Designing CAR T cells for HIV: A link between cancer and infectious disease therapy

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Disclosures

• I have no significant financial interest or other relationship with ViiV Healthcare.

• I am a founder and hence own equity in Juno Therapeutics, a company that is devoted to developing genetically engineered T cells for cancer.

• In the last few months, Juno has agreed to utilize its technologies to initiate pre-clinical studies of HIV infection.
Examining the battlefield

• HIV is a disseminated infection in which, even with ART, one can detect HIV RNA in every lymphoid organ; in the nodes, the spleen, the liver, bone marrow, and likely in most of the tertiary lymphoid structures in the rectal and genital tract.

• In tissue based studies, major reservoir of HIV-1 is CD4+ T cells in LN; in untreated outside GC – in ART treated inside GC.

• Peripheral Tfh cells are also enriched in HIV-1:
  – easily infectable, especially PD1+ Tfh
  – likely PD1+ a marker of HIV-1 in Tfh
Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production

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Simian Immunodeficiency Virus Infects Follicular Helper CD4 T Cells in Lymphoid Tissues during Pathogenic Infection of Pigtail Macaques

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Peripheral T Follicular Helper Cells Are the Major HIV Reservoir within Central Memory CD4 T Cells in Peripheral Blood from Chronically HIV-Infected Individuals on Combination Antiretroviral Therapy

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B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers

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Spatial dynamics of HIV infection

- HIV infection occurs in focal areas where CD4+ T cells are “packed together”.

- HIV spreads via cell to cell spread; infection spreads well before clinical “eclipse phase”.

- The K_i between HIV-1 env and CD4 is very, very strong and gp120 causes dysfunction / infection of both T cells and B cells. Thus, cells that are next to reactivating cells and cells that migrate into the milieu become dysfunctional quickly.
Corollary of the above

• Strategies for aborting these localized bursts of HIV reactivation / infection requires rapid recognition and a very potent antiviral effect.

• Strategies that are post-HIV entry or don’t handle “collateral spread” will likely be inefficient.

• Whether one can induce enough “collateral damage” to effectively eliminate the frequency of such bursts is unclear.

• How complete such an effect is, also unclear.
  – Should plasma viremia reduction or “functional cure” be the way to evaluate initial efficacy?
A very basic conundrum

- Endogenous CD8+ T cells are not located in GCs.

- Approaches that induce endogenous CD8+ T cells are unlikely to alter this God given fact of spatial identity.

- Seems like we need a strategy to alter this.
  - genetically engineer a trafficking factor that would allow them to enter or give the cells that normally traffic there anti-HIV activity
CXCR5 for follicular homing

What are Chimeric Antigen Receptor (CAR) T cells?

Basic Concept:

• Link an extracellular ligand recognition domain, typically a single chain fragment variable (scFV), to an intracellular signaling molecule that includes CD3ζ to induce T cell activation upon Ag binding.
  • first generation CAR T cells only CD3ζ – no efficacy

• Second generation that links the signaling endodomains of CD28 4-1BB:OX40 to CD3ζ to provide a second signal; one that mimics co-stimulating provided during normal TCR recognition by Ag presenting cells and required for full T cell activation.

• Current CAR T cells provide co-stimulating in cis – third generation CARS are adding cytokines, e.g. IL-12, IL-15, etc.
Process for generation of CAR T cells for clinical use

Step 1. Leukapheresis +/- Selection of T Cell Subsets

Step 2. Lentiviral or non viral transfer of CAR transgene

Step 3. Expansion/QC Testing

Step 4. Re-infusion

Step 5. Patient/Immune Monitoring
A short review of CAR-T cell therapy for hematologic malignancy

- Impressive data for CD19 CAR T cell in refractory ALL
- High efficacy in refractory ALL - CR> 80% of patients
- Both adult and children
- High response rates in aggressive refractory NHL
Resolution of advanced lymphoma with a low dose of CD-19 CAR T cells comprised of CD8\(^+\) T\(_{CM}\)/CD4\(^+\) Th

Cell Pellet Infused – 2x10\(^5\) cells/kg (CD8 TCM/CD4)

Pre CD-19 CAR-T cell infusion

28 Days Post CD-19 CAR-T cell infusion
CAR T cells and B cell malignancies

- MHC molecules on normal T cells can identify even a single agonist. MHC complex can trigger cytokine production from naïve T cells.
  - one can increase the sensitivity to an antigen by enhancing the co-stimulating signal

- The success of CD19 CAR T cells in B cell malignancies appears to be the high density of CD19 on malignant B cells.

- CD19 expressed at high copy numbers – 10,000 molecules / B cell; CD20 CAR killed at 200 copies.

- Will Ag density of HIV infected T cells be high enough to elicit good killing?
Potency of CARs can lead to side effects

• Cytokine Release Syndrome:
  – seen during rapid proliferation, usually 2-5 hours post infusion
  – hypotension, pulmonary edema, DIC Syndrome
  – higher tumor burden - higher frequency, e.g. seen more commonly in ALL than in NHL
    • 30-40% of persons, 5% mortality (getting better)

• Neurological side effects perhaps dose related
  – pathogenesis poorly understood; incidence quite variable by disease and perhaps by T cell preparation
Other Issues that need solving

• Conditioning with cytotoxic agents such as Cytoxan +/- Fludarabine required for proliferation.
• Functional durability of the cells is highly variable and little understood
• Cell growth rate and conditions/ types of cells utilized are critical and the number and “health” of the cells infused markedly influences efficacy and toxicity
CAR-T cells are detected at higher levels in blood from patients with high tumor burden.
Kinetics of CAR-T cell expansion, migration, and peak blood levels in relation to cell dose
Anti-HIV-1 CAR-T Cells

• Have potential to kill HIV-1 infected cells that have escaped endogenous immune response:
  – targeting viral epitope minimizes the potential for off target side effects
  – CAR T cells not fazed by MHC downregulation strategies of HIV-1

• Can potentially persist for years.

• Can enter the reservoirs (including CNS and LN).
Several groups have constructed anti-HIV-1 CAR-T cells that kill autologous viral infected cells \textit{in vitro}.

Methods to gene protect these cells, including CD4+ CAR-T cells, have been devised.

\textit{Ex vivo} killing of latently reactivated T cells has been demonstrated.
HIV-specific CAR T cells have been given to humans

- First generation CAR T cells using sCD4 as binding site and only a first generation product.
  - linked to CD3ζ

- No significant anti-HIV activity, but long term persistence and safety.
Decade-Long Safety and Function of Retroviral-Modified Chimeric Antigen Receptor T Cells

John Scholler, Troy L. Brady, Gwendolyn Binder-Scholl, Wei-Ting Hwang, Gabriela Plesa, Kristen M. Hege, Ashley N. Vogel, Michael Kalos, James L. Riley, Steven G. Deeks, Ronald T. Mitsuyasu, Wendy B. Bernstein, Naomi E. Aronson, Bruce L. Levine, Frederic D. Bushman, Carl H. June

Fig. 1. Persistence of CD4ζ-modified CAR T cells over 11 years after infusion. (A) Total samples tested at annual visits and the corresponding number of samples with detectable CD4ζ. (B to D) Persistence of CAR T cells for the 43 individual patients in the (B) Mitsuyasu (8), (C) Deeks (9), and (D) Aronson (clinicaltrials.gov NCT01013415) trials at annual visits beginning at 1 year after infusion. The limit of detection (LOD) for the assay is plotted as a dotted reference line.
Gene protect the CD4+ CAR T cell from HIV infection
Figure 4: Selective Killing by Anti-HIV CAR in a mixed population HIV(+) & HIV(-) cells

- **Control CAR (anti-CD19)**
- **Anti-HIV CAR without ΔCCR5**
- **Anti-HIV CAR with independent ΔCCR5**
- **ΔCCR5 Anti-HIV CAR generated by HDR**

**Graph:**
- **Y-axis:** Viral Capsid (μg/mL p24)
- **X-axis:** Day

The graph illustrates the viral capsid production over time for different CAR treatments. The control CAR (anti-CD19) shows the highest viral capsid production, followed by Anti-HIV CAR without ΔCCR5, Anti-HIV CAR with independent ΔCCR5, and finally, ΔCCR5 Anti-HIV CAR generated by HDR, which shows the lowest production.
Interesting second generation HIV-specific CAR T cells have been designed
Novel CD4-Based Bispecific Chimeric Antigen Receptor Designed for Enhanced Anti-HIV Potency and Absence of HIV Entry Receptor

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HIV-1-Specific Chimeric Antigen Receptors Based on Broadly Neutralizing Antibodies

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FIG 8 Efficiencies of novel CAR-transduced primary CD8⁺ T cells against a panel of HIV-1 isolates. CAR-transduced primary CD8⁺ T cells were tested against a panel of 4 subtype B viruses and one subtype C virus (TZA246) to determine the percent efficiency of log suppression, as shown in Fig. 7. For each virus, bars represent the medians for all replicates across 1 to 6 (mean, 2.9) independent experiments, each with triplicates, with standard-error bars. Note that a 30% log efficiency of suppression for a typical experiment with control viral replication of 5 log₁₀ pg/ml would correspond to a reduction of 1.5 log₁₀ units, or 96.8% suppression of viral replication.
Figure 7. Suppression of HIV replication in ex vivo expanded CD4+ T cells by autologous CD8+ CAR T cells

A. Antiviral activity in 2 representative subjects. All 8 studies demonstrate anti-HIV activity.

B. Differences in cytokine production between CD28 and 4-1BB CAR T-cell constructs. Top row, IFN-γ; middle row, TNF-α; bottom row, MIP1-b. Y-axis units x 1000.
Time to move onto answering the more difficult questions

- Will these cells traffic to germinal centers?
- Will they work with low antigen expression?
- Will they really persist?
- Will resistance emerge?
NHP studies

• Studies to utilize anti-HIV sCFv using SHIV model.

• Studies to construct SIV scfV using SIV model.

• Gene protect the cells from HIV infection using CCR5 disruption or inhibit fusion.

• Express CXCR5 to traffic to GC.

• Determine if they can persist and exert antiviral effects for prolonged time period.
Larry’s hypotheses

• Any form of effective immunotherapy of HIV-1 will require the presence of persistent HIV-1 specific CD4+ T cell help.
  - I think this will only occur if one protects these cells from HIV envelope (knock out CCR-5 on both alleles or protect CD4 or fusion site).

• CD4+ T cell help required to enhance CD8+ T cell responses in tissue; this will dial down viral load – necessary for persistence and to deliver effective immunotherapy to replicating virus.

• To eliminate HIV-1 reservoir one will need a resident memory gene protected HIV-1 specific CAR T cell:
  - gains entry to the reservoir site
  - able to recognize and kill early in viral reactivation
  - engineered to stay put and to persist
  - can a gene protected CD8+ T cell do it alone or will one need both CD4 and CD8 gene protected cells?
Conclusion

• HIV-1 CAR specific T cells can be designed to eliminate HIV-1 infected T cells in a way that likely can overcome immune escape.

• They will need to be “protected” from HIV-1 infection.

• They will need to get into immunologically privileged sanctuaries.

• They will need to persist.

• We will need them to not produce severe CRS syndrome or neural toxicity.
Hypothesis continued

• If we can accomplish the above with little toxicity, we will have a novel medically useful form of therapy.

• The technologies to do this are now here. This will require a wide variety of adaptation to the NHP system.

• We will learn by doing.
# Acknowledgements

**Most of My Colleagues and Friends**

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