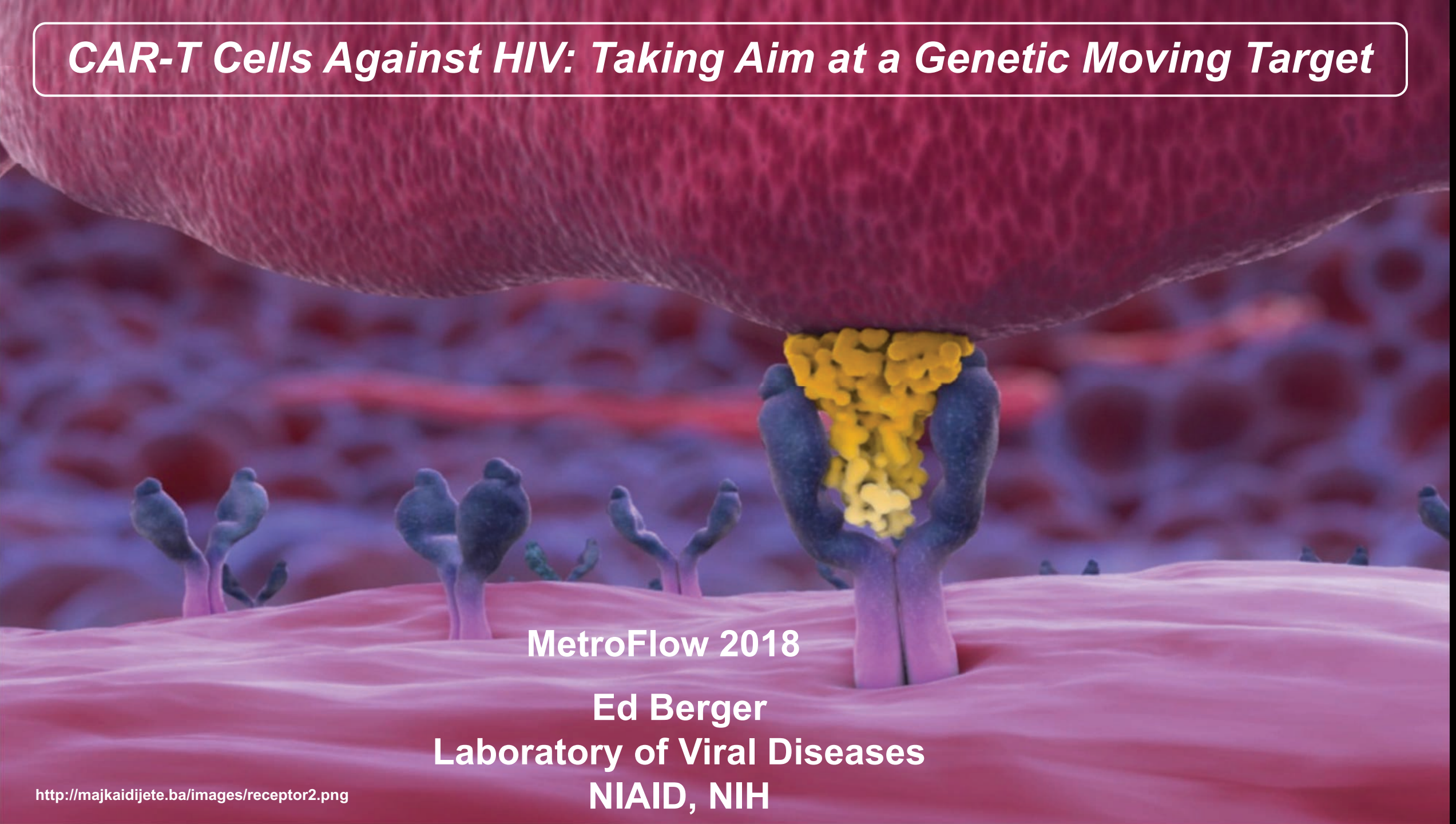
CAR-T Cells Against HIV: Taking Aim at a Genetic Moving Target



MetroFlow 2018

Ed Berger
Laboratory of Viral Diseases
NIAID, NIH

http://majkaidijete.ba/images/receptor2.png

My Credentials for MetroFlow

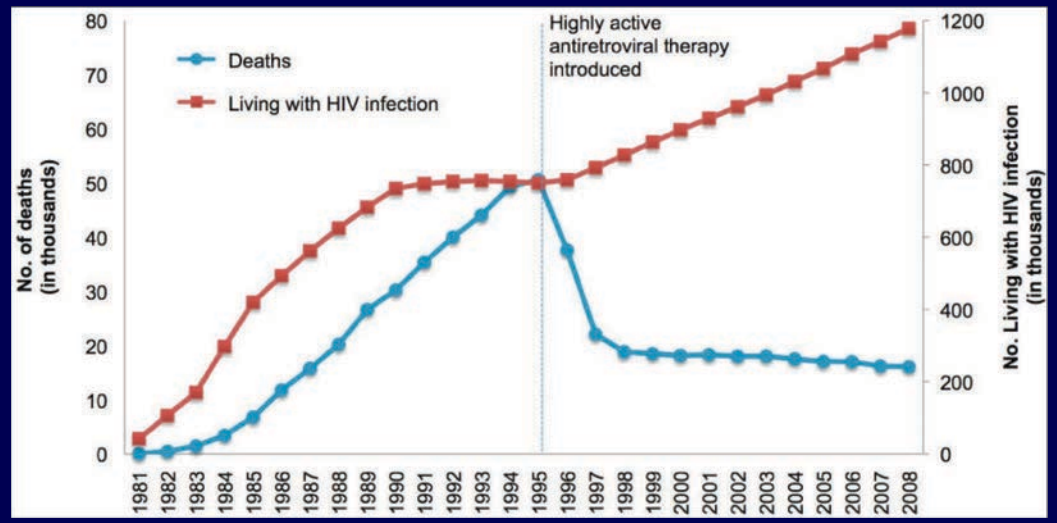
1973 – 1976:
Postdoctoral Fellow
Stanford University School of Medicine
Department of Genetics (Eric Shooter)

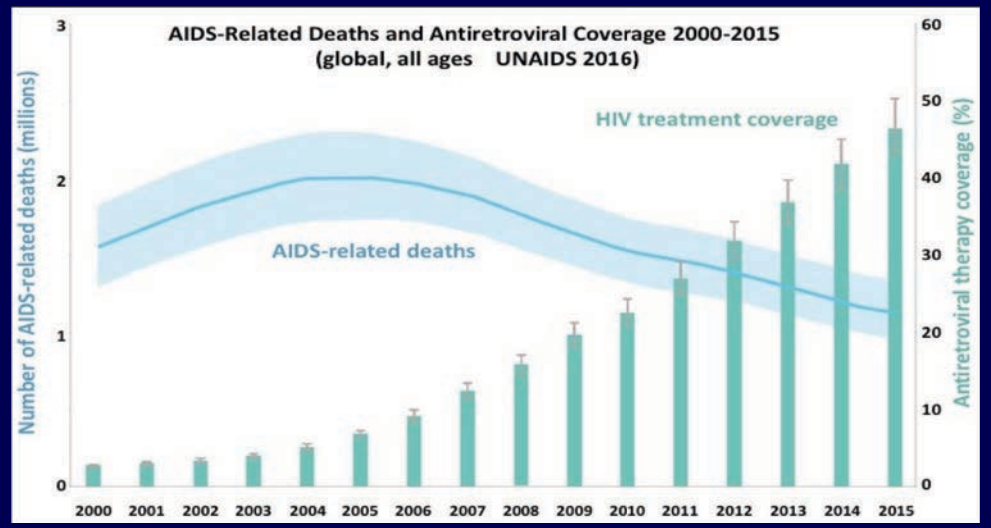
Down the hall from
Len and Lee Herzenberg

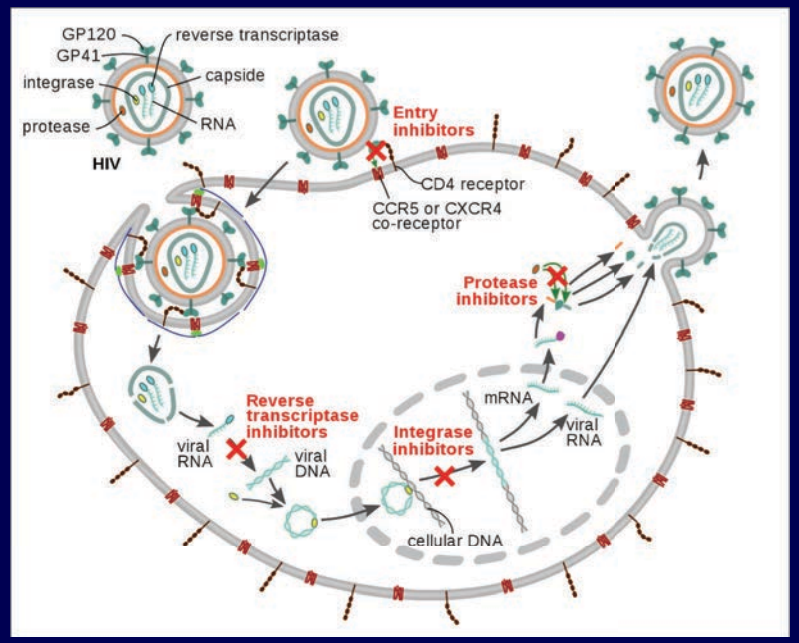
1999:
Member, Inaugural Faculty Search Committee
NIH Vaccine Research Center

Dinner host for
Mario Roederer

Combination Antiretroviral Drug Therapy: 30+ years and still going!



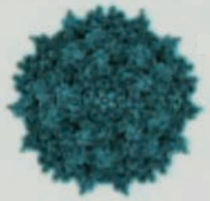




Antiretrovirals: The Next Generation?

Implantable (& removable) antiretrovirals

Vectored delivery of combination antibodies, proteins



Recombinant AAV

But, combo-ART is NOT a cure of HIV Infection

- Reservoirs of infected cells persist
- Antiretrovirals must be taken for life

Despite highly efficacious combination Antiretroviral Therapy, there is great interest in developing a Cure for HIV Infection

Recent developments in the effort to cure HIV infection: going beyond N = 1
Janet D. Siliciano[1] and Robert F. Siliciano[1,2] — The Journal of Clinical Investigation 2016

HIV reservoirs as obstacles and opportunities for an HIV cure
Tae-Wook Chun, Susan Moir & Anthony S Fauci — NATURE IMMNUNOLOGY 2016

The New York Times
The Opinion Pages
Toward an AIDS-Free World
By FRANCOISE BARRE-SINOUSSI and ADEEBA KAMARULZAMAN
Published: November 19, 2012
"More than ever, we need a fourth pillar: an H.I.V. cure."

COUNTDOWN TO A CURE FOR AIDS BY 2020
amfAR

towards an HIV cure
people focused science driven
International AIDS Society

THE RESEARCH FOUNDATION TO CURE AIDS

Grand Challenges | EXPLORATIONS
Design New Approaches to Cure HIV Infection
August 2010
NIH/NIAID Collaboration with Gates Foundation

Martin Delaney Collaboratories for HIV Cure Research (UM1)
RFA-AI-15-029 Dec. 7, 2015
The cure of HIV infection is one of the highest priorities of the NIAID

HARNESSING NOVEL IMAGING APPROACHES TO GUIDE HIV PREVENTION AND CURE DISCOVERIES
NIAID Headquarters, Rockville, Maryland USA — May 8-9 2017

CGT 4 HIV Cure
Cell & Gene Therapy for HIV Cure 2017

IAS HIV Cure & Cancer Forum
22 & 23 July 2017
Institut Curie, Amphi C. BURG
Paris, France

"Sterilizing cure": eradicate all infectious HIV (virus, infected cells) – the "Berlin patient"
"Functional cure": durable remission; enable cessation of HAART for (life-)long periods

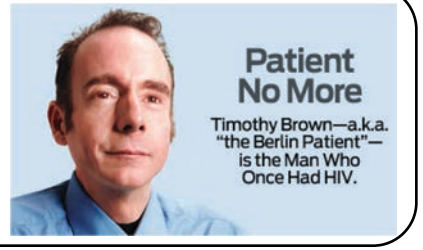
Is an HIV Cure Possible?

✓ **"Berlin patient"**
CCR5 Δ32 HSCT
"HIV-resistant"
immune system

Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation

Kristina Allers,[1] Gero Hütter,[2] Jörg Hofmann,[3] Christoph Loddenkemper,[4] Kathrin Rieger,[2] Eckhard Thiel,[2] and Thomas Schneider[1]

Blood 2011



✗

"Mississippi baby"

Absence of Detectable HIV-1 Viremia after Treatment Cessation in an Infant

Deborah Persaud, M.D., Hannah Gay, M.D., Carrie Ziemniak, M.S., Ya Hui Chen, B.A., Michael Piatak, Jr., Ph.D., Tae-Wook Chun, Ph.D., Matthew Strain, M.D., Ph.D., Douglas Richman, M.D., and Katherine Luzuriaga, M.D.

NEJM 2013

Viremic Relapse after HIV-1 Remission in a Perinatally Infected Child Luzuriaga et al., NEJM 2015

"very early treatment may restrict but not eradicate HIV-1 reservoirs."

An HIV Cure is possible, but the challenges are great (scientific, commercial, social, legal, ethical, etc.)

CURRENT OPINION

Toward an HIV cure based on targeted killing of infected cells: different approaches against acute versus chronic infection

Barna Dey and Edward A. Berger Curr. Opin. HIV/AIDS 2015

Why Targeted Cell Killing as Complement to cART to Achieve and HIV Cure?

- **What cART <u>does</u> achieve:**

  Potent inhibition of viral replication, viral load ⬇

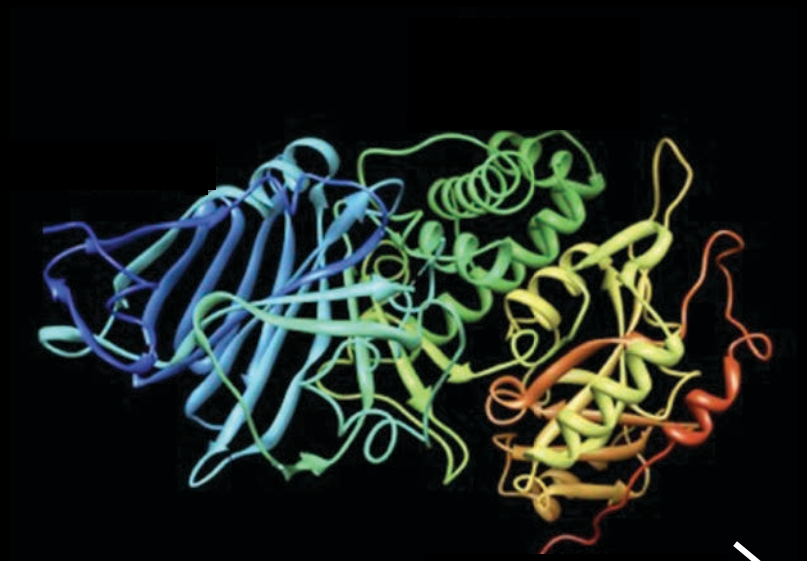
- **What cART <u>does not</u> achieve:**

  Killing of already-infected cells (at least not directly)

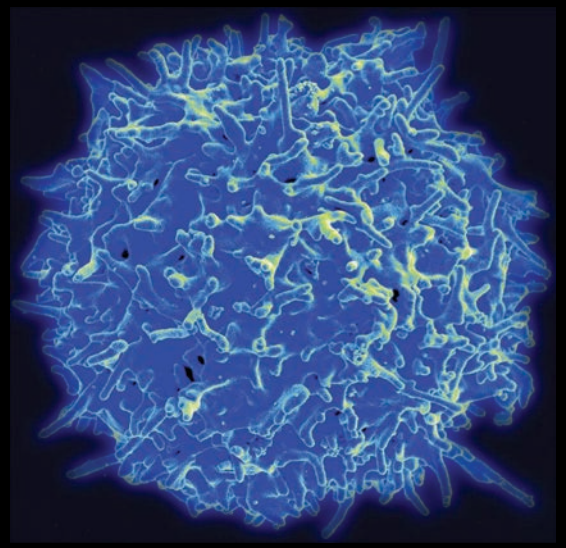
Hence:

Why not complement cART with a treatment that directly kills HIV-infected cells?

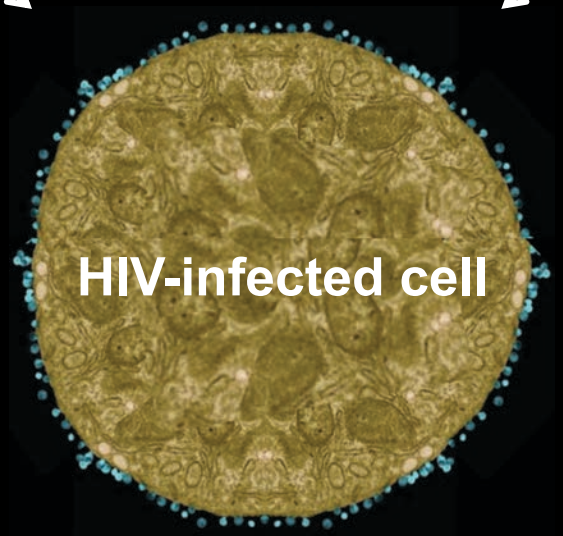
More than 1 Way to Kill an HIV-Infected Cell: Can Any Lead to a Cure?



Targeted cytotoxic protein
e.g. Immunotoxin



Reprogrammed T cell
e.g. Chimeric Antigen Receptor

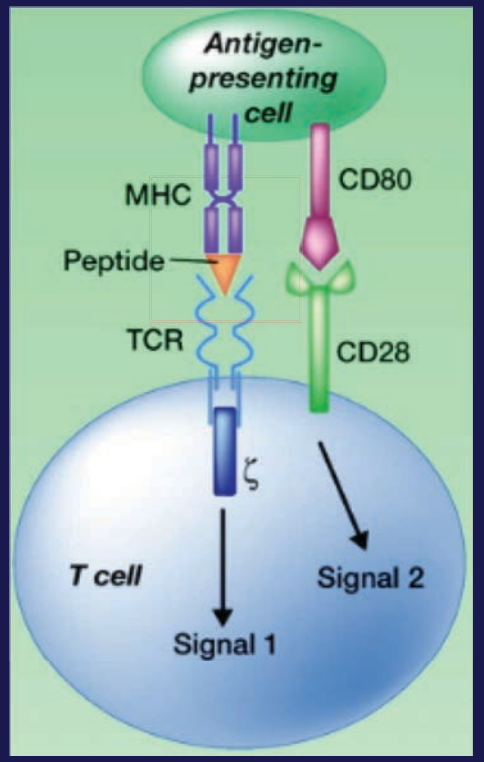


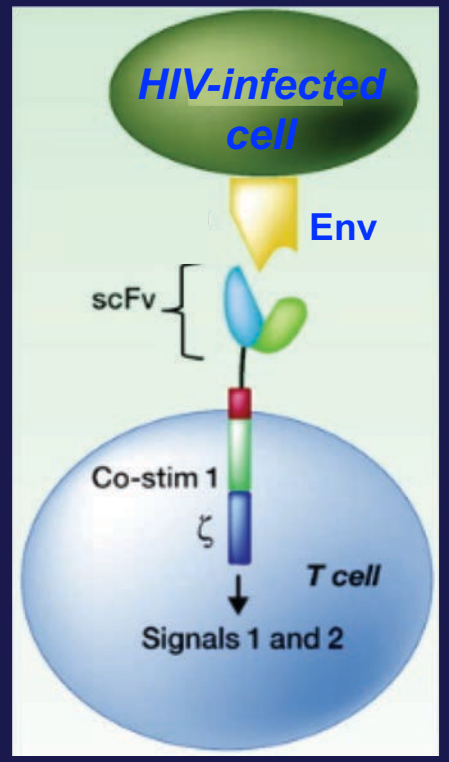
HIV (Functional) Cure: Durable Targeted Cell Killing

Genetically Reprogrammed T Cell

Cloned TCR

HLA-restricted, Ag processing





CAR (2nd Gen)

HLA-independent, no Ag processing

CAR-T Cells: HIV vs. Cancer

Lesser challenge for HIV

- For HIV, there is only 1 molecular target (HIV Env glycoprotein).
  And that target molecule is completely absent from normal human cells.
- For HIV, loss/down-regulation of molecular target = loss of pathogenicity.
  Cancer cells can escape by losing/down-regulating/mutating target molecule.

Greater challenge for HIV

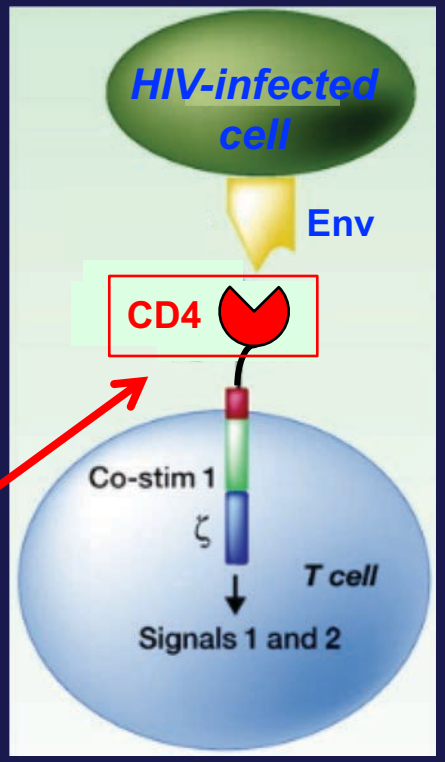
- HIV latency demands very long (life-long?) persistence of CAR function.
  Cancer cure may be achievable even with loss of CAR function.
- With HIV, number of "disease cells" expands not only by cell proliferation, but also by explosive viral bursts and infection of new cells.

Shared challenges

- Location, Location, Location:
  Solid tumors: Hostile local environment
  HIV: persistence in B cell follicles

HIV (Functional) Cure: Durable Targeted Cell Killing

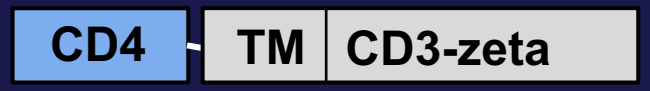
CAR-T Cell



Targeting Moiety:
Ideal features for durable (life-long) activity

- Escape-Proof (extreme breadth)
- Non-immunogenic
- Highly potent

But:
CD4 CARs (1st gen} were tested clinically (early 2000s).



No effects on viral load

CAR-T Cells as a Potential Functional Cure: Our Guiding Principles for Optimal Anti-HIV CAR Design

A functional cure of HIV infection requires durable (life-long?) virus suppression. To achieve this:

CAR must be designed for:

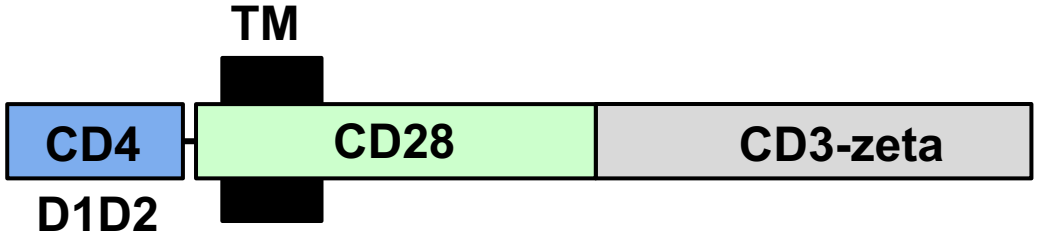
- **High potency**
- **HIV-specificity (no off-target activity)**

CAR design must also strive to approach the ideal qualities of:

- **Inescapable (universal breadth)**
- **Non-immunogenic ("all-human" components)**

Satisfies some requirements, but

- **Sub-optimal potency**
- **CD4 can promote HIV entry**

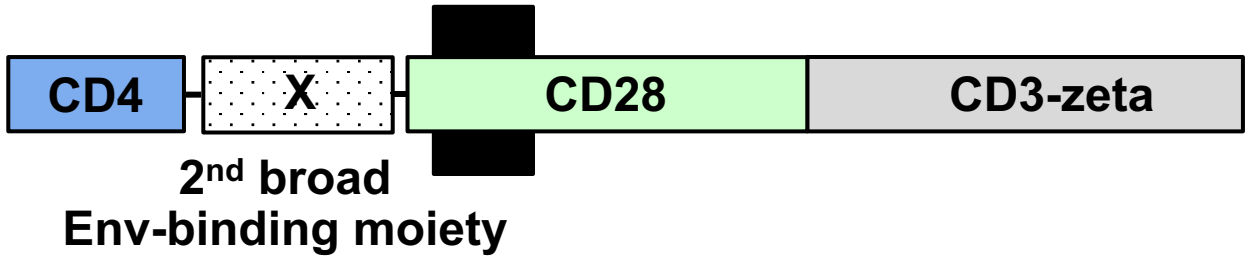


CD4 CAR (2nd gen)

Bispecific CD4-Based CAR

2nd moiety intended to

- **Increase CAR potency**
- **Block entry receptor activity of the CD4 moiety**



Bispecific CD4-X CAR

CD4-scFv Bispecific CAR

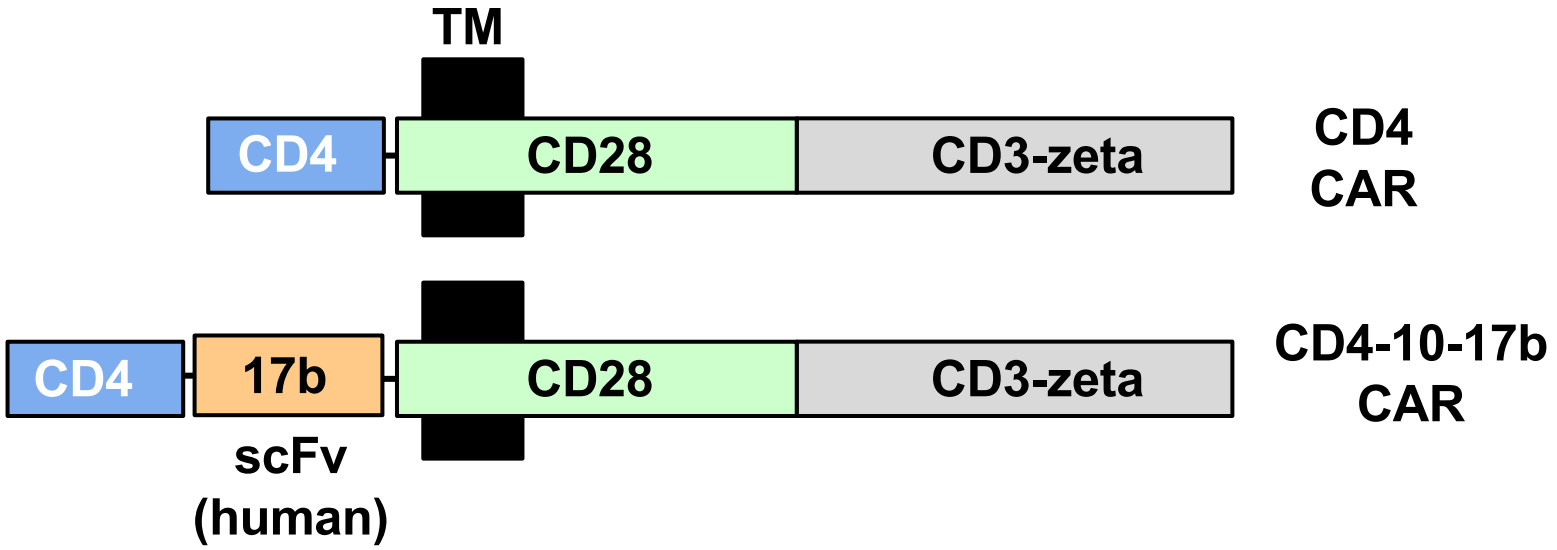
Novel CD4-Based Bispecific Chimeric Antigen Receptor Designed for Enhanced Anti-HIV Potency and Absence of HIV Entry Receptor Activity

Li Liu,[a*] Bhavik Patel,[a] Mustafa H. Ghanem,[a] Virgilio Bundoc,[a] Zhili Zheng,[b] Richard A. Morgan,[b*] Steven A. Rosenberg,[b] Barna Dey,[a] Edward A. Berger[a]

J.Virol. 2015

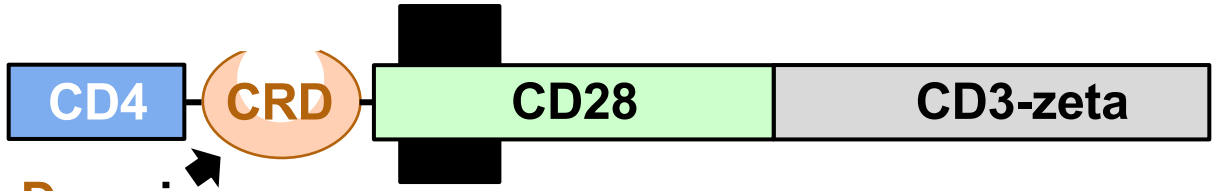
Observed improvements

- ✓ Enhanced potency
- ✓ Breadth retained
- ✓ Blocked CD4-mediated entry receptor activity



✗ **Major Concerns**: Immunogenicity of scFv V regions
Mutational escape from scFv

CD4-CRD Bispecific CAR (all human)



CD4-CRD CAR

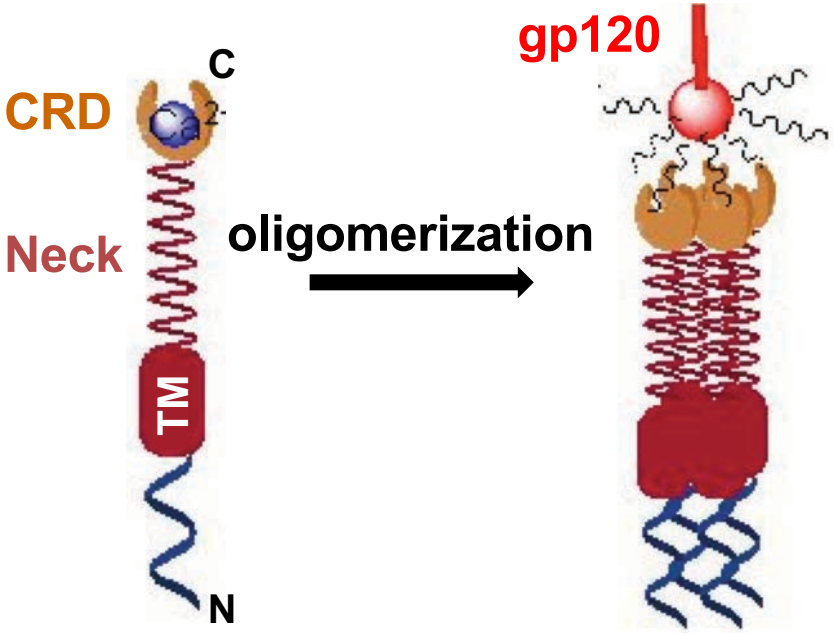
CHO Recognition Domain from human C-type lectin

Anticipated Improvements

- Minimal-immunogenicity (no V-region equivalent)
- Glycan shield essential

Retain

- High potency and High breadth
- No entry receptor activity
- HIV-specificity



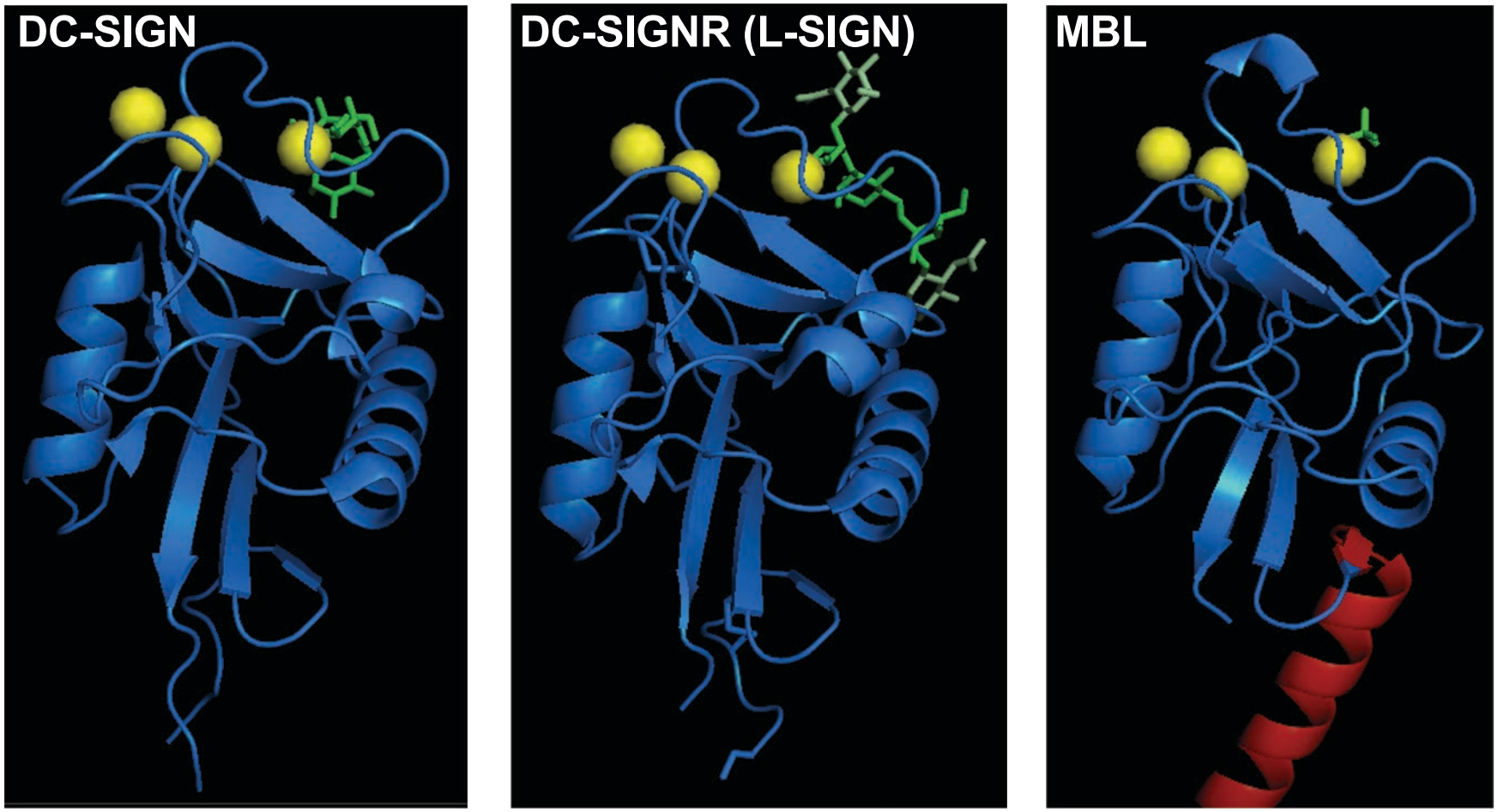
high mannose glycans

human C-type lectin

Multiple Options for CD4-CRD CARs

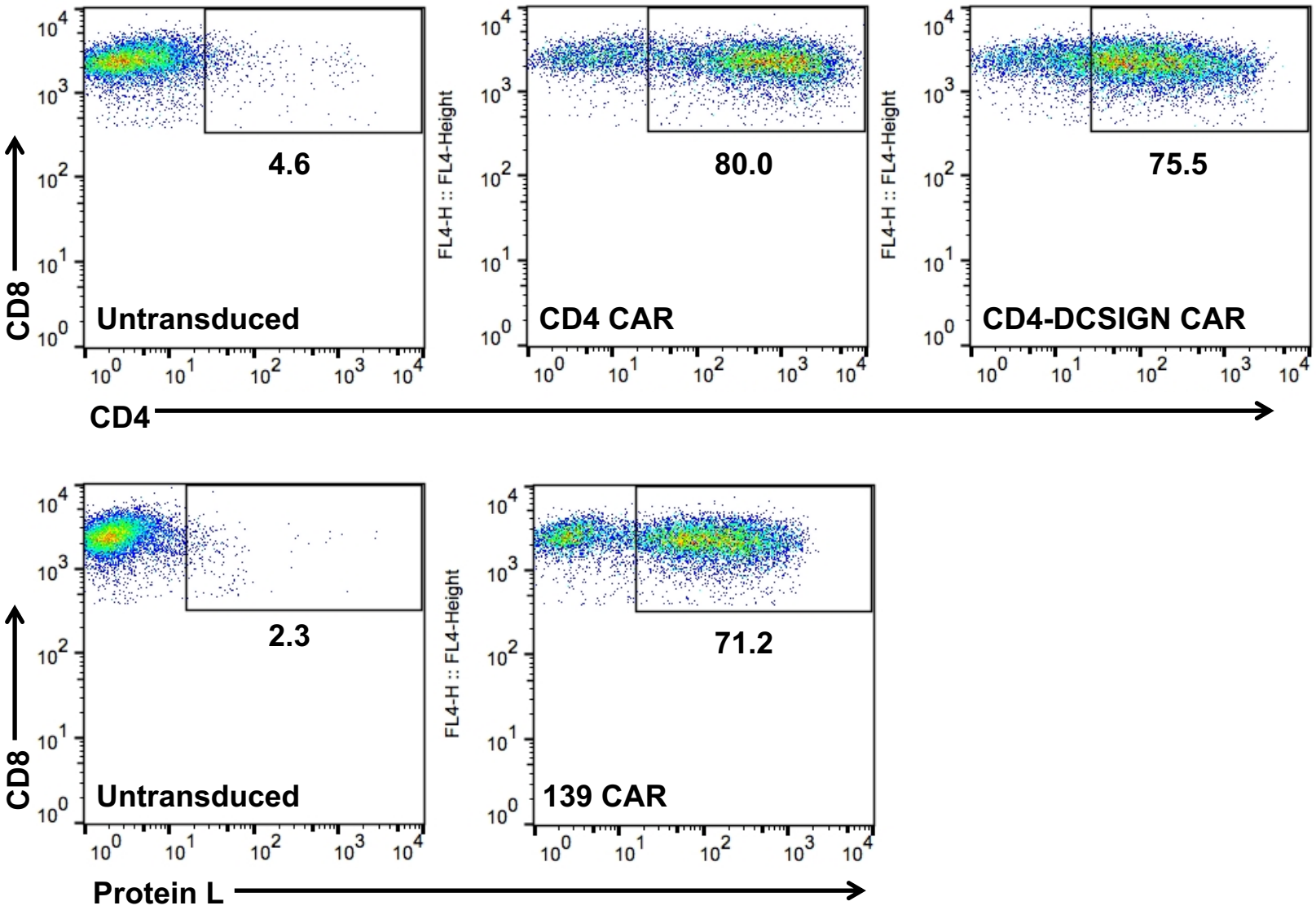
Carbohydrate Recognition Domains (CRDs)

CRD options
- DC-SIGN
- L-SIGN
- MBL
- Langerin



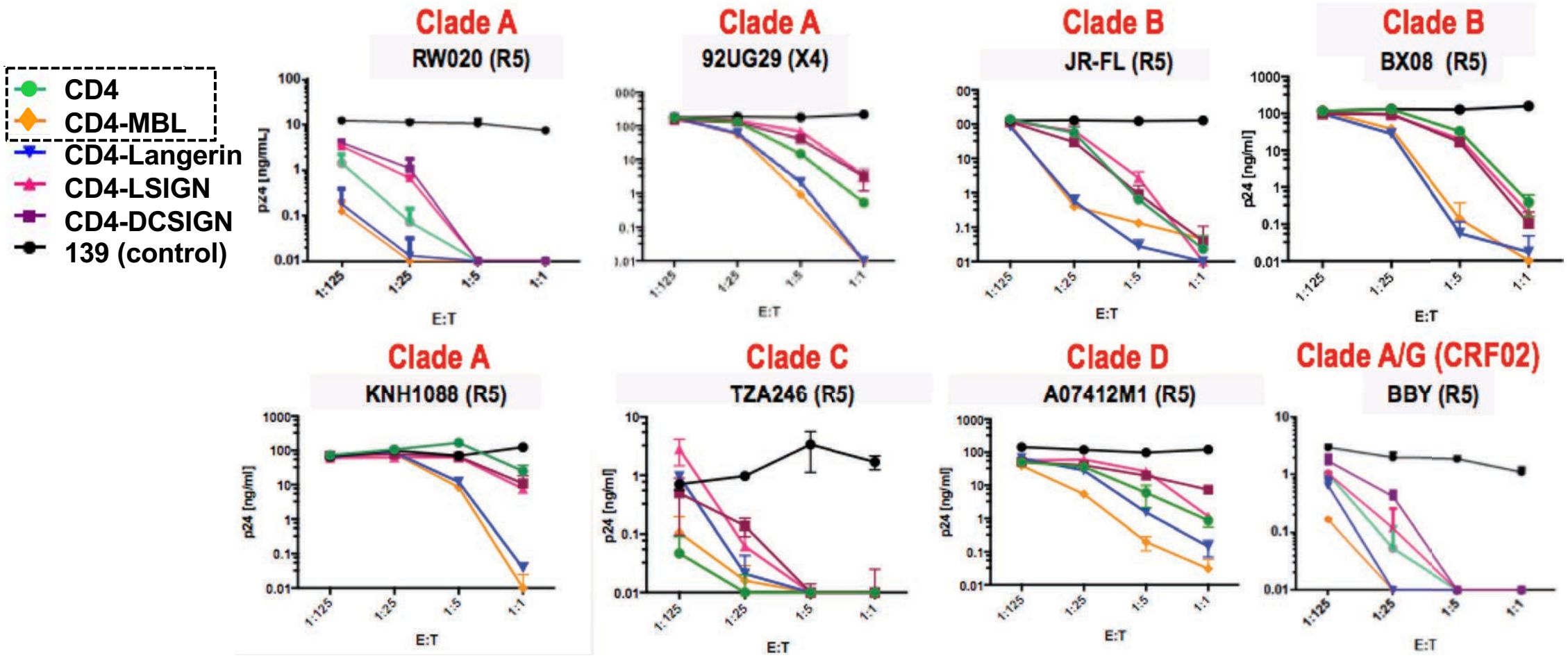
CAR Expression at the Cell Surface

Gtated on CD8 T cells



Potency and Breadth of CD4-CRD CARs Against Spreading Infection by Genetically Diverse HIV-1 Isolates

(Ghanem et al. Cytotherapy 20: 407- 419, 2018)

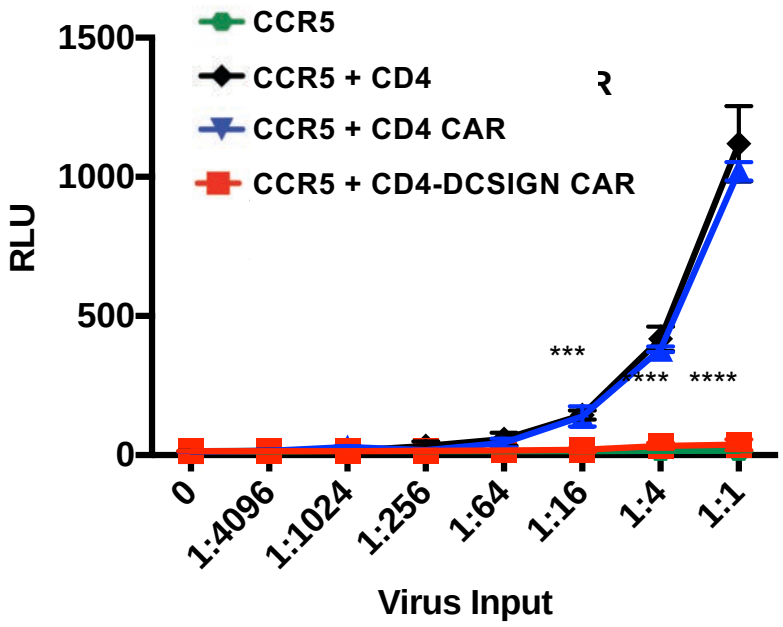


- All CD4-based CARs show potent activity against genetically diverse HIV-1 isolates (2-4 log suppression).
- The CRD moiety blocks the CD4 component from acting as an entry receptor (not shown).
- CD4-MBL CAR is consistently the most potent; in all cases CD4-MBL ≥ CD4 CAR.

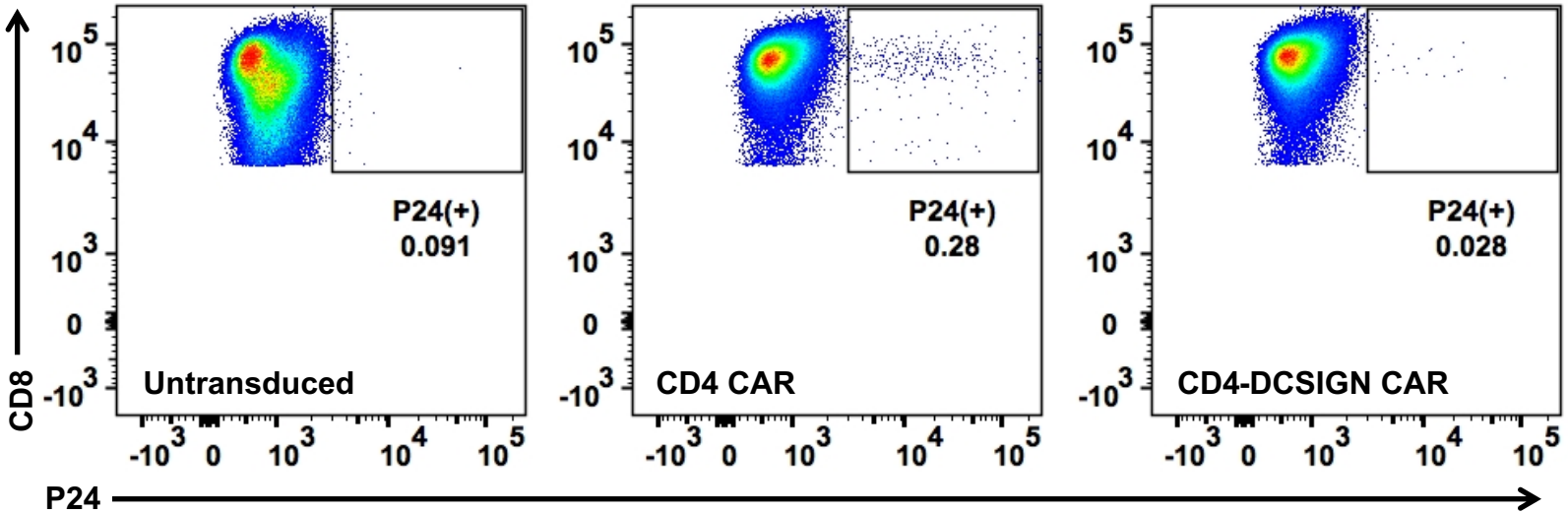
Lectin CRD Moiety Blocks HIV Entry Activity of CD4 Moiety

(Ghanem et al. Cytotherapy 20: 407- 419, 2018)

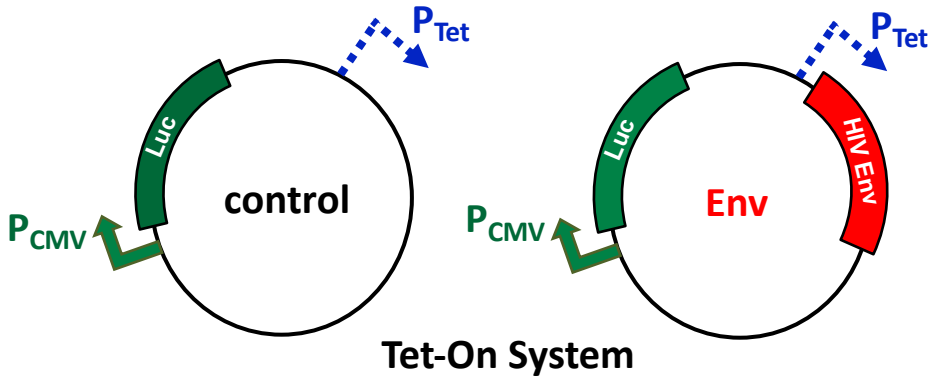
Pseudovirus infection of CAR-transduced targets

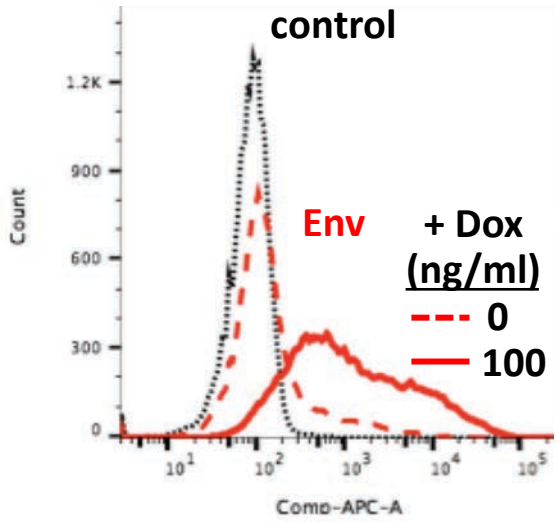


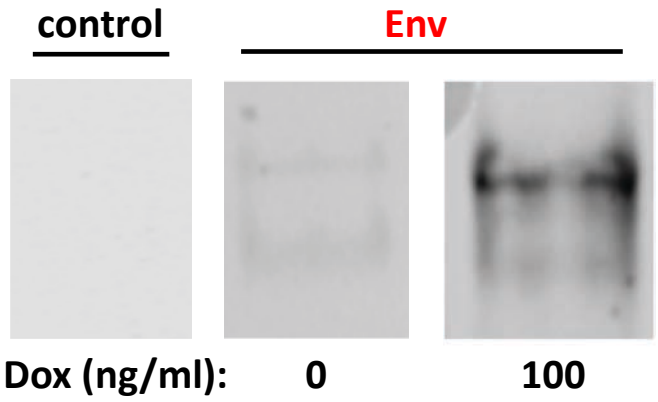
HIV-1 infection of CAR-transduced CD8⁺ T cells

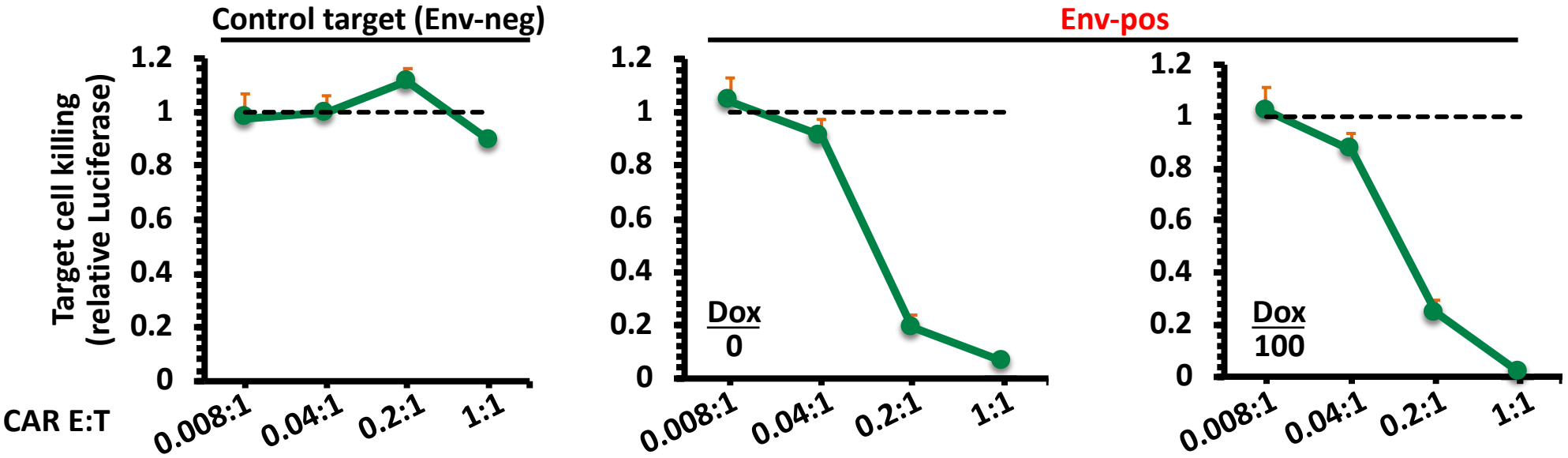


Effect of Env Expression Level on Sensitivity to CAR-T Killing



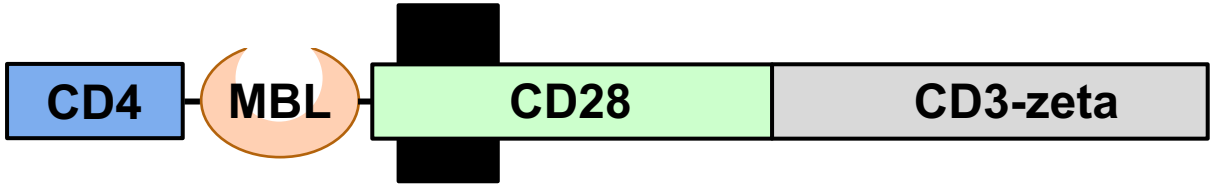




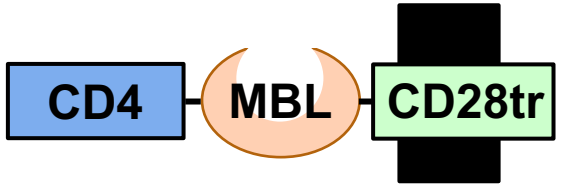


Is HIV Suppression by CAR-T Cells Due to Killing of Infected Cells?

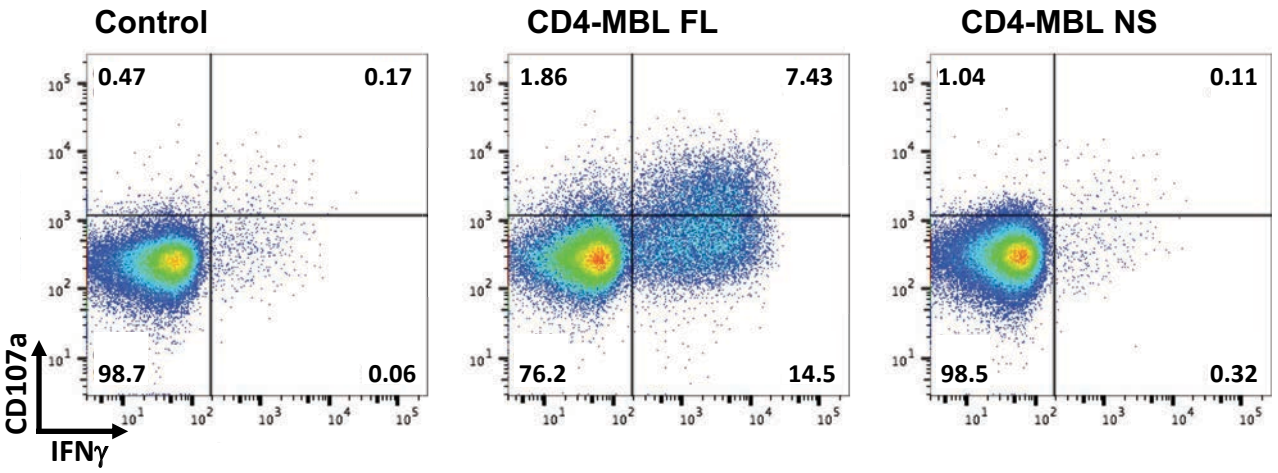
Full Length: CD4-MBL (FL)



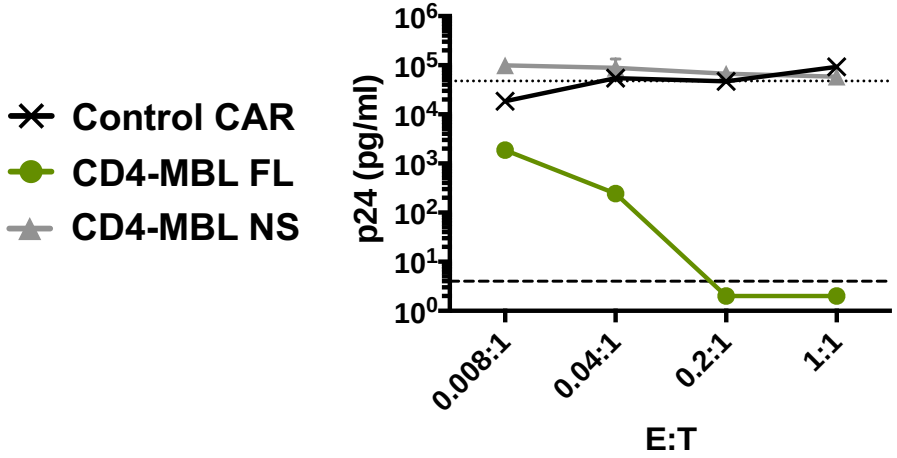
Non-Signalling: CD4-MBL (NS)



Activation



HIV Suppression



- The signaling domains required for CAR-T cell activation are essential for HIV suppression.
- HIV suppression by CAR-T cells reflects killing of infected cells (not simply binding of free virus or shed gp120).

CD4-MBL emerging as the favored CAR for ongoing studies

- Potency: CD4-MBL consistently > CD4, against genetically diverse HIV-1 isolates.
- CD4 entry receptor potential is strongly blocked by lectin moiety.
- Approaches inescapable and non-immunogenic ("all-human").
- Native MBL has additional favorable features for use in a CAR:

  - Soluble protein (not cell surface), at high concentrations in circulation

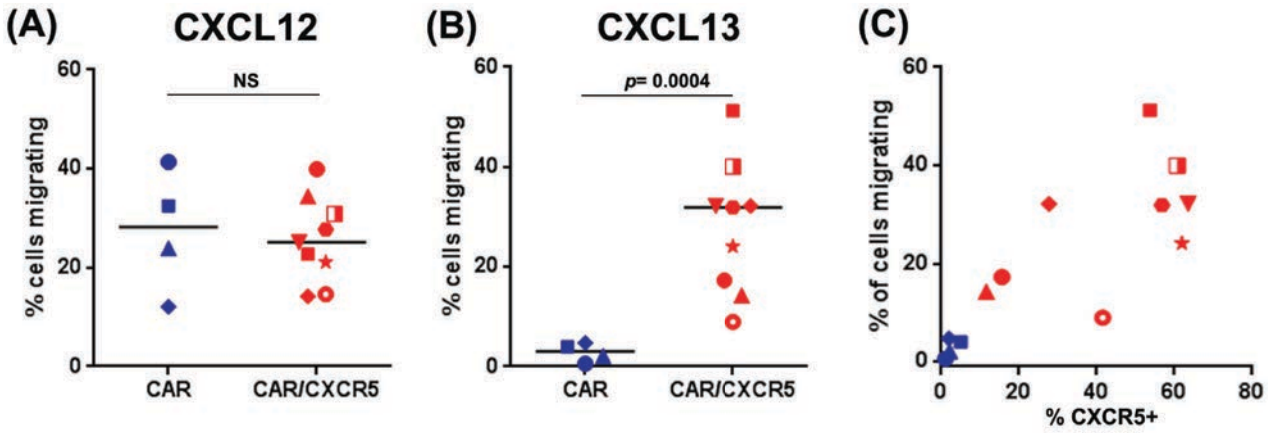
  - Reportedly does not bind to any normal human molecules

    (unlike the other lectins tested)

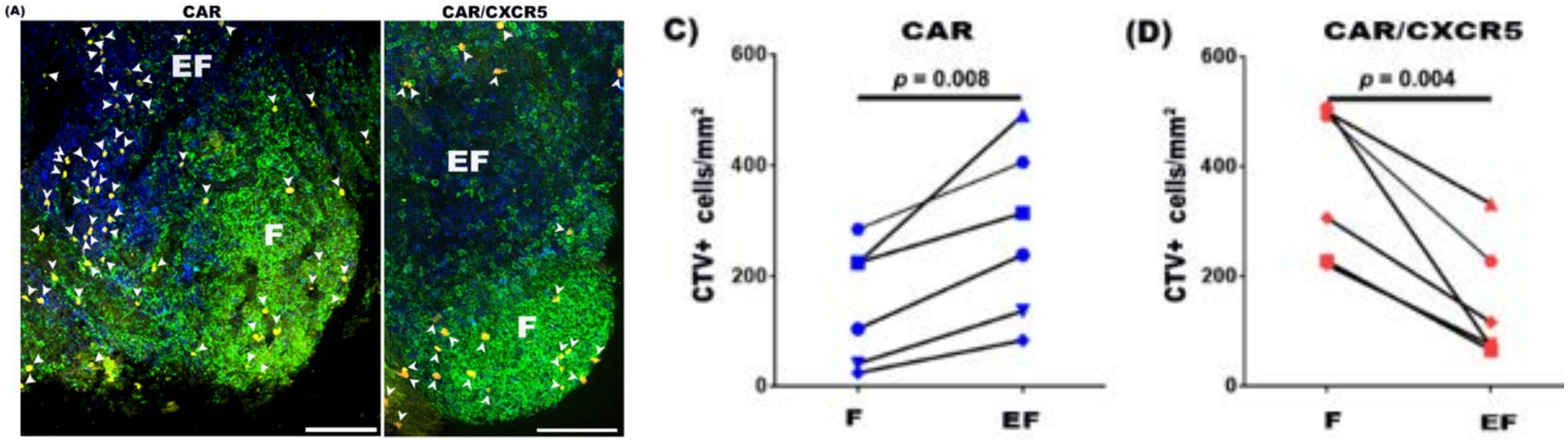
Engineering Preferential Trafficking of CAR-T Cells

(Haran et al. Front. Immunol. 2018)

***In vitro* chemotaxis assay: CXCR5 promotes migration toward CXCL13**



***Ex vivo* lymphoid migration assay: CXCR5 promotes CAR-T cell accumulation in B cell follicles**



From *in* vitro to *in* vivo: Many Challenges

Collaborative Studies: Martin Delaney Collaboratories

BELIEVE – Cornell/Weill, NYC

defeatHIV – FHCRC, Seattle

- **Animal models:** HIV in humanized mice, SIV/SHIV in NHP
- **Location, Location, Location:** Getting CAR-T cells to right place(s)
  - T_{FH} cells in B cell follicle; CNS; other cryptic sites
- **Potency, inescapability, and non-immunogenicity**
  - Will CD4 bispecific CARs make the grade? Direct side-by-side comparisons
- **Persistence of functional CAR-T cells**
  - Durable suppression of HIV - much more demanding than cancer?
  - Many factors:
    - Alternative signaling domain(s)
    - CAR gene insertion – mode, recipient cell type
    - Mode of activation – *ex vivo, in vivo*
    - etc. etc. etc.

CAR-T Cells: Toward an HIV Functional Cure

LVD, NIAID

Agi Hajduczki
Virgilio Bundoc
David Elias
David Danielson
Kaelin Amaya
Li Liu
Bhavik Patel
Mustafa Ghanem
Sara Bolivar-Wagers
Barna Dey
Diego Vargas-Inchaustegui

NCI

Rick Morgan
Steve Rosenberg

BELIEVE MDC Collaboratory

Pam Skinner, U.Minn.
Liz Connick, U. Az.
Brad Jones, George Washington U.

Questions/Directions – Basic (*in vitro*)

CAR potency: Mechanisms/Modifications

- Explanation for extremely high potency of CAR compared to IT?
- Can killing occur based on Env introduced by infecting virion?
- Oligomerization state: Dimerization
- Role of Hinge/TM regions
- Influence of linker length
- Alternate intracellular co-stimulation domains

Mode of CAR-mediated killing

- Can CAR promote killing of cells harboring bound virions (FDC)?
- CAR-expressing $CD4^+$ T cells – How to protect from infection?

Murine Models
(BELIEVE Collaboratory)

R. Brad Jones (GWU)

NSG mice infused with human $CD4^+$ memory T cells (from HIV-pos or HIV-neg donors"

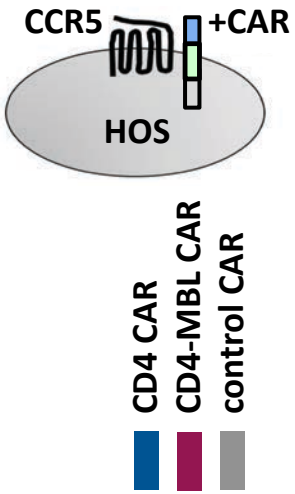
Cath Bollard (National Childrens Hosp)

HXT-NEETs (expanded HIV-specific T cells against 'non-escaped' epitopes

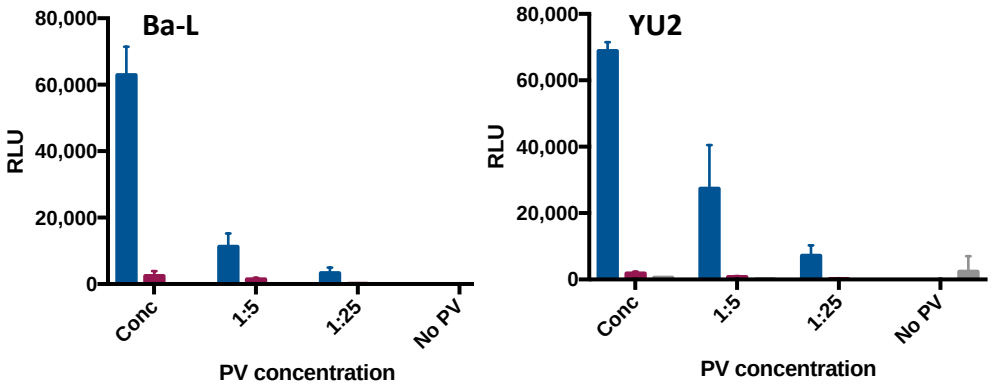
- Test HIV suppression by:
  - CAR-T cells (syngeneic)
  - CAR-T cells vs. HST-NEETs
  - CARs expressed on expanded antigen-specific T cells (HIV, CMV, etc.)

- What rules govern:
  - Efficacy of HIV suppression
  - In vivo expansion and persistence of adoptively transferred cells
  - Effects of variations in CAR design
  - etc. etc. etc.

Can the CD4-MBL CAR Act as an HIV Entry Receptor?



MBL moiety inhibits entry receptor activity of CD4 moiety (HIV pseudovirus particles)



CD4-MBL emerges as the favored CAR for ongoing studies

- **Potency: CD4-MBL consistently > CD4, against genetically diverse HIV-1 isolates**
  CD4-MBL is superior to our previous CD4-scFv CAR (CD4-17b)
- **Potential for the CD4 to act as an entry receptor strongly blocked by the MBL**
- **Potentially inescapable and non-immunogenic ("all-human"). Also have "all-rhesus" version**
- **Native MBL has additional favorable features for use in a CAR:**
  - **Soluble protein (not cell surface), at high concentrations in circulation**
  - **Reportedly does not bind to any normal human molecules (unlike the other lectins tested)**