Abstract
Adaptive cell therapies featuring or using expanded autologous T cells engineered to express tumor-targeting chimeric antigen receptors (CARs) have demonstrated the potential for treatment of a broad range of cancer patients. 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 fusin glycoprotein and engineered to express T cell binding activating, and costimulatory ligands. We evaluated multiple surface engineering approaches, including incorporation of an anti-CD3 single chain fragment complexed with a panel of T cell costimulatory ligands, such as CD80, into the particles to initiate T cell activation in conjunction with stimulation and particle binding to facilitate efficient transduction. CAR T cells generated with VivoVec particles exhibited a less-differentiated and memory-like phenotype and CAR antigen specific polyclonality, including proliferation and tumor cell killing in vitro. Finally, we observed that VivoVec particles generated CAR T cells in vivo with potent antitumor activity by genetically engineered NSG mouse model of B cell malignancies. Overall, our results support a collective mechanism of action of VivoVec particles to initiate in vivo CAR T cell generation and subsequent antitumor immune responses is enabled by particle surface-displayed ligands that promote T cell binding, activation, and costimulation, rendering T cells competent for treatment while optimizing their immunomodulatory functions.

Graphical Abstract

VivoVec introduces a CAR T cell generation platform with viral particle incorporating additional costimulatory molecules increasing cytokine production and cytotoxicity in vivo xenograft model

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+CD28 or CD80 were added to PBMC donors. 7 days later activation on T cells was assayed by CD25 expression (Left) and 7 days after transduction total CAR+ T cells we examined (Right). Similar data seen for CD8 T cells.

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

The indicated VivoVec particles packaging an anti-CD3scFv and 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. Particles incorporating costimulatory molecules increase transduction frequency and T cell expansion

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

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 generate 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 vitro cytokine production and killing assays
• Mice that received enhanced VivoVec particles had greater numbers of CAR+ cells in the blood and inhibited increased tumor clearance - including a robust rechallenge response

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

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

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

Left: anti-CD3 CAR+ T cells were generated using the indicated CARtransgene containing particles. Anti-CD19 CAR T cells were cultured with Nalm6 tumor cells for 22 hours followed by supernatant cytokine analysis by MSD. Right: anti-CD3 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.