clinical scale, ~10% of cells are CCR5 deficient

C34-CXCR4- Potent fusion inhibitor that blocks all stains of HIV effectively

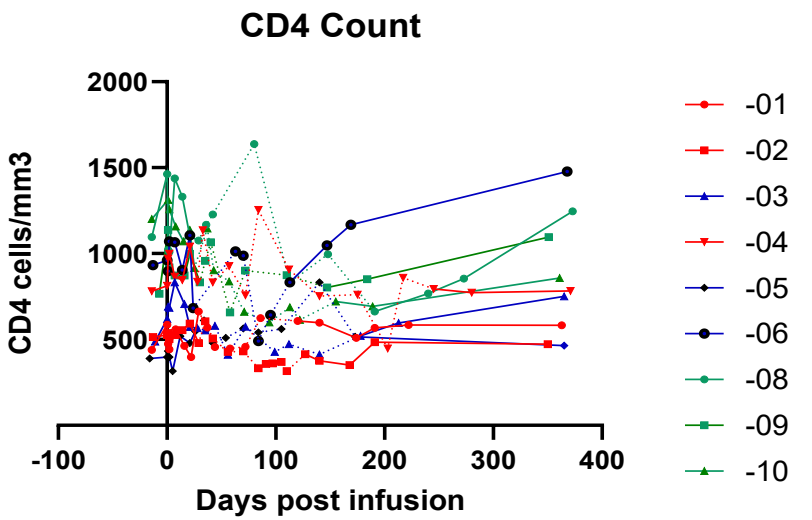


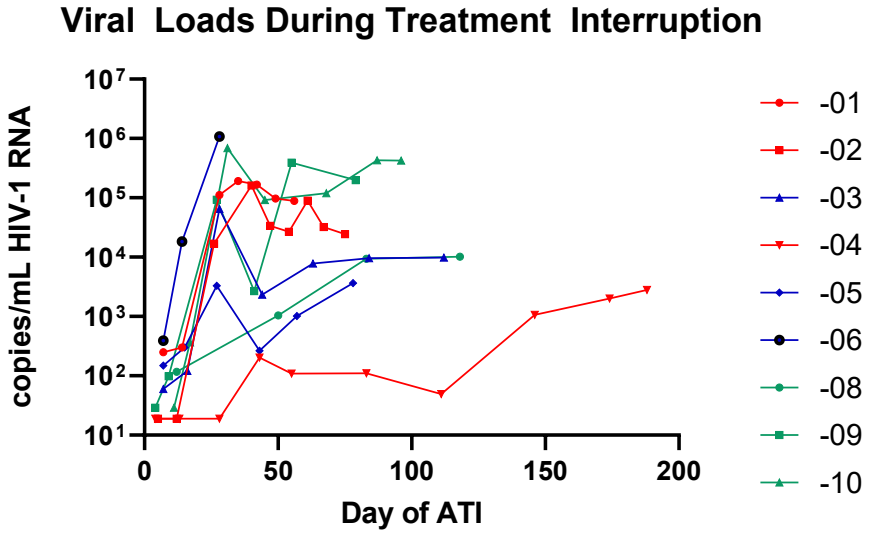
- Advantages
  - Higher expression
  - Concentration at the virologicial synapse
  - Better positioned near the membrane to place the inhibitor so it can block fusion
- Disadvantages
  - May make previously non-immunogenic C34 immunogenic due to C34 fusion with CCR5 or CXCR4
  - Disregulated chemokine receptor expression may alter T cell trafficking

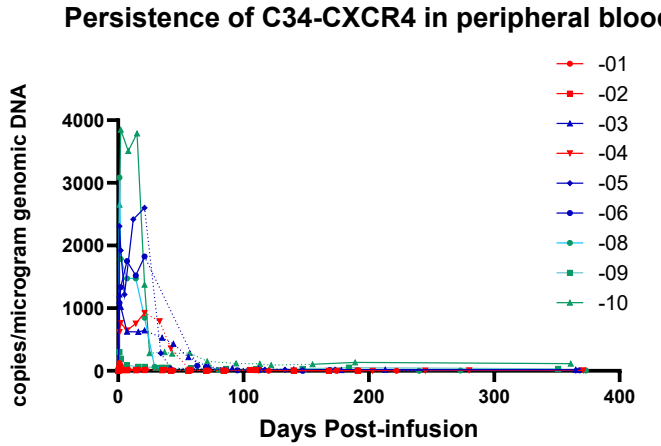
Clinical Trial Results Provide the Rationale to Protect Dual HIV-specific T Cells with a Signaling-Defective HIV Fusion Inhibitor



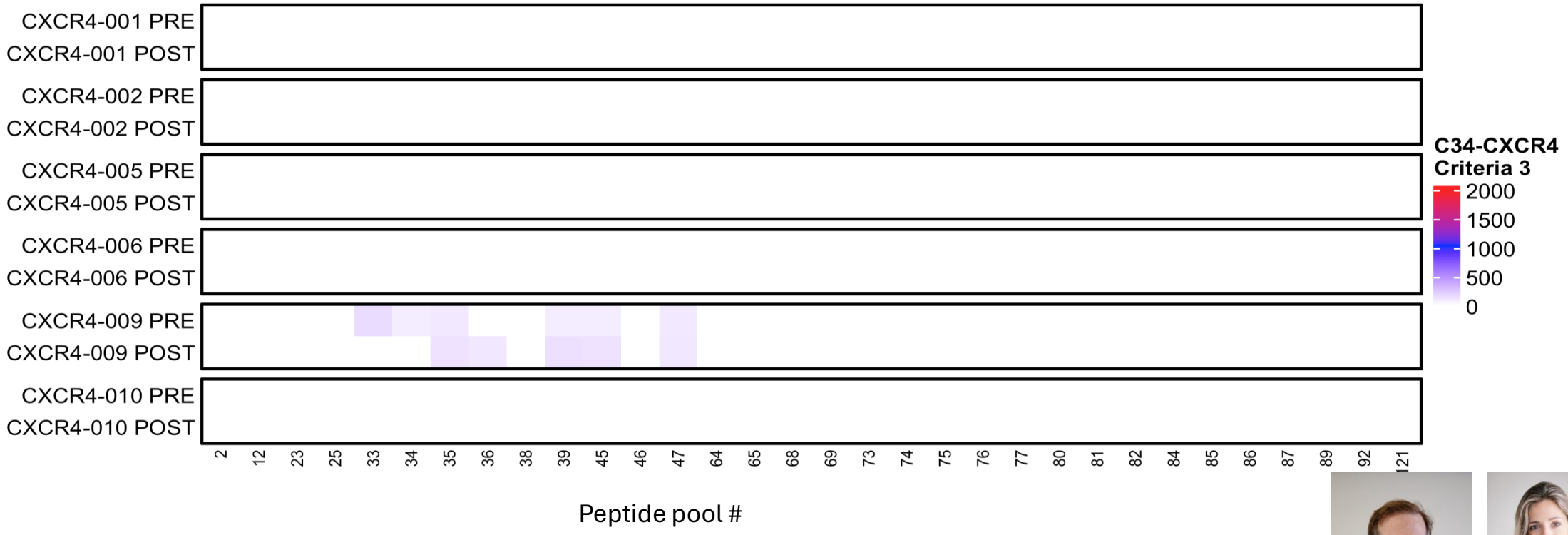
Dose Escalation
1, 3, 10 billion C34-CXCR4 expressing T cells

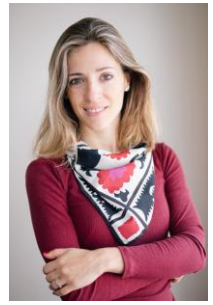






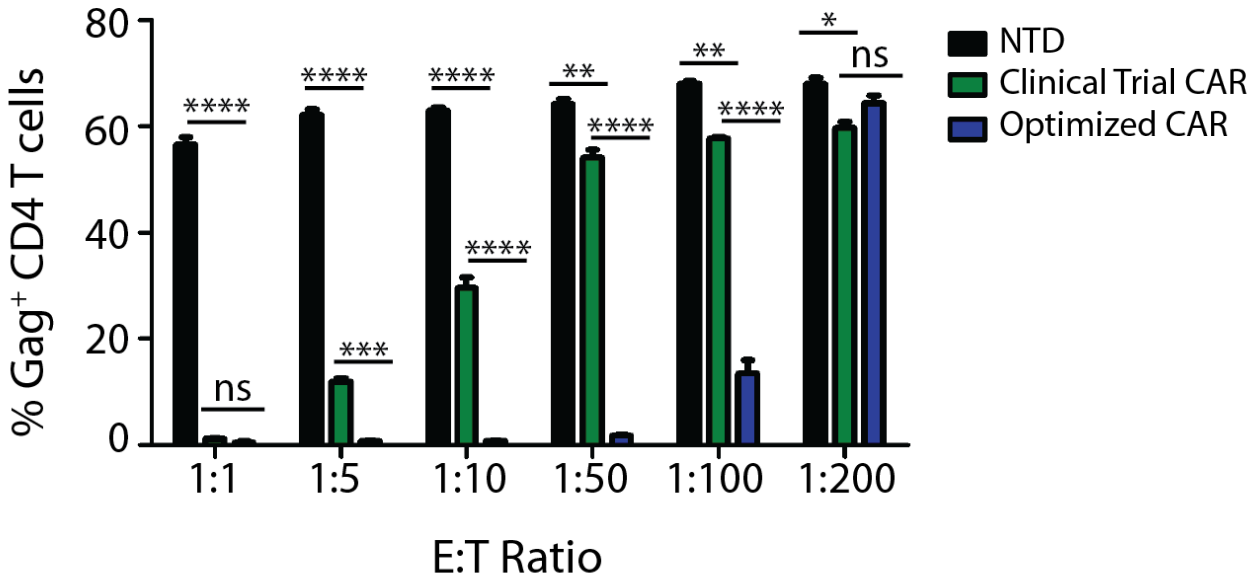
No Re-invigoration of HIV-specific CD8 T Cell Responses





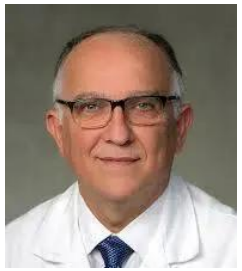
Optimized CD4 CAR controls HIV-1 replication *in vitro* 50-fold better than original CAR construct

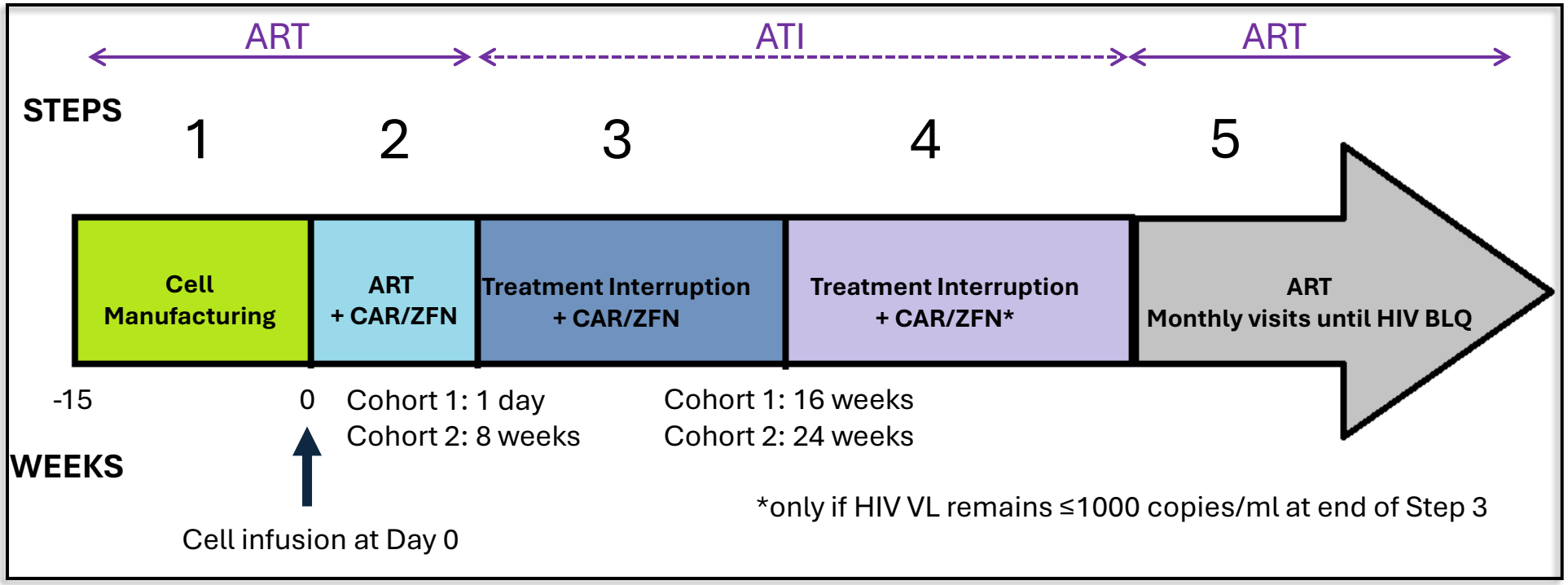
Component	Clinical Trial Construct	Optimized Constructs
Viral Vector	Murine γ-retrovirus	Lentivirus
Promoter	PGK	EF1α
Hinge	None	CD8α
Transmembrane domain	CD4	CD8a or CD28
Stimulatory domains	CD3ζ	CD3ζ and others (4-1BB, CD28, etc.)
Extracellular domain	CD4	CD4



Leibman et al PloS Pathogens 2017

A Pilot Study of T Cells Genetically Modified by CCR5-specific ZFNs and CD4 Chimeric Antigen Receptor in HIV-infected Subjects (NCT03617198)





1: To what extent does ongoing HIV replication contribute to the maintenance of the HIV reservoir?

2: Can engineered T cells restore functionality to endogenous HIV-specific T cell populations?

3. Can engineered T cells provide durable control of HIV replication?

4. When is the best time to do the ATI?

Cohort 1- engraftment (step 2) of 1 day before ATI
Cohort 2- engraftment (step 2) 8 weeks before ATI

BEAT-HIV
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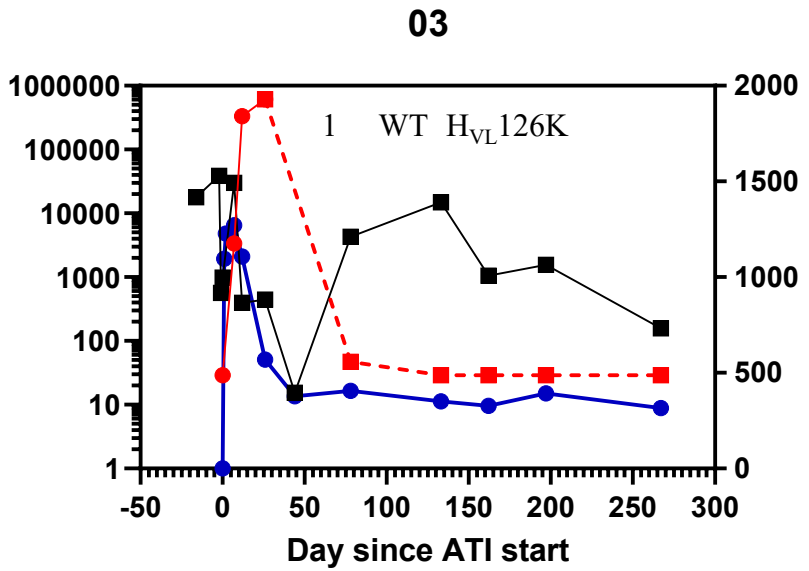
2



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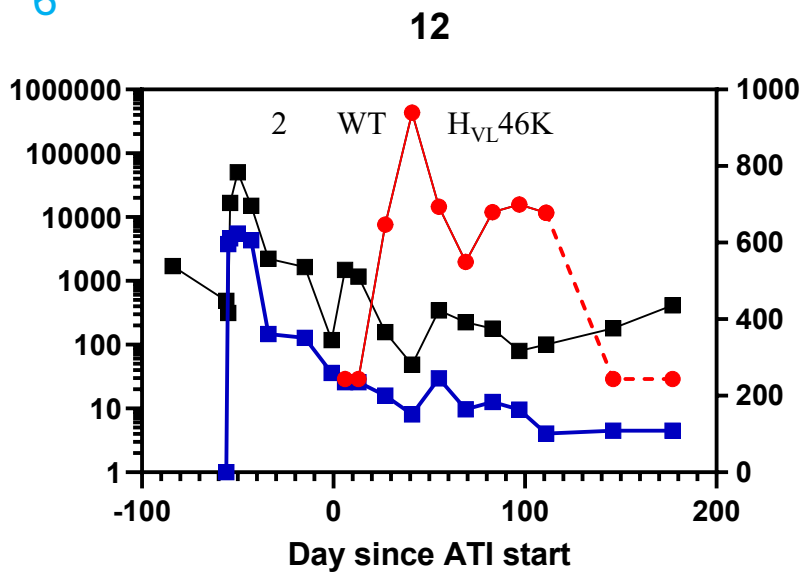

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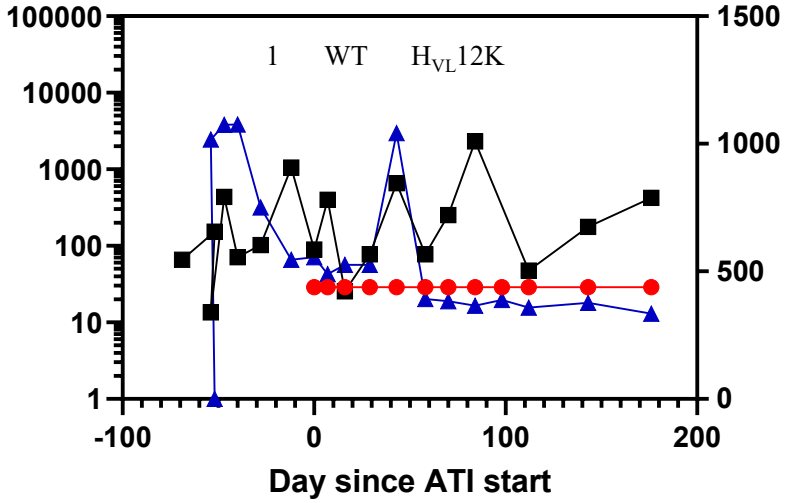

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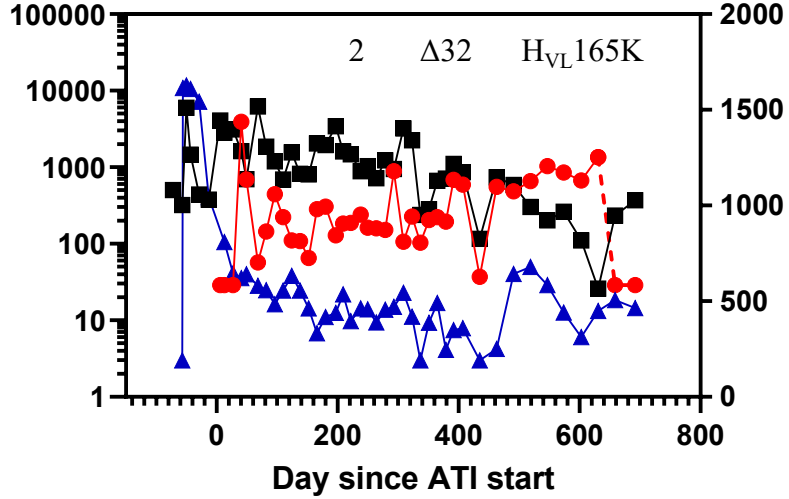
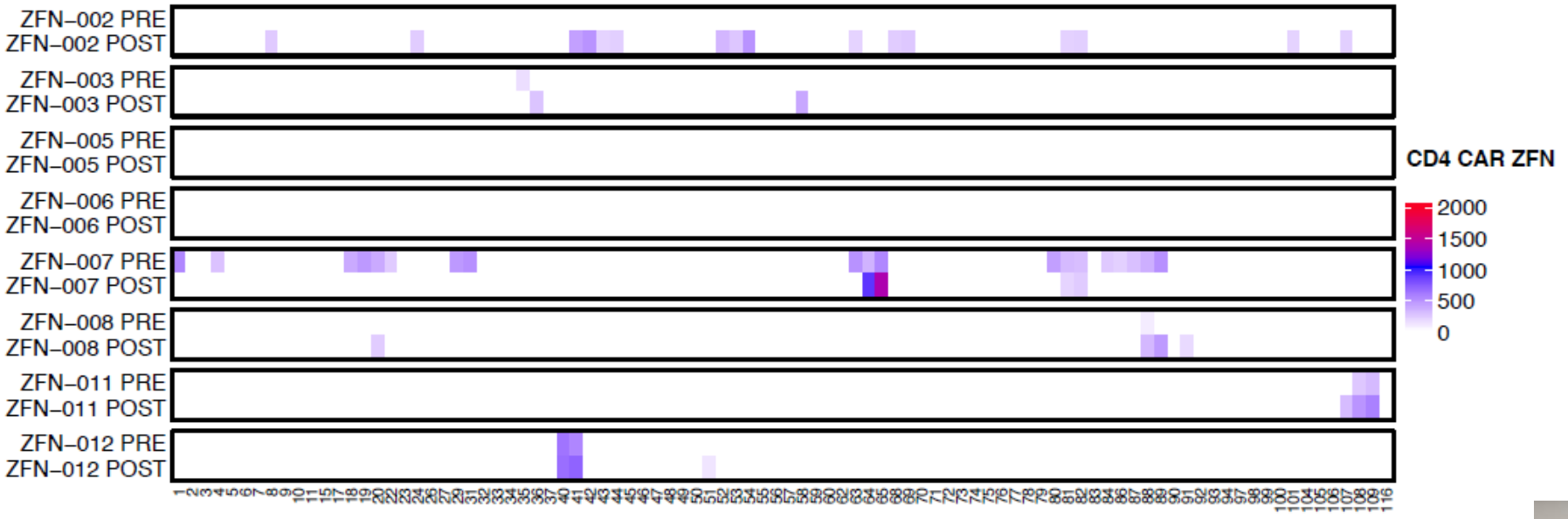
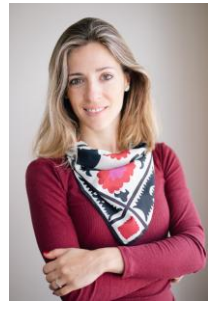


Viral Load (Red) Vector Copies (Blue)

CD4 Counts (Black)

Re-invigoration of HIV-specific CD8 T cells





HIV CARs do not expand in the PB in the presence of virus

Three Explanations:

1. Suboptimal CAR Design
   Dual CAR T cell

2. HIV CARs are becoming infected
   Re-evaluate C34-CXCR4

3. HIV CARs are not persisting well in the absence of antigen ART
   Env expressing Vaccine

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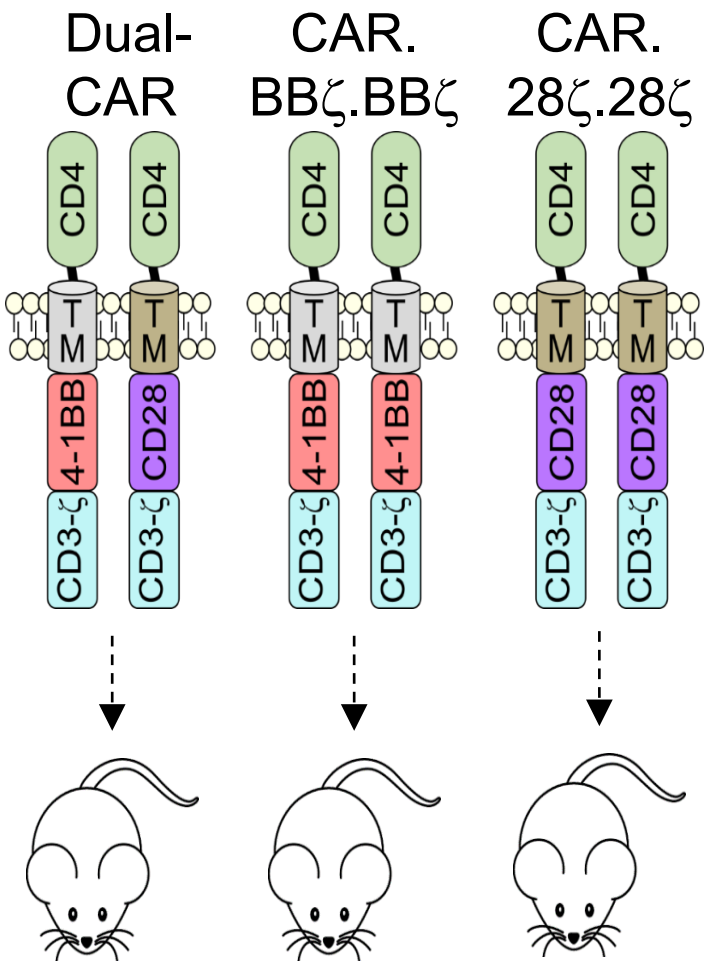
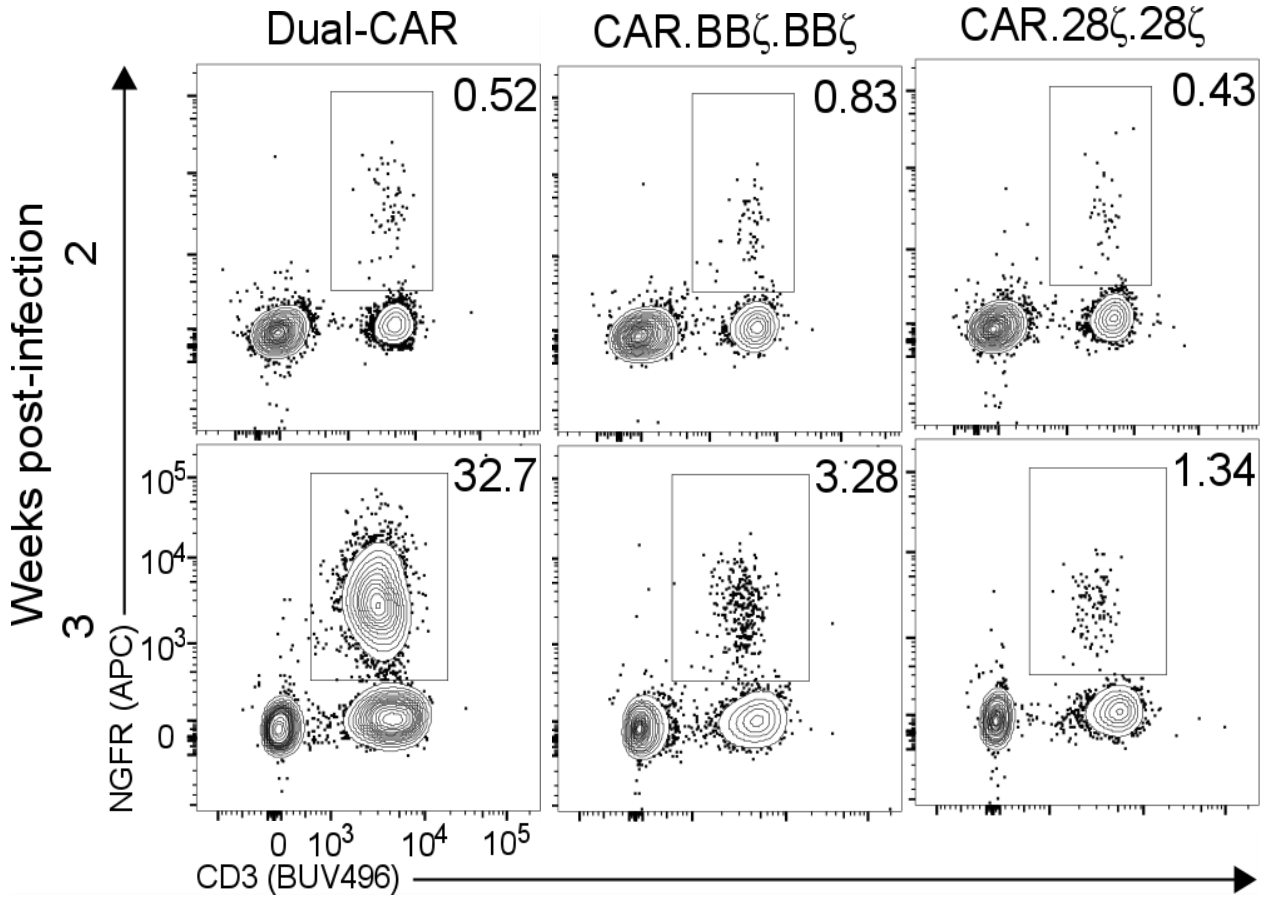
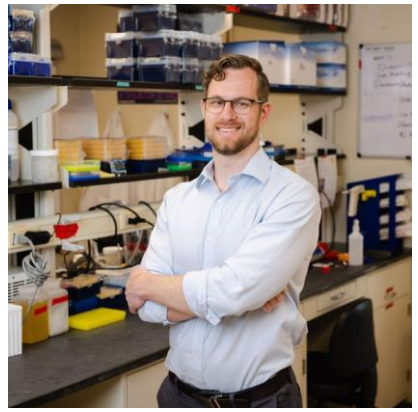
DELANEY COLLABORATORY

Why Two CARs are Better than One



HIV-infected BLT mice

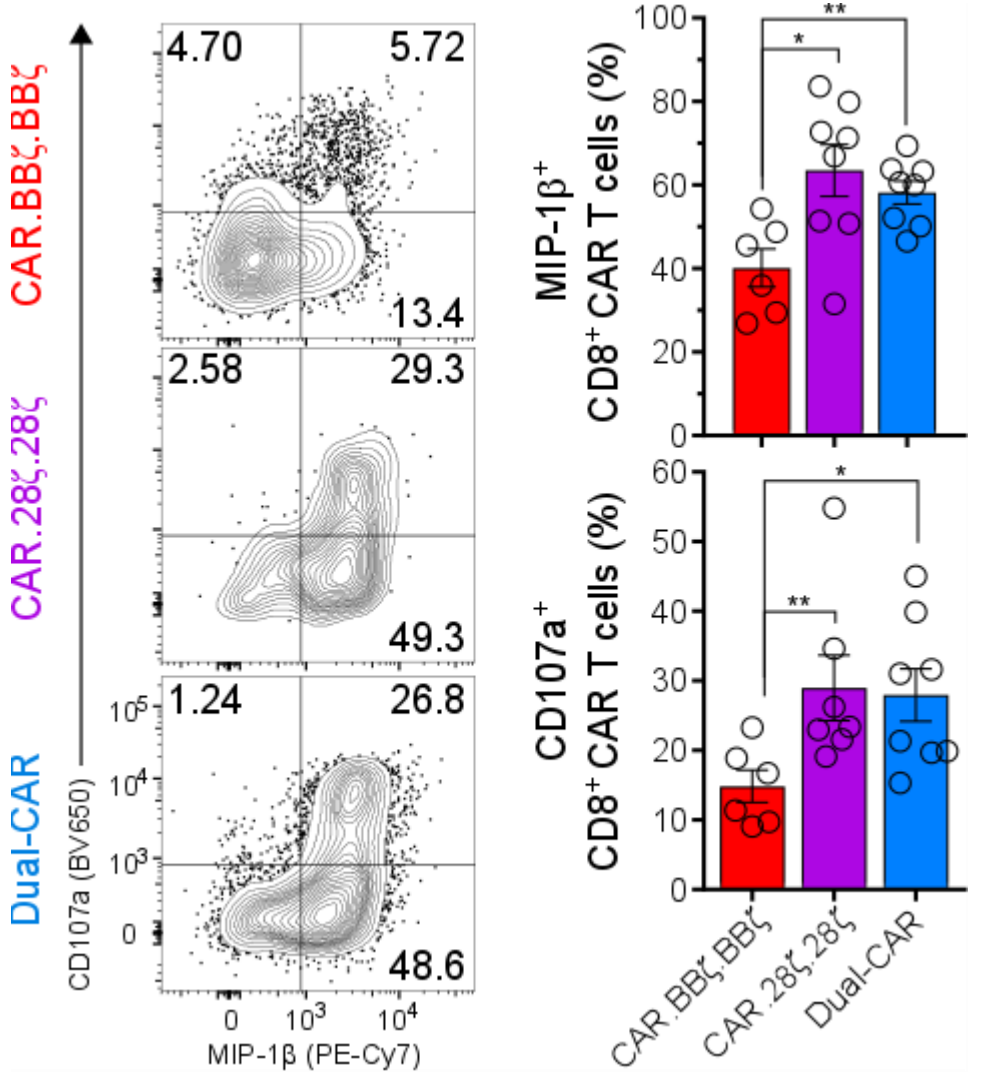


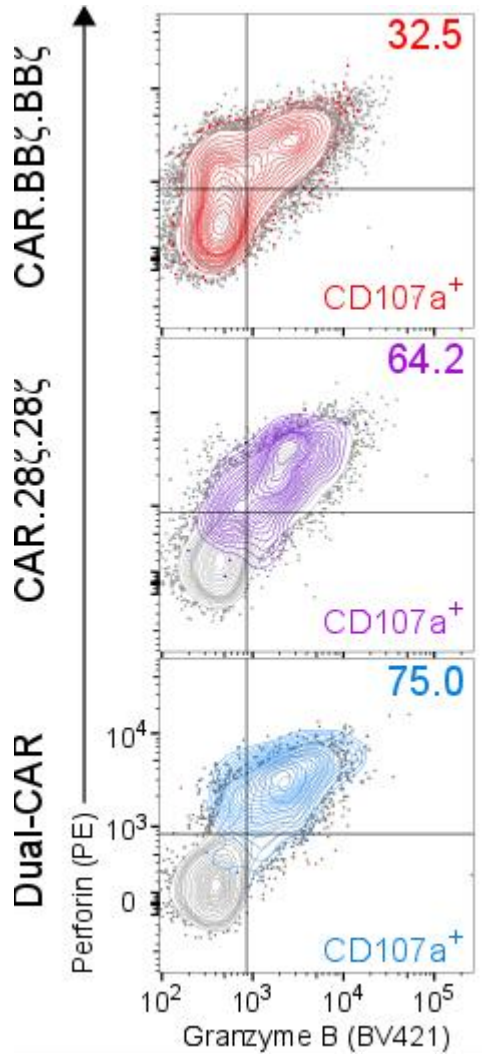


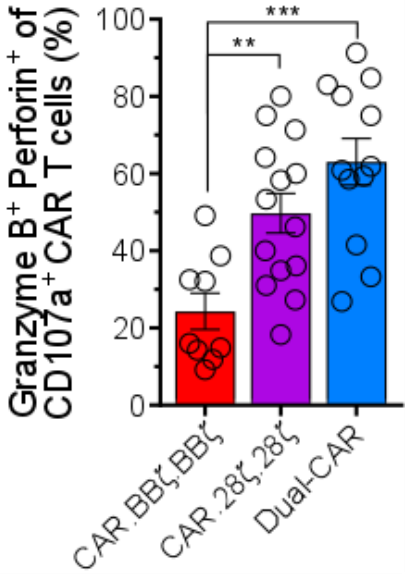
BEAT-HIV
DELANEY COLLABORATORY

Maldini Nature Medicine 2020

Dual-CAR T cells exhibit greater effector functions than 4-1BB-costimulated CAR T cells

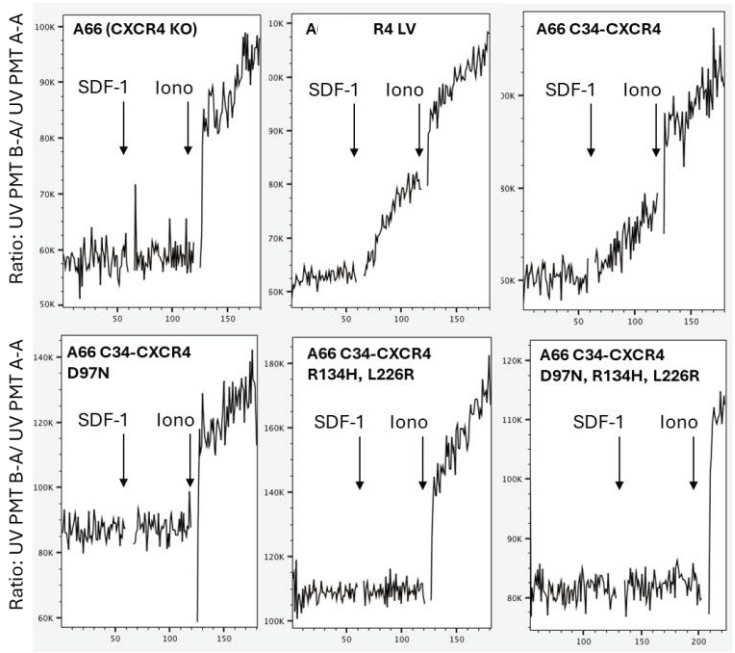


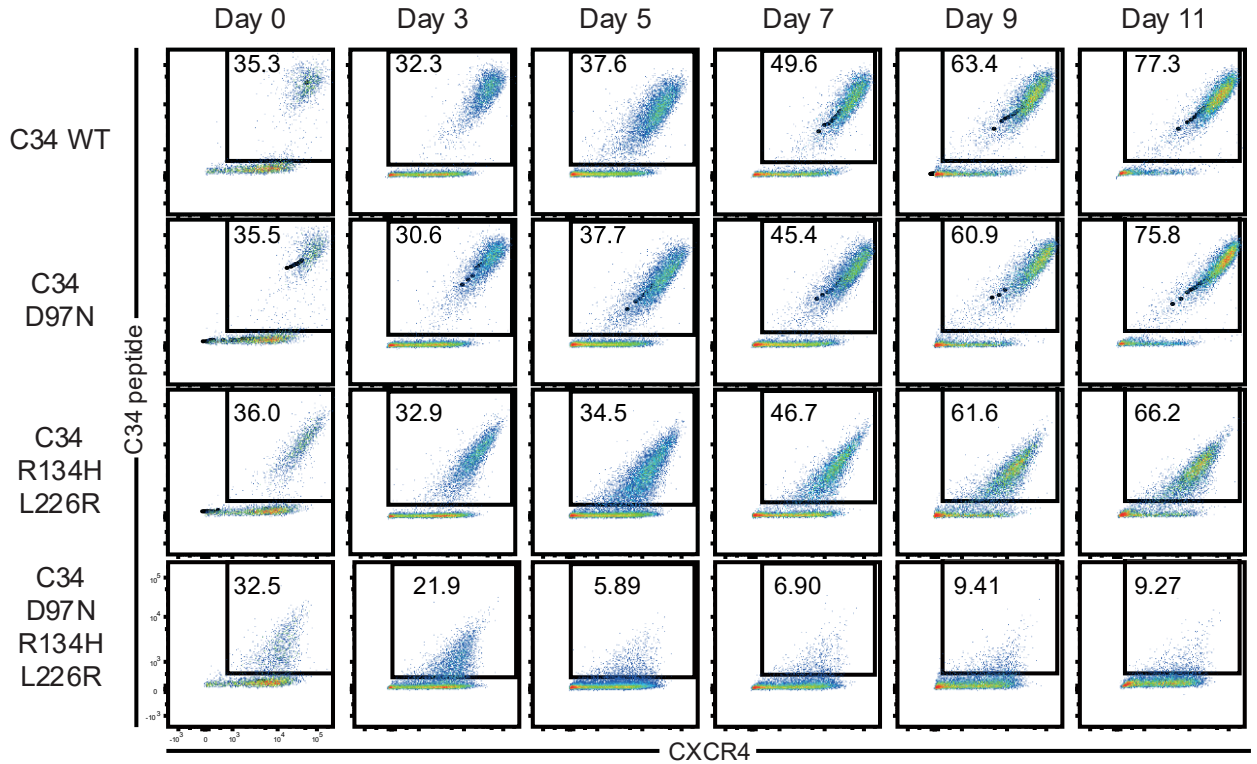




- Generated a novel CAR T cell product that combines the attributes of 4-1BB and CD28
- First evidence of therapeutic efficacy
- No impact on HIV replication

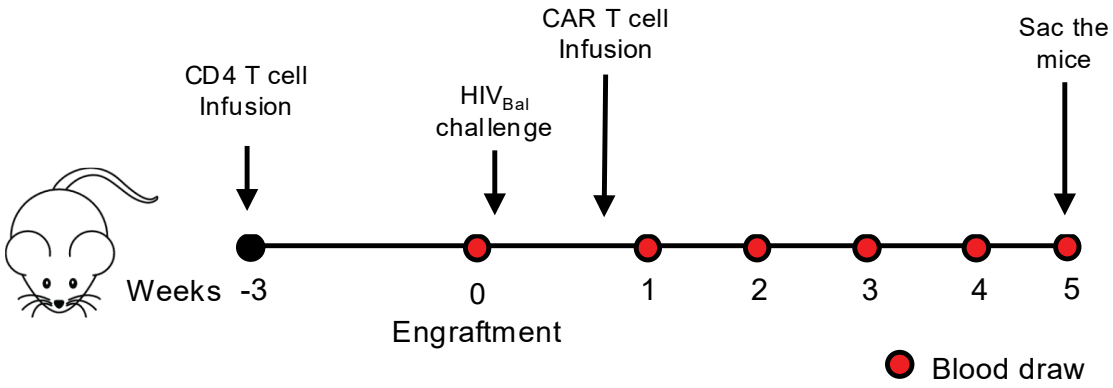
Is CXCR4 signaling required for C34-CXCR4 antiviral activity

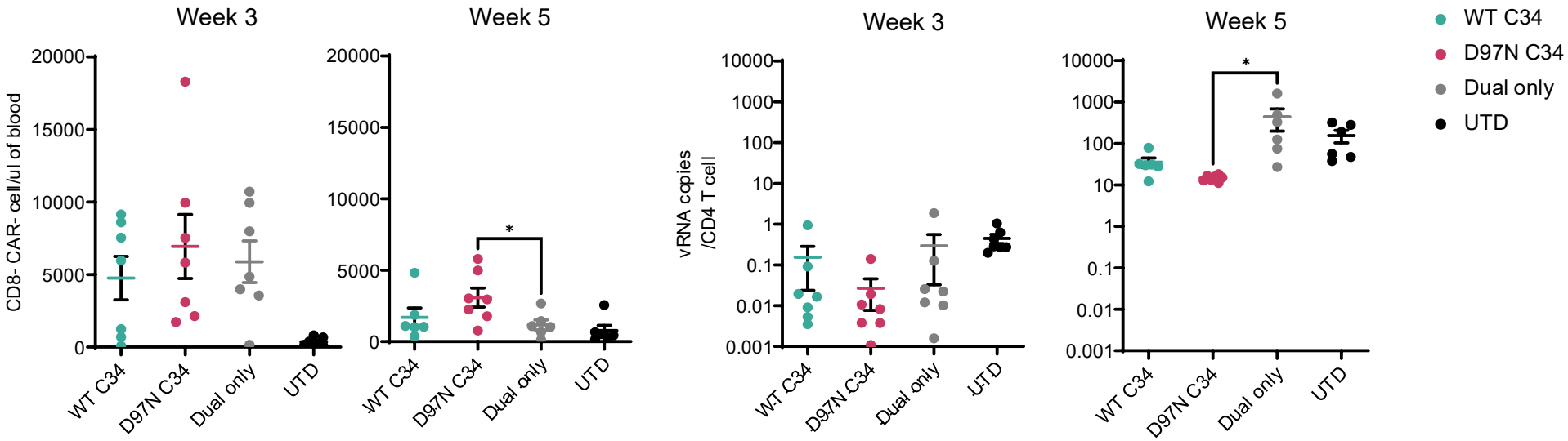




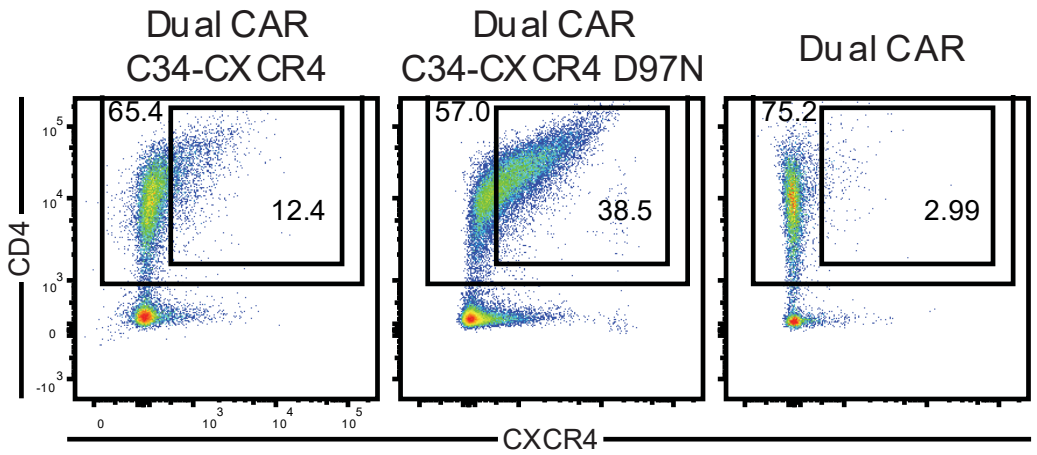
Tebas et al Molecular Therapy 2026

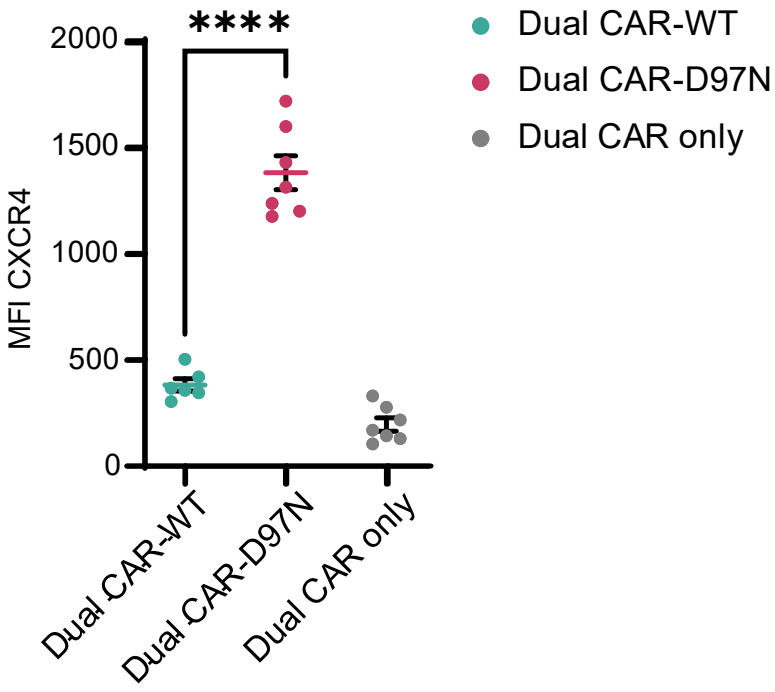
C34-CXCR4 D97N expression protects HIV-specific Dual CAR T cells in vivo





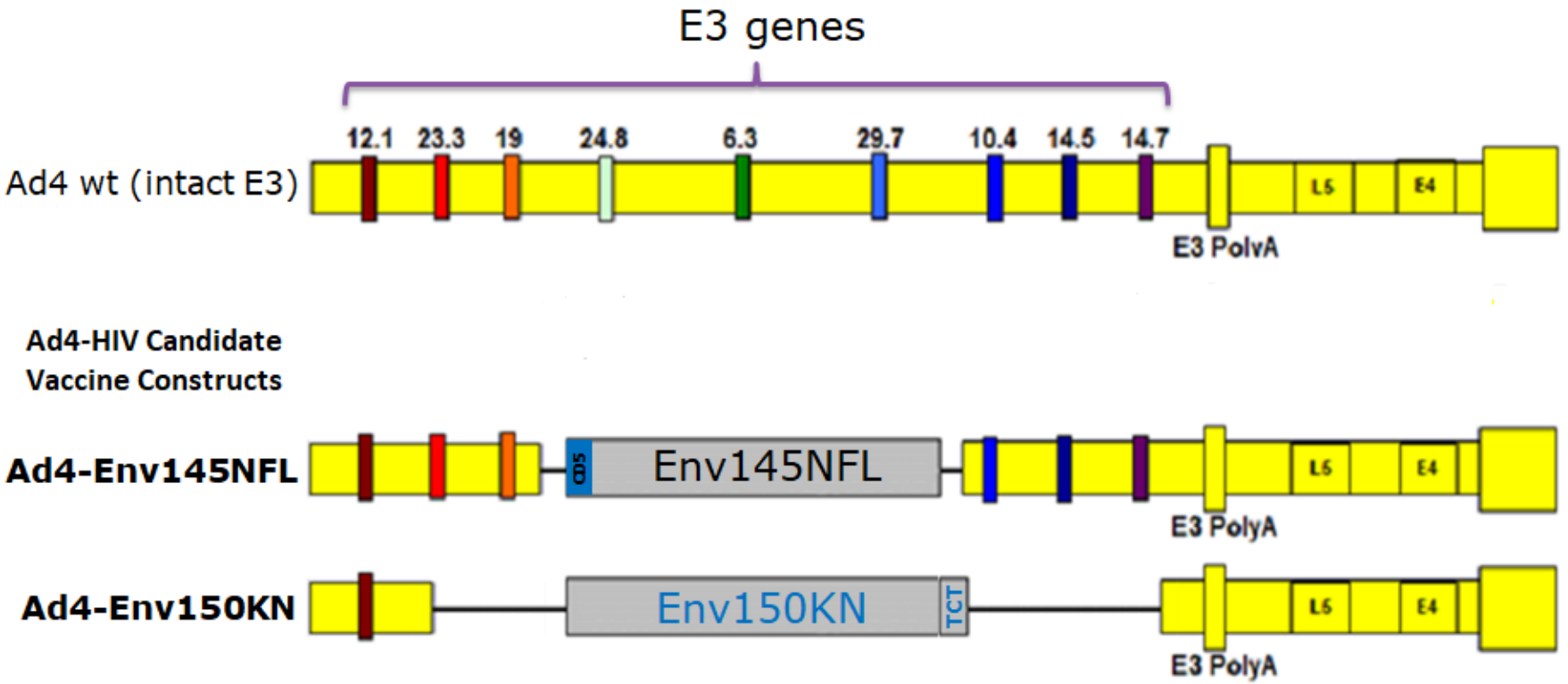
Signaling Deficient C34-CXCR4 retains high expression in vivo





How can we boost CAR T cell levels prior to ATI?

HIV-1 Adenovirus Type 4-Vectored (A549 cells) Vaccines, Live, Ad4-Env150KN and Ad4-Env145NFL (Native flexibly linker)



a stabilized version of the 1086 clade C Env antigen of the HIV-1 virus with a native flexible linker between gp41 and gp120 subunits that obviates the need for furin-mediated cleavage

1086 Clade C

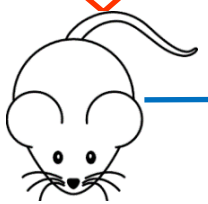
Mark Connors

BEAT-HIV DELANEY COLLABORATORY

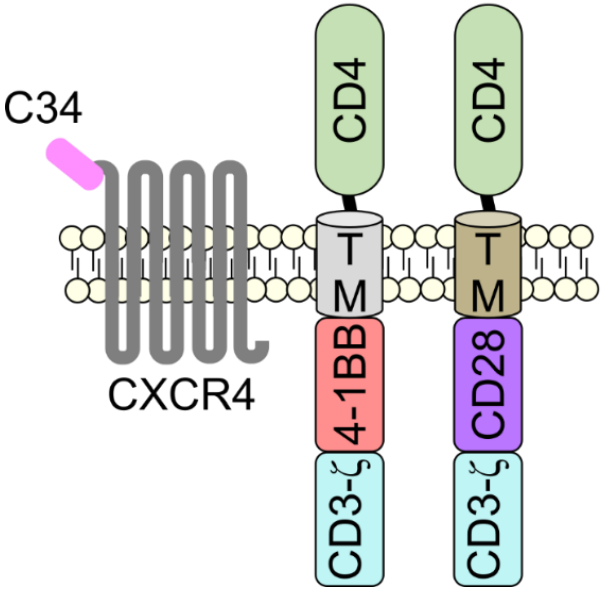
Which Vaccine can Promote the Expansion of HIV-specific Dual CAR T Cells

Timeline

Day0
10 million
CAR-T
(15%)
Cells
(i v)



NSG MHC 1/II KO



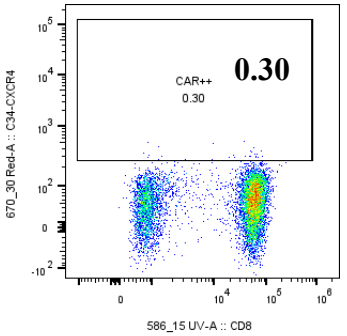
Week:3
Mouse Bleed
prior to vaccination

Week:3
Vaccination
IntraNasal
10^8

Weekly Mouse Bleeds

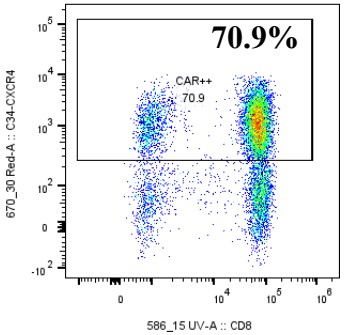
Week 3 | Week 4 | Week 5 | Week 6

UTD

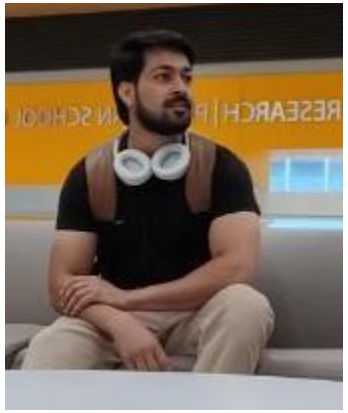


C34

CARTs

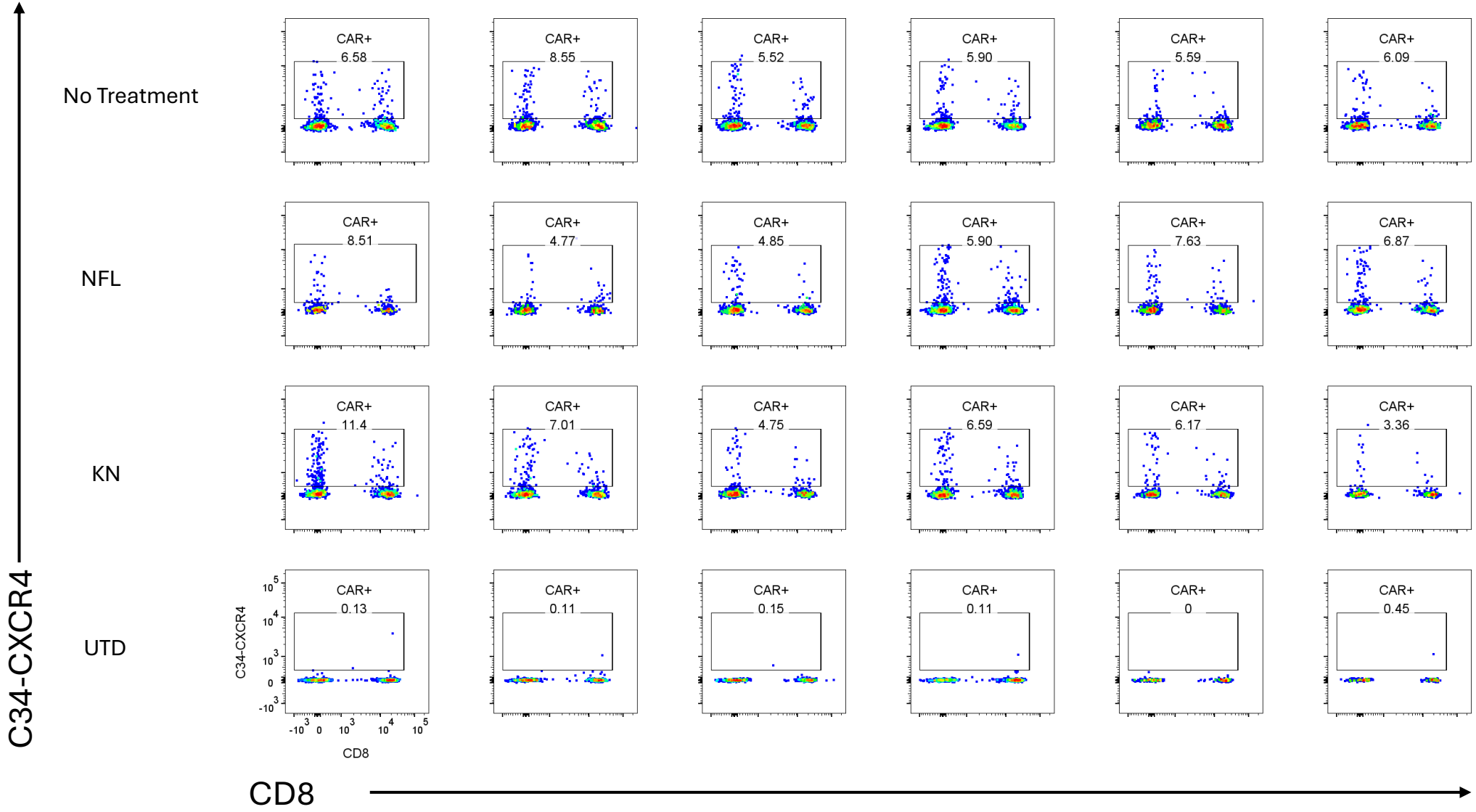


CD8

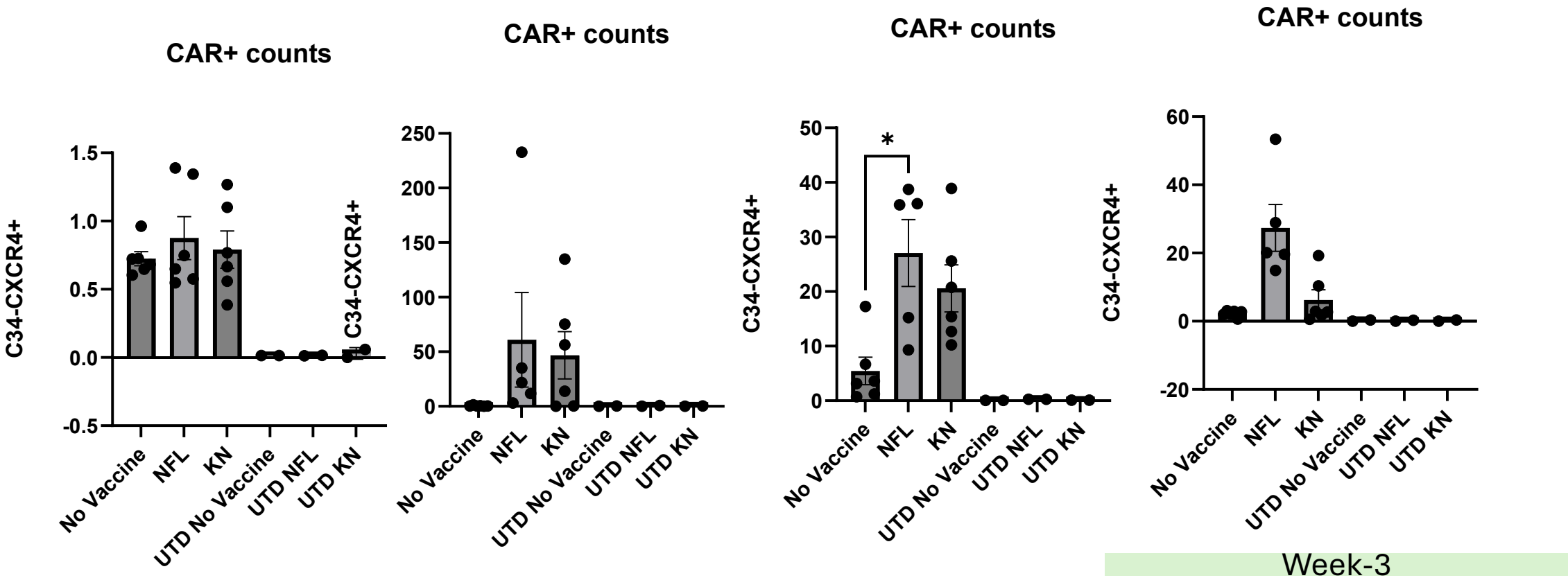


Vipin
Bhardwaj

Pre-vaccination



Stabilized HIV Env Vaccination Results in a More Rapid and Sustained CAR T cell Expansion



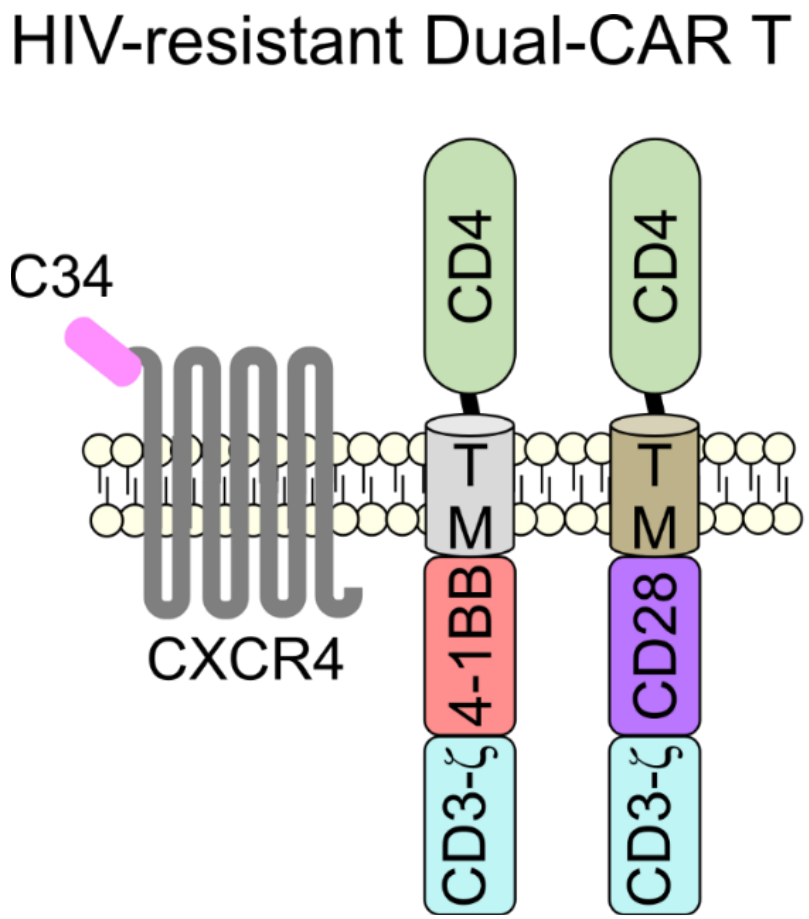
A5419

Two interventions:

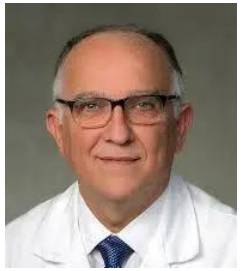
1. Dual Car T cells

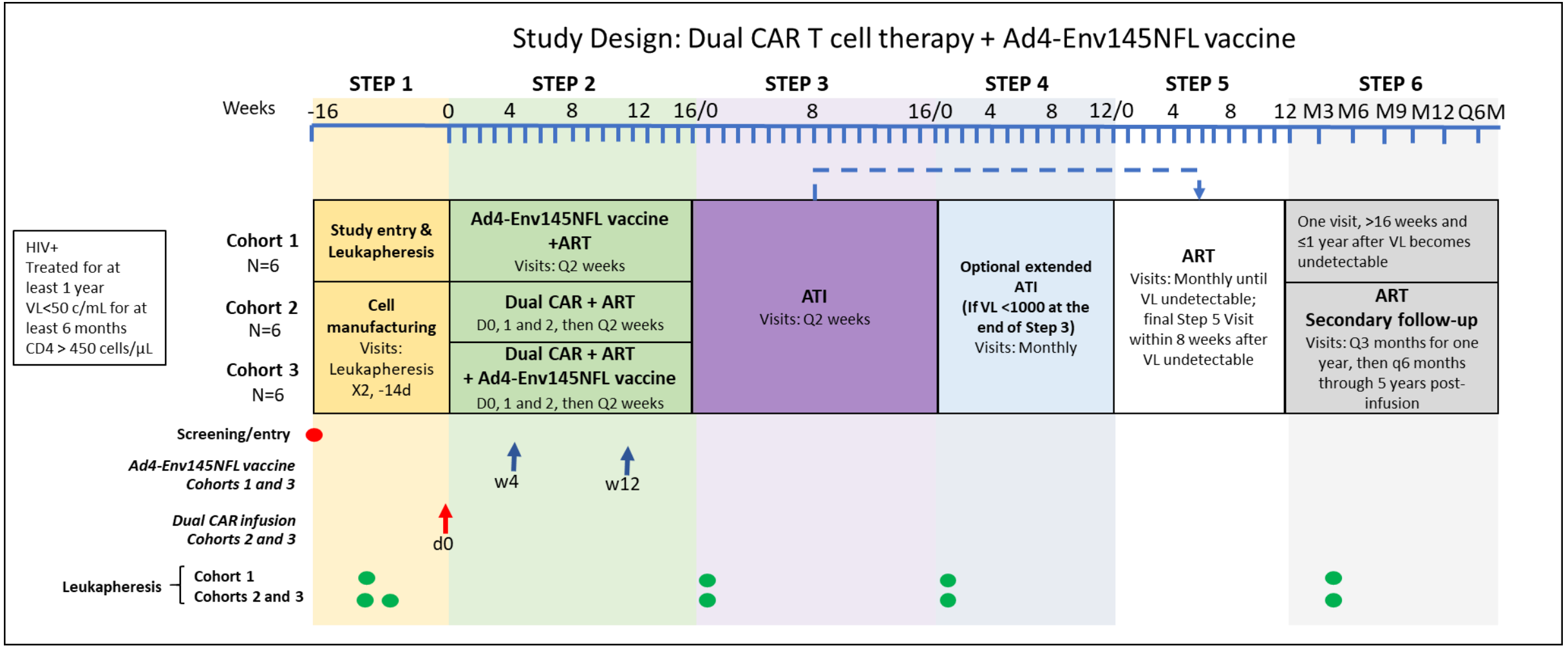
- Better in vivo expansion
- Can recognize lower levels of HIV Env on the cell surface
- Better in vivo activity (Maldini et al Nature Medicine 2020)

- C34-CXCR4 signaling defective (protected from HIV infection) HIV-specific



2. Ad4-Env145NFL vaccine (Ad4 vaccine)




Study Design: Dual CAR T cell therapy + Ad4-Env145NFL vaccine
STEP 1
STEP 2
STEP 3
STEP 4
STEP 5
STEP 6
Weeks
-16
0
4
8
12
16/0
8
16/0
4
8
12/0
4
8
12
M3
M6
M9
M12
Q6M
HIV+
Treated for at least 1 year
VL<50 c/mL for at least 6 months
CD4 > 450 cells/µL
Cohort 1
N=6
Cohort 2
N=6
Cohort 3
N=6
Study entry & Leukapheresis
Cell manufacturing
Visits: Leukapheresis X2, -14d
Ad4-Env145NFL vaccine +ART
Visits: Q2 weeks
Dual CAR + ART
D0, 1 and 2, then Q2 weeks
Dual CAR + ART + Ad4-Env145NFL vaccine
D0, 1 and 2, then Q2 weeks
ATI
Visits: Q2 weeks
Optional extended ATI
(If VL <1000 at the end of Step 3)
Visits: Monthly
ART
Visits: Monthly until VL undetectable; final Step 5 Visit within 8 weeks after VL undetectable
One visit, >16 weeks and ≤1 year after VL becomes undetectable
ART
Secondary follow-up
Visits: Q3 months for one year, then q6 months through 5 years post-infusion
Screening/entry
Ad4-Env145NFL vaccine Cohorts 1 and 3
w4
w12
Dual CAR infusion Cohorts 2 and 3
d0
Leukapheresis
Cohort 1
Cohorts 2 and 3

Acknowledgements

Riley Lab

- Yipin Bhardwaj
- Nancy Liu
- Colby Maldini
- Rachel Leibman
- Yuqi Zhou

Human Immunology Core

- Max Eldabbas
- Emileigh Maddox
- Tanishk Sinha
- Jiayi Shu

CFAR Center for AIDS Research
University of Pennsylvania
Children's Hospital of Philadelphia
The Wistar Institute

George Leslie
Jim Hoxie

Funding:
NIAID U19 Engineering Durable Control of HIV

PO1 Immunoengineering Durable Control of HIV

Carl June
Anne Chew
Bruce Levine

Pablo Tebas
Julie Jadlowsky

BEAT-HIV DELANEY COLLABORATORY

Mike Betts
John Wherry
Rick Bushman
Todd Allen

Luis Montaner
Bob Siliciano

ACTG

Penn Medicine
Center for Cellular Immunotherapies