Enhancement of the CD4 Chimeric Antigen Receptor against HIV infection

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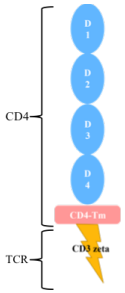
Abstract

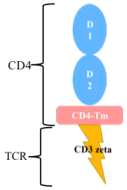
Cell-based gene therapy has quickly become the new frontier for treatment of HIV infection. Clinical trials have shown CD4 chimeric antigen receptor (CD4CAR) modified T cells to be moderately effective against HIV and safe when introduced through adoptive T cell transfer. This CAR expresses CD4 attached to an intracellular CD3 zeta-domain. When bound to the HIV envelope glycoprotein 120 (gp120), CD4 CAR expressing CD8 cells elicit an immune response against infected cells. Despite its success in vitro, T cells modified with the CD4 CAR showed limited efficacy against HIV infection in clinical trials. Studies suggest that CD4 CAR modified CD8 cells may have lost their efficiency due to their susceptibility to HIV infection via CD4 expression, excessive ex vivo activation and cell processing, and immune exhaustion/premorbidity.

To address these problems and attempt to improve CAR-driven T cell responses and prevent cellular infection, we constructed a truncated CD4 CAR molecule that contains solely the D1 and D2 domains of the CD4 molecule. In addition, we incorporated a PD-1 specific shRNA into the vector to knock down check-point inhibitor PD-1 expression on CAR modified cells to attempt to enhance their function. Humanized mice were constructed and infected with HIV and received autologous T cell transplants of modified CD4 and CD8 cells with either a control non-specific shRNA containing D1D2CD4CAR lentiviral vector or a PD1 shRNA containing D1D2CD4CAR lentiviral vector.

We found that the D1D2CD4CAR maintains the HIV Env binding site but does not allow HIV infection when introduced into CD8 T cells. The PD-1 shRNA effectively knocked down PD-1 expression on modified T cells both in vitro and in vivo. Further, we found that the PD-1 shRNA containing D1D2CD4CAR cells have improved function and proliferation. Mice that received PD1 shRNA D1D2CD4CAR cells showed improved viral control as compared to mice that received control CAR modified T cells. Thus, specific knockdown of PD-1 expression on CAR expressing cells can enhance anti-HIV efficacy of the CAR modified cells and highlights the potential of targeting immune check point inhibitors to enhance T cell gene therapy.

Background: CD4zeta Chimeric Antigen Receptor



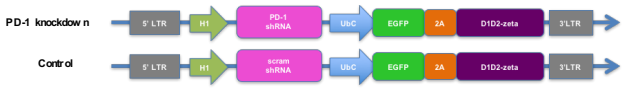


- Extracellular CD4 molecule and transmembrane domains, CD3 zeta signaling domain.
- Recognizes HIV gp120 **independent of HLA restriction.**
- Ligation induces T cell receptor signaling/activation.
- Previously used in peripheral T cells in multiple clinical trials: stable, safe engraftment and persistence >10 years
- Modest clinical efficacy due to functional defects in modified peripheral T cells
- Excessive ex vivo activation and expansion may have rendered CD4 CAR T cells exhausted
- **Allows infection of cells expressing the CAR**

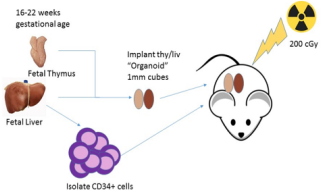
- **Aim: Use a truncated version containing Domain 1 and Domain 2 of the CD4 receptor:**
  - **D1D2CAR protects T cells from HIV infection**
  - **Preserved ability to recognize HIV envelope**
  - **Incorporate a PD-1 knockdown component**
  - **Test effectiveness against HIV infection *in vivo***

Methods

Vector constructs expressing D1D2-zeta CAR:



Mouse model of HIV infection: Humanized BLT mice



- NOD/SCID mice co-transplanted with human liver and thymus tissue along with autologous CD34+ hematopoietic stem cells
- Development of a robust human hemato-lymphoid system
- T cells educated on autologous thymic tissue
- Detectable T and B cell responses to HIV infection

PD-1 is upregulated on anti-HIV modified T cells in HIV infected mice

Fig. 1A: Analysis of PD-1 expression from T cells modified with an anti-HIV TCR during HIV infection

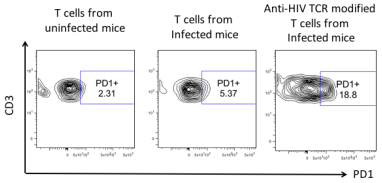
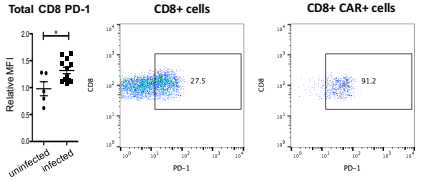


Fig. 1B: Analysis of PD-1 expression of CD8+ T cells modified with a CD4zeta CAR during HIV infection



PD-1 short hairpin RNA is effective in knocking down PD-1

Fig. 2A: Screening for an effective PD-1 shRNA

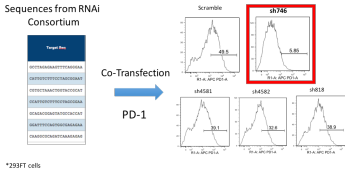
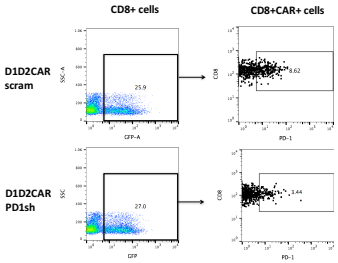
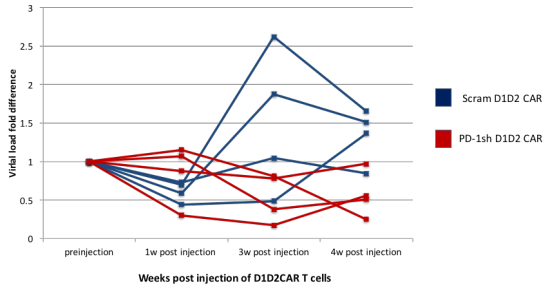


Fig. 2B: PD-1 shRNA knockdown of PD-1 expression in CAR transduced CD8+ T cells



Knockdown of PD-1 on CAR T cells reduces viral load in HIV infected mice

Fig. 4: Viral load fold difference in HIV infected mice treated with scram D1D2 CAR or PD-1shRNA D1D2 CAR T cells



Efficacy of PD-1shRNA D1D2 CAR during HIV infection

Fig. 5A: PD-1shRNA D1D2 CAR enhances viral suppression

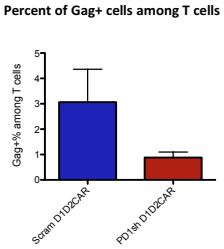
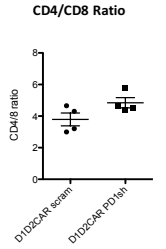


Fig. 5B: PD-1 shRNA D1D2 CAR protects CD4+ T cells from HIV mediated depletion



Detection of D1D2 CAR in peripheral blood during HIV infection

Fig. 3A: *In vivo* experimental approach to test efficacy of PD-1 shRNA D1D2 CAR cells

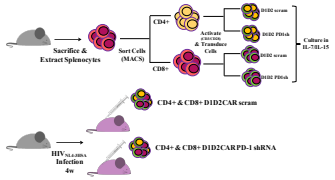


Fig. 3B: D1D2 CAR T cells are maintained in vivo during HIV infection

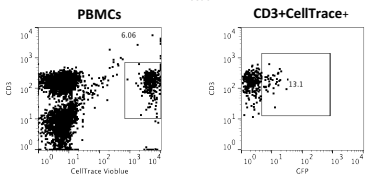
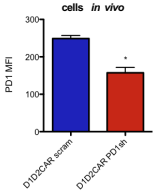


Fig. 3C: Effective knock down of PD-1 on D1D2 CAR cells *in vivo*



Conclusions and Future Directions

- PD-1 shRNA effectively knocks down PD-1 expression on CAR-modified T cells
- Knockdown of PD-1 on CAR-modified cells can enhance viral suppression and protect CD4 cells from HIV mediated depletion
- Perform in depth analysis of D1D2 CAR-modified cells with PD-1 shRNA for *in vivo* proliferation, effector/memory differentiation
- Target other immunosuppressive receptors (Tim-3, LAG-3)
- 2nd generation D1D2 CARs containing costimulatory domains

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