VivoVec lentiviral vector particles surface-engineered with T cell activating and co-stimulatory ligands enhance *in vivo* CAR T cell generation and antitumor activity

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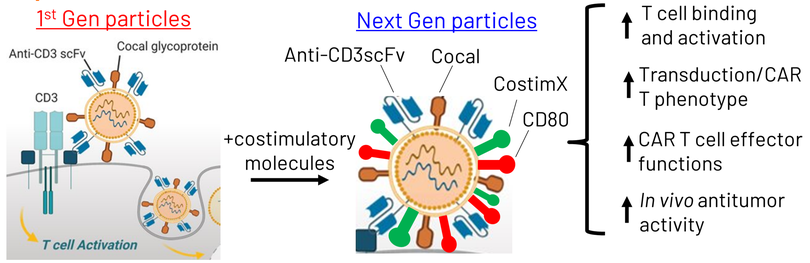
Abstract

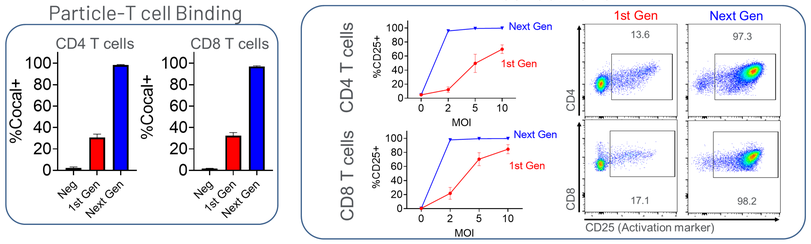
Autologous chimeric antigen receptor (CAR) T cell therapies have revolutionized the treatment of B cell malignancies, leading to long-term remission in 30-40% of certain patient populations. Despite the promising clinical efficacy of CAR T cells in hematologic malignancies, major limitations hinder their widespread application, including challenges for patient access, complex manufacturing, and high cost. To overcome these challenges, we have developed VivoVec, a surface-engineered lentiviral vector-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* T cell transduction, VivoVec particles are pseudotyped with the Cocal fusion glycoprotein and an anti-CD3 single chain variable fragment (scFv), and we have previously shown that these first-generation particles generate CAR T cells *in vivo* that mediate antitumor activity. We have advanced the VivoVec platform through incorporating costimulatory molecules into the particle surface, in addition to the anti-CD3 scFv and Cocal fusion glycoprotein. These next generation VivoVec particles exhibit enhanced T cell binding and activation, resulting in increased transduction and greater numbers of CAR+ T cells *in vitro*. In addition, CAR T cells generated with next generation VivoVec particles exhibited a less-differentiated, central memory-like phenotype and enhanced CAR-antigen-specific polyfunctionality, including cytokine production, proliferation, and tumor cell killing. Finally, in a humanized NSG mouse model of B cell malignancy we observed that next generation VivoVec particles generated greater numbers of CAR T cells in the blood, resulting in enhanced antitumor activity at lower doses compared to first-generation particles. Our results indicate that incorporation of costimulatory molecules onto the surface of VivoVec particles increases both the overall number and functionality of the resulting CAR T cells, greatly augmenting VivoVec mediated CAR T cell generation and antitumor activity *in vivo*. Overall, these data demonstrate that next generation VivoVec particles efficiently generate large numbers of highly functional CAR T cells able to mediate durable tumor control in a preclinical model of B cell malignancy. VivoVec particles have the potential to overcome many of the limitations associated with the current class of CAR T cell therapies.

Graphical Abstract



1st Gen 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*. Next Gen VivoVec particles incorporate T cell costimulatory molecules, in addition to the anti-CD3scFv and Cocal, resulting in enhanced T cell activation and transduction and increased CAR T cell anti-tumor effector function both *in vitro* and *in vivo*.

Fig 1. Next Gen particles demonstrate increased binding to T cells and enhance T cell activation



Left: Vector Binding Assay. Particles cultured with PBMCs for 6 hours followed by surface staining for Cocal glycoprotein on T cells. 3 PBMC donors. **Right:** CD25 expression on T cells 3 days after transduction with VivoVec particles. 3 PBMC donors. Flow plots taken from representative samples at MOI=2.

Fig 2. Next Gen Particles incorporating costimulatory molecules increase transduction frequency and T cell expansion *in vitro*

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.

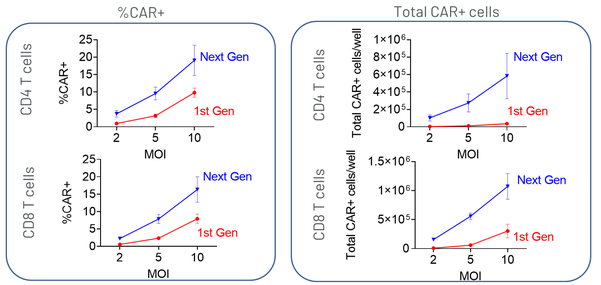
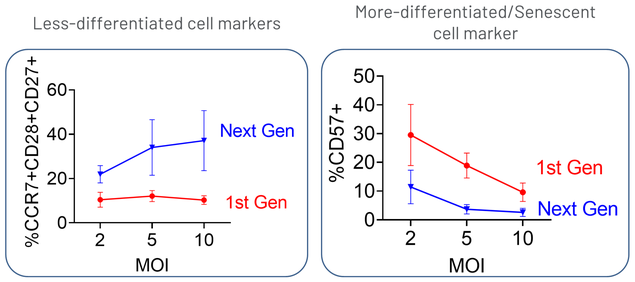
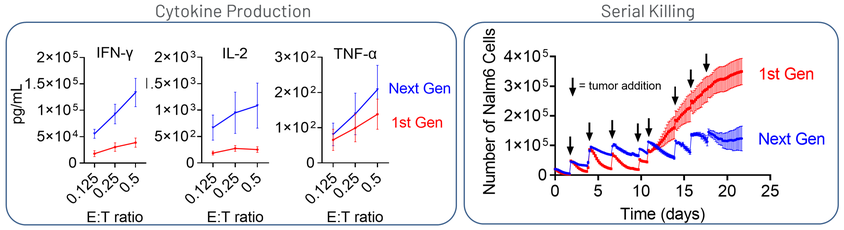


Fig 3. CAR T cells generated with Next Gen Surface-engineered particles display a less differentiated phenotype



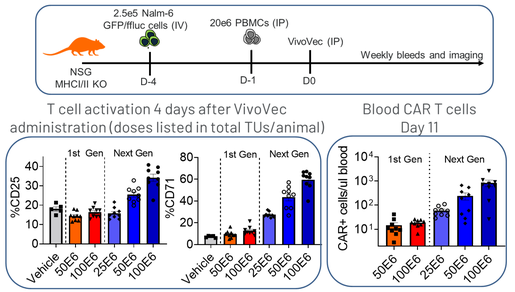
VivoVec particles packaging an anti-CD19 CAR were added to PBMCs from 3 donors. 7 days later phenotype of CAR+ CD8 T cells was assessed using CCR7, CD27, CD28, and CD57 surface markers.

Fig 4. CAR T cells generated with Next Gen VivoVec particles exhibit increased functional responses *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 serial-stimulated with Nalm6 tumor cells every 2-3 days. Total Nalm6 tumor cells were tracked over time on an IncuCyte®.

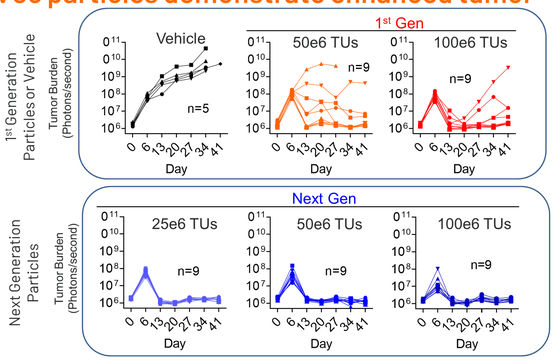
Fig 5. Next Gen VivoVec particles drive dose-dependent T cell activation and transduction *in vivo* in a tumor xenograft model



Top: Study outline. Luciferase+ Nalm6 Tumor-bearing NSG MHCI/II KO mice were humanized by injecting 20e6 PBMCs into the intraperitoneal cavity. Mice were then given varying doses of VivoVec lentiviral vector particles packaging an anti-CD19 CAR transgene. **Left:** Activation makers on CD3+ T cells in the blood 4 days after VivoVec administration. Doses in TUs (Transducing Units). **Right:** Total anti-CD19 CAR+ CD3+ T cells found in the blood on Day 11.

Fig 6. Next Gen VivoVec particles demonstrate enhanced tumor control *in vivo*

Luciferase+ Nalm6 Tumor-bearing NSG MHCI/II KO mice were humanized by injecting 20e6 PBMCs into the intraperitoneal cavity. Mice were then given varying doses of VivoVec lentiviral vector particles packaging an anti-CD19 CAR transgene. Tumor burden was assessed as total flux and tracked over time using an *In vivo* imaging system (IVIS®).



Summary

- Next Gen VivoVec particles incorporating additional T cell costimulatory molecules enhance particle binding and T cell activation
- Next Gen VivoVec particles improve T cell transduction and produce greater numbers of T cells *in vitro*
- CAR T cells generated with Next Gen VivoVec particles exhibit phenotypic properties associated improved persistence
- CAR T cells generated with Next Gen VivoVec particles have greater antigen-specific cytokine production and *in vitro* tumor killing capacity
- Next Gen VivoVec particles generated more CAR+ T cells in the blood and enhanced antitumor activity *in vivo*, at lower doses than 1st Gen particles, potentially enabling lower clinical doses
- See more exciting work from Umoja at Posters **#366** and **#375**

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