VivoVec: A novel lentiviral-based *in vivo* CAR T cell generation platform with viral particle surface engineering incorporating T cell activating and co-stimulatory ligands

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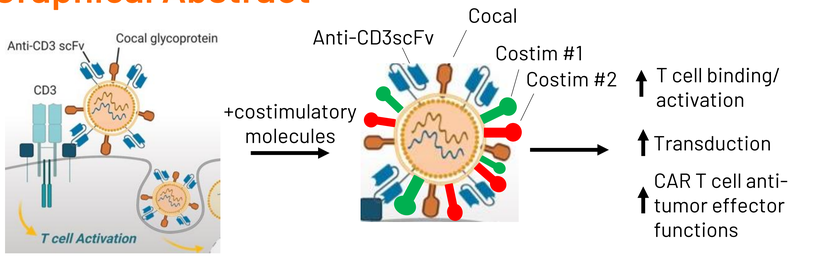
Abstract

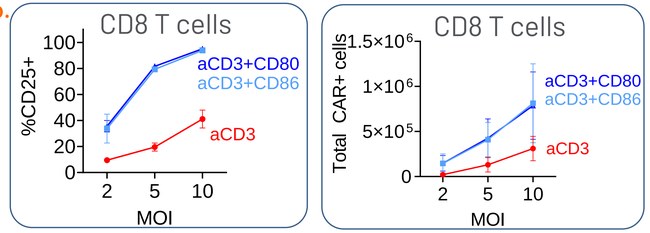
Adoptive cell therapies featuring *ex vivo* expanded autologous T cells engineered to express tumor-targeting chimeric antigen receptors (CAR Ts) have revolutionized the treatment of B cell malignancies, leading to long-term remission in 30-40% of certain patient populations. Despite the promising clinical efficacy of CAR T cells in hematologic malignancies, major limitations hinder their widespread application, including challenges to patient access, complex manufacturing, and high cost. To overcome these challenges, we have developed VivoVec, an engineered lentiviral particle-based platform harboring a CAR transgene that is being developed for off-the-shelf use for the generation of CAR T cells *in vivo*. To achieve specific and efficient *in vivo* transduction, VivoVec particles are pseudotyped with the Cocal fusion glycoprotein and engineered to express T cell binding, activating, and costimulatory ligands. We evaluated multiple novel surface engineering approaches, including incorporation of an anti-CD3 single chain variable fragment combined with a panel of T cell costimulatory ligands, such as CD80, into the particles' surfaces to initiate T cell activation in conjunction with co-stimulation and particle binding to facilitate efficient transduction. CAR T cells generated with VivoVec particles exhibited a less-differentiated, central memory-like phenotype and CAR-antigen specific polyfunctionality, including proliferation and tumor cell killing *in vitro*. Finally, we observed that VivoVec particles generated CAR T-cells *in vivo* with potent antitumor activity in a humanized NSG mouse model of B cell malignancies. Overall, our results suggest that the collective mechanism of action of VivoVec particles to initiate *in vivo* CAR T cell generation and subsequent anti-tumor immune responses is enabled by particle surface-displayed ligands that promote T cell binding, activation, and costimulation, rendering T-cells competent for transduction while optimizing their immunophenotype and function.

Graphical Abstract



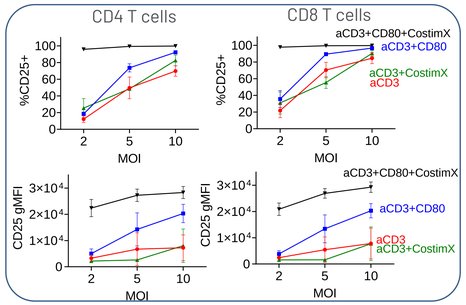
VivoVec lentiviral vector particles contain an anti-CD3scFv and Cocal glycoprotein to facilitate transduction and delivery of chimeric antigen receptors (CARs) to T cells *in vivo*. VivoVec particles can be further augmented by the addition of T cell costimulatory molecules, resulting in enhanced activation and transduction of T cells and increased anti-tumor effector functions of the resulting CAR-T cells.

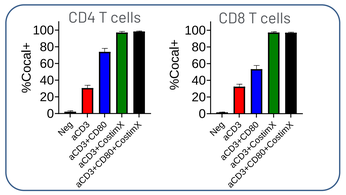
Fig 1. Particles incorporating CD28 costimulatory ligands activate T cells better than particles with CD3scFv alone and generate increased numbers of CAR+ T cells invitro.



VivoVec particles packaging an anti-CD19 CAR containing either aCD3scFV alone or aCD3scFV+CD80 or CD86 were added to 2 PBMC donors. 3 days later activation on CD8 T cells was assed by CD25 expression (**Left**) and 7 days after transduction total CAR+ CD8 T cells we examined (**Right**). Similar data seen for CD4 T cells.

Fig 2. Incorporation of additional costimulatory molecules further enhances T cell activation and binding by VivoVec particles





Left: CD25 expression on T cells 3 days after transduction with VivoVec particles. 3 PBMC donors. **Right:** Viral Binding Assay. Particles cultured with PBMCs for 6 hours followed by surface staining for viral Cocal glycoprotein on T cells. 3 PBMC donors.

Fig 3. Particles incorporating costimulatory molecules increase transduction frequency and T cell expansion

The indicated VivoVec particles packaging an anti-CD19 CAR were added to PBMCs from 3 donors. 7 days later % of T cells (**Left**) and total number of CAR+ T cells/well (**Right**) were analyzed.

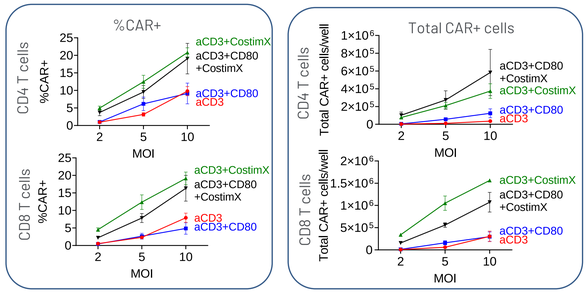
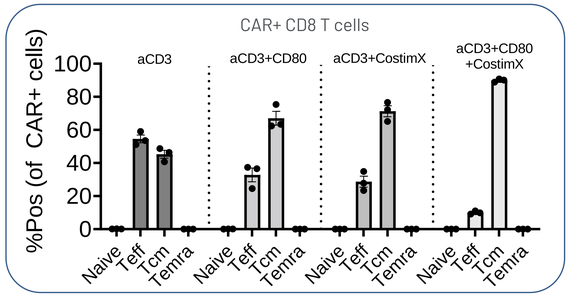


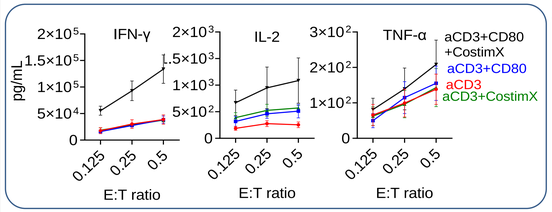
Fig 4. Particles incorporating costimulatory molecules favor generation of T cells with a central memory (Tcm) phenotype

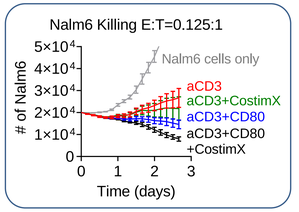


VivoVec particles packaging an anti-CD19 CAR were added to PBMCs from 3 donors. 7 days later phenotype of CAR+ CD8 T cells was assessed using CD45RA and CCR7 surface markers. Phenotypical categories are defined as:

Naïve - CD45RA+CCR7+
Teff - CD45RA-CCR7-
Tcm - CD45RA-CCR7+
Temra - CD45RA+CCR7-

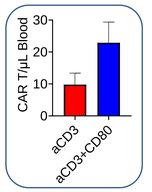
Fig 5. CAR T cells generated with enhanced VivoVec particles exhibit increased cytokine production and cytotoxicity *in vitro*

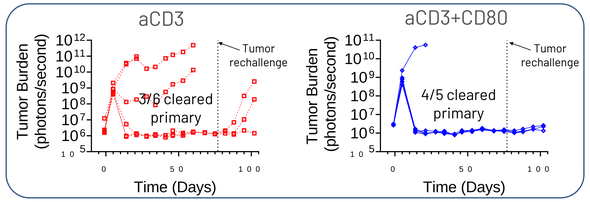




Left: anti-CD19 CAR+ T cells were generated using the indicated VivoVec particles. Anti-CD19 CAR T+ cells were cultured with Nalm6 tumor cells for 22 hours followed by supernatant cytokine analysis by MSD. **Right:** anti-CD19 CAR+ T cells generated with indicated VivoVec particles were cultured with Nalm6 tumor cells at an E:T ratio of 0.125:1. Total Nalm6 tumor cells were tracked over time on an IncuCyte®.

Fig 6. Particles containing costimulatory molecules demonstrate enhanced tumor control in primary tumor response and re-challenge in an *in vivo* tumor xenograft model





NSG MHCI/II KO mice were humanized by injecting 20e6 PBMCs into the intraperitoneal cavity. Mice were then given 50e6 transducing units of the indicated VivoVec lentiviral vector particles packaging an anti-CD19 CAR transgene followed by challenge with 5e5 luciferase+ Nalm6 tumor cells intravenously. 75 days after primary challenge survivor mice were rechallenged with 5e5 Nalm6 cells. The left panel depicts total CAR+ cells per ul blood on Day 11. The right panel depicts tumor burden over time as measured by flux.

Summary

- VivoVec particles incorporating costimulatory molecules in addition to an aCD3scFv enhance particle binding and activation of T cells
- Enhanced VivoVec particles improve T cell transduction and produce greater numbers of T cells in vitro
- CAR T cells generated with enhanced VivoVec particles exhibit a central memory-like phenotype
- CAR T cells generated with enhanced VivoVec particles were more functional in *in vitro* cytokine production and killing assays
- Mice that received enhanced VivoVec particles had greater numbers of CAR+ cells in the blood and exhibited increased tumor clearance – including a robust rechallenge response

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