Potent *in vivo* CAR T cell generation and durable antitumor activity in preclinical models using VivoVec, a surface-engineered lentiviral vector platform

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Poster # 1441

Abstract

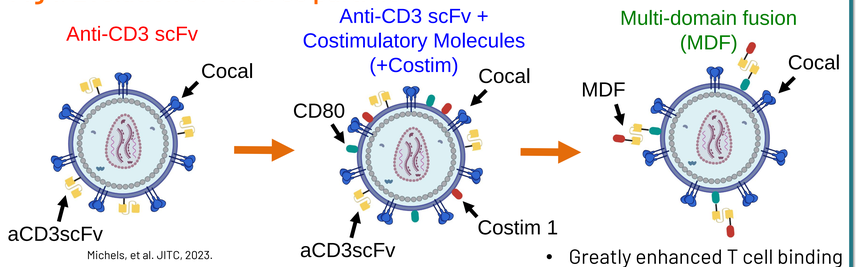
Rationale: Autologous chimeric antigen receptor (CAR) T cell therapies have revolutionized the treatment of B cell malignancies, leading to long-term remission in a substantial number of patients. Despite the promising clinical efficacy of CAR T cells in hematologic malignancies, major limitations hinder their widespread application, including challenges for patient access, complex manufacturing, and high cost. Therefore, novel CAR T cell therapies are necessary to enhance patient access to this powerful new form of immunotherapy.

Goals: Umoja Biopharma is developing a novel off-the-shelf surface-engineered lentiviral vector platform for *in vivo* CAR T cell generation, termed VivoVec. To achieve specific and efficient *in vivo* T cell transduction, VivoVec particles are surface-engineered with the cocal fusion glycoprotein and incorporate an anti-CD3 single chain variable fragment (scFv) and T cell costimulatory ligands on the particle surface to promote T cell binding, activation, and transduction.

Results: VivoVec particles have greatly enhanced T cell binding and CAR T cells generated with VivoVec particles exhibited a less-differentiated, central memory-like phenotype and enhanced CAR-antigen-specific polyfunctionality, including cytokine production, proliferation, and tumor cell killing *in vitro*. In a humanized NSG mouse model of B cell malignancy we observed that VivoVec particles generated substantial numbers of CAR T cells in the blood, resulting in potent and durable antitumor activity at low doses following direct *in vivo* administration.

Furthermore, the high avidity of VivoVec particles to T cells allows for VivoVec particles to be delivered via an extracorporeal gene delivery (ECGD) system. The ECGD system allows for controlled delivery of VivoVec to T cells and is compatible where lymphodepleting chemo may be required. We show that the ECGD system can produce substantial numbers of CAR T cells in the blood, resulting in potent antitumor activity in vivo.

Fig 1. Evolution of VivoVec particles

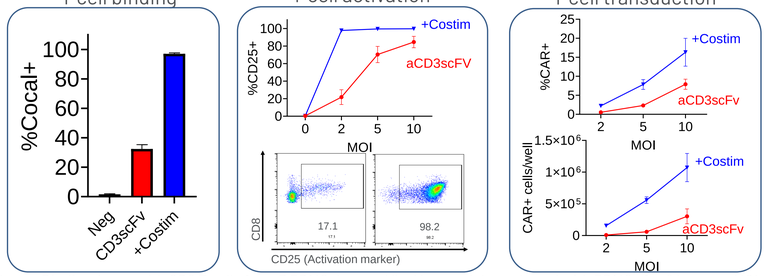


- T cell activation and transduction
- *In vivo* efficacy

- Enhanced T cell activation and transduction
- Greater T cell functionality
- Greater *In vivo* efficacy

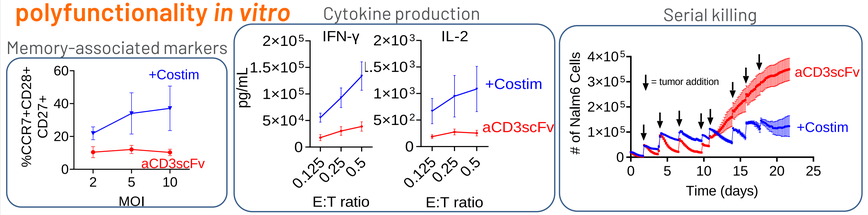
- Greatly enhanced T cell binding and transduction
- *In vivo* antitumor efficacy at lower doses
- Enables extracorporeal delivery

Fig 2. Addition of costimulatory molecules to VivoVec increased particle-T cell binding, activation, and transduction of T cells *in vitro*



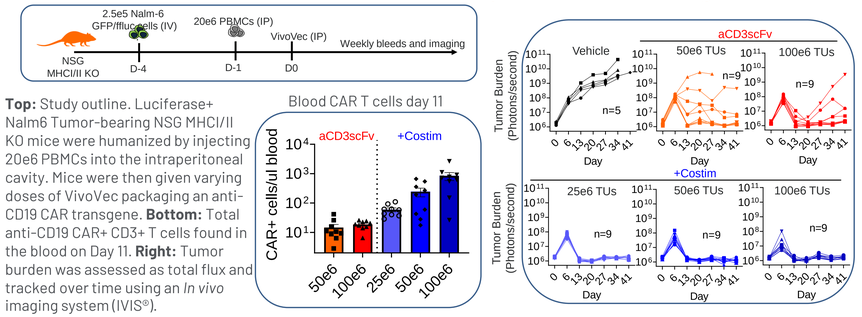
Left: Vector Binding Assay with CD8 T cells from 3 PBMC donors. VivoVec particles cultured with PBMCs for 6 hours at MOI=10 followed by surface staining for Cocal glycoprotein on CD8 T cells. **Center:** CD25 expression on CD8 T cells from 3 PBMC donors 3 days after transduction with VivoVec particles. Flow plots taken from representative samples at MOI=2. **Right**: VivoVec particles packaging an anti-CD19 CAR were added to PBMCs from 3 donors. 7 days later CAR expression was assessed on CD8 T cells.

Fig 3. CAR T cells generated with VivoVec containing costimulatory molecules display a less differentiated phenotype and enhanced polyfunctionality *in vitro*



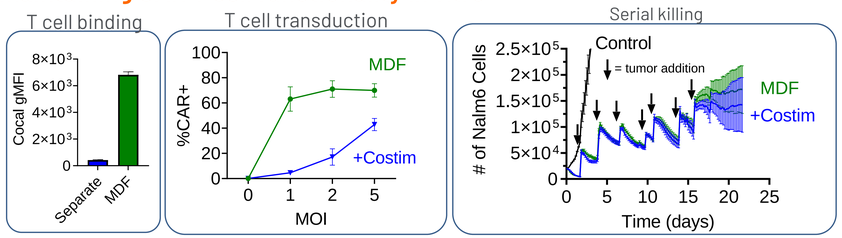
Left: 7 days after transduction, anti-CD19 CAR+ CD8 T cells were assessed for CCR7, CD28, and CD27 expression by flow cytometry. **Center:** anti-CD19 CAR+ T cells were generated using the indicated VivoVec particles and cultured with Nalm6 tumor cells for 22 hours followed by supernatant cytokine analysis 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 4. VivoVec containing costimulatory molecules drive dose-dependent T cell transduction and antitumor immunity in an *in vivo* xenograft model



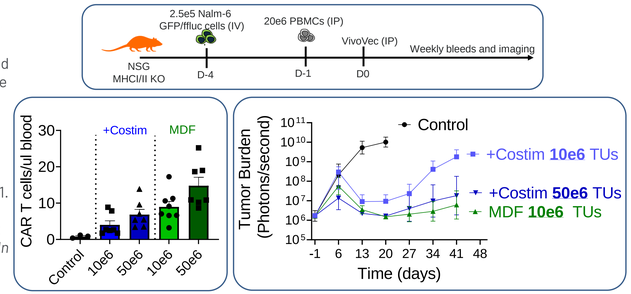
Top: Study outline. Luciferase+ Nalm6 Tumor-bearing NSG MHCI/II KO mice were humanized by injecting 20e6 PBMCs into the intraperitoneal cavity. Mice were then given varying doses of VivoVec packaging an anti-CD19 CAR transgene. **Bottom:** Total anti-CD19 CAR+ CD3+ T cells found in the blood on Day 11. **Right:** Tumor burden was assessed as total flux and tracked over time using an *In vivo* imaging system (IVIS®).

Fig 5. VivoVec particles incorporating a multidomain fusion (MDF) molecule enhance particle-T cell binding and transduction while maintaining CAR T cell functionality *in vitro*



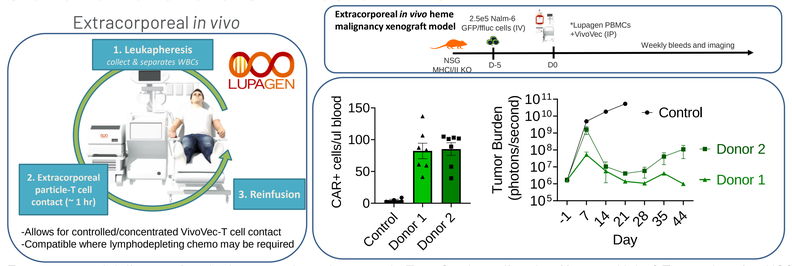
Left: Binding Assay. VivoVec particles cultured with PBMCs (3 donors) at MOI=10 for 1 hour followed by surface staining for Cocal glycoprotein on T cells. CD8 T cells shown. **Center:** VivoVec particles packaging an anti-CD19 CAR were added to PBMCs from 3 donors. 6 days later CAR expression was assessed on CD8 T cells. **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 6. Multidomain fusion VivoVec particles demonstrate improved anti-tumor responses, allowing for lower dosing, 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 received varying doses of VivoVec particles packaging an anti-CD19 CAR transgene. **Left:** Total anti-CD19 CAR+ CD3+ T cells found in the blood on Day 11. **Right:** Tumor burden was assessed over time as total flux and tracked over time using an *In vivo* imaging system (IVIS®).

Fig 7. Extracorporeal delivery of VivoVec-bound T cells allows for generation of functional CAR T cells *in vivo*



Left: Extracorporeal delivery using the Lupagen-based approach. **Top:** Study outline. Luciferase+ Nalm6 Tumor-bearing NSG MHCI/II KO mice were infused with PBMCs treated for 1 hour with VivoVec particles at MOI 2. **Bottom:** Total anti-CD19 CAR+ CD3+ T cells found in the blood on Day 14 and tumor burden assessed over time as total flux and tracked over time using an *In vivo* imaging system (IVIS®).

Summary

- VivoVec particles incorporating T cell costimulatory molecules enhance particle binding, T cell activation, and transduction
- VivoVec particles containing costimulatory molecules generated T cells that exhibit phenotypic properties associated improved persistence and have enhanced functional properties *in vitro*
- VivoVec particles containing costimulatory molecules generated more CAR+ T cells in the blood and had enhanced antitumor activity *in vivo*
- VivoVec incorporating a multidomain fusion (MDF) molecule had greatly enhanced binding and transduction of T cells
- MDF VivoVec particles exhibited similar in vivo antitumor effects at lower doses compared to particles containing separate costimulatory molecules
- MDF VivoVec particles delivered using the extracorporeal system led to *in vivo* CAR T cell generation and potent antitumor effects
- See a great talk by Umoja Scientist **Alyssa Sheih, Wednesday 3:45, Room 403B**

Poster presented at the American Society for Gene and Cell Therapy (ASGCT) Annual Meeting, May 16 – 20, 2023, Los Angeles, California, US