In vivo generation of universal CAR T cells that mediate durable anti-tumor immunity through combinatorial targeting with bispecific small molecule adapters

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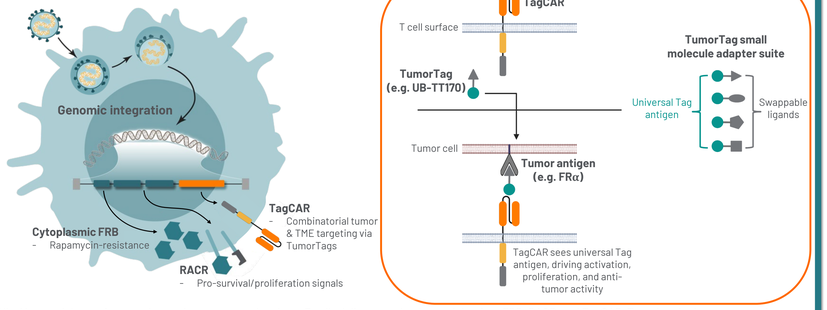
Poster #375

Background

Chimeric antigen receptor (CAR) T cell therapies have demonstrated limited efficacy against solid tumors, in part due to challenges overcoming solid tumor heterogeneity and CAR T cell exhaustion associated with the immunosuppressive tumor microenvironment (TME). Our integrated platform aims to overcome these roadblocks by engineering T cells in vivo to express a universal **TagCAR** which binds to a common tag on bispecific adaptor **TumorTags**, bridging TagCAR T cells to TumorTag-bound tumor- and TME-associated antigens, including folate receptor (FR) which is upregulated on many tumor types as well as immunosuppressive tumor-associated macrophages. The TagCAR payload is delivered to T cells using **VivoVec particles**, which are surface-engineered lentiviral vectors pseudotyped with the Cocal fusion glycoprotein, an anti-CD3 single chain variable fragment (scFv), and costimulatory molecules to achieve specific and efficient T cell activation and transduction. Additionally, our TagCAR T cells are engineered to express a **rapamycin-activated cytokine receptor (RACR)** which selectively provides survival signals to TagCAR T cells in the presence of rapamycin. Here, we identify a universal TagCAR that demonstrates potent and persistent in vitro and in vivo anti-tumor polyfunctionality against FR$^+$ target cells with a folate receptor-targeting TumorTag (UB-TT170). These data support development of this platform as a new cellular therapy approach against solid tumors, using combinatorial targeting of tumor- and TME-associated antigens with in vivo-generated universal TagCAR T cells and multiple TumorTags.

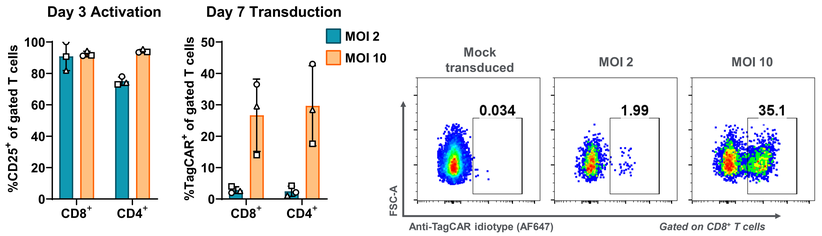
Graphical abstract

VivoVec particles containing TagCAR-RACR payload
(See Poster #1230 by Chris Nicolai for more on Umoja's surface engineering platform)
- Activate/expand T cells



Left. VivoVec particles surface-engineered to engage and activate T cells deliver a payload containing free FRB, RACR, and TagCAR. Together, in the presence of rapamycin, FRB-RACR drives expansion of transduced TagCAR T cells while additionally inhibiting tumor proliferation and potential immune responses against VivoVec particles. **Right.** Expression of the universal TagCAR results in T cells that can be targeted to various antigens of the tumor and TME with TumorTags. These bispecific molecules contain on one end the universal Tag recognized by the TagCAR, and on the other end, a ligand that engages tumor/TME-associated antigen(s).

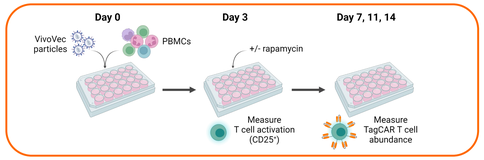
Fig 1. VivoVec-mediated activation and transduction of primary human T cells results in TagCAR expression in the absence of additional stimuli



Left. Peripheral blood mononuclear cells (PBMCs) from 3 healthy donors were cultured with VivoVec particles with a TagCAR-RACR payload at indicated multiplicity of infection (MOI) in the absence of T cell activating reagents, such as αCD3/CD28α beads. Activation and transduction of gated T cell populations was assessed by flow cytometry on Day 3 and Day 7 post-transduction, respectively. Data are represented as mean of biological replicates ± SD. **Right.** Representative flow plots on Day 7 post-transduction showing TagCAR expression in CD8$^+$ T cells.

Fig 2. Rapamycin-mediated RACR activation enriches and expands TagCAR T cells in vitro

In vitro assay method:



PBMCs from 2 healthy donors were cultured with VivoVec particles with a TagCAR-RACR payload at MOI 10. Cells were split into 50U/mL IL-2 alone or 50U/mL IL-2 + 10nM rapamycin on Day 3 post-transduction.
Bottom left. %TagCAR$^+$ of total T cells was measured by flow cytometry at the indicated days post-transduction.
Bottom right. Total TagCAR T cells were enumerated by flow cytometry with counting beads.
Data are represented as mean of biological replicates ± SD.

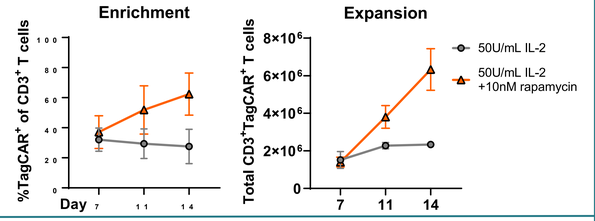
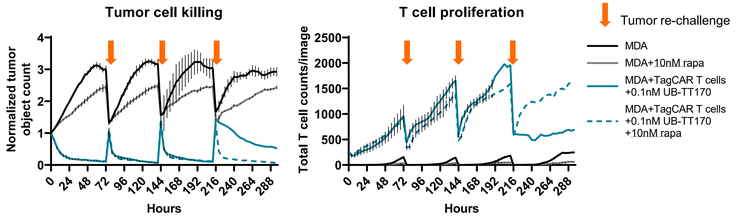
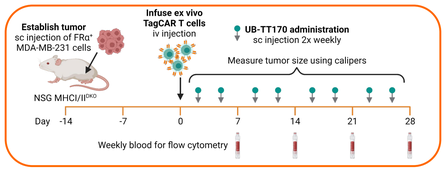


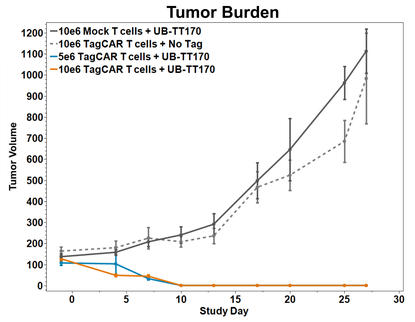
Fig 3. TagCAR T cells repeatedly kill FRα$^+$ tumor targets in vitro in the presence of UB-TT170, with improved activity with rapamycin treatment



On Day 11 post-transduction, rapamycin-expanded TagCAR T cells were co-cultured with mCherry$^+$FRα$^+$ MDA-MB-231 tumor targets in the presence of 0.1nM UB-TT170 +/- 10nM rapamycin at a 1:1 TagCAR T cell:target cell ratio. Tumor cells (**Left**) and total T cells (**Right**) were quantified over time using the Incucyte® Live-Cell Analysis System. At timepoints indicated with the orange arrows, remaining TagCAR T cells were transferred to plates containing freshly plated mCherry$^+$FRα$^+$ MDA-MB-231 tumor targets. Data are represented as mean of technical replicates ± SD. Graphs show results from TagCAR T cells generated from one healthy donor. Data were recapitulated independently with 2 additional healthy donors.

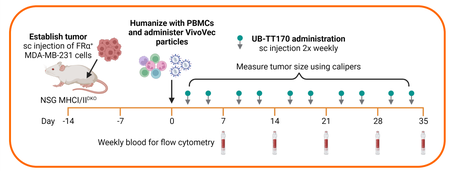
Fig 4. Ex vivo-generated TagCAR T cells rapidly clear FRα$^+$ solid tumors in vivo in mice receiving UB-TT170

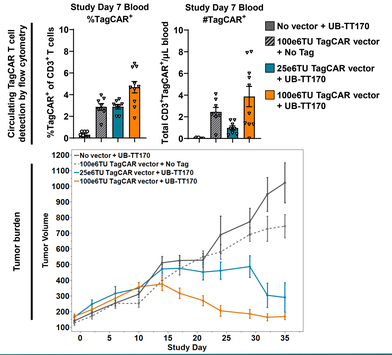




Left. Ex vivo TagCAR proof-of-concept study design. Female NSG MHCI/II^{DKO} mice were engrafted with FRα$^+$ MDA-MB-231 tumors in the flank, followed by administration of ex vivo-manufactured Mock transduced or TagCAR T cells (5e6 or 10e6 cells) after tumors reached a size of 100mm^3. Mice were dosed 2x weekly dosing with UB-TT170. Efficacy was measured by tumor regression using digital calipers and plotted as seen on the **Right.** Data are represented as mean of biological replicates ± SEM.

Fig 5. In vivo VivoVec particle delivery generates TagCAR T cells which control FRα$^+$ solid tumors in mice dosed with UB-TT170

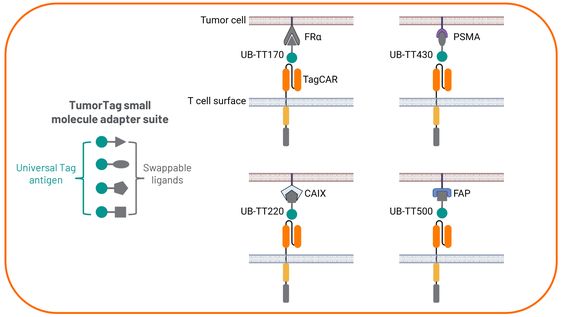




Left. In vivo-generated TagCAR T cell efficacy study design. Female NSG MHCI/II^{DKO} mice were engrafted with FRα$^+$ MDA-MB-231 tumors in the flank and tumors were allowed to grow until 100-200mm^3 in size. Mice were humanized with healthy human donor PBMCs followed by administration of VivoVec particles containing the TagCAR payload. Mice were treated with UB-TT170 2x weekly. **Top right.** TagCAR T cells were detected in circulation as early as Day 7 after particle administration. **Bottom right.** Efficacy was measured by tumor regression using digital calipers. Data are represented as mean of biological replicates ± SEM.

Fig 6. Additional TumorTags that bind to other tumor- and TME-associated antigens are being developed for combinatorial use with TagCAR T cells to target solid tumors

Multiple TumorTags are being evaluated for combinatorial use with TagCAR T cells to increase therapeutic efficacy. TumorTags currently in development bind to tumor- and TME-associated antigens found in a high percentage of solid tumors, establishing a path by which a diversity of tumors can be targeted.



Summary

- TagCAR T cells can be generated in vitro with Umoja's surface-engineered VivoVec particles alone, in the absence of additional T cell activating agents
- Rapamycin engages RACR and selectively enriches and expands TagCAR T cells in vitro
- TagCAR T cells repeatedly kill FRα$^+$ tumor targets in vitro with UB-TT170 and expand in the presence of antigen, both functions of which are enhanced with addition of rapamycin
- Ex vivo manufactured TagCAR T cells demonstrate robust and rapid clearance of solid FRα$^+$ tumors in mice dosed with UB-TT170
- TagCAR T cells can be generated in vivo with administration of VivoVec particles and mediate tumor control and regression with UB-TT170
- Umoja's universal TagCAR T cells function in the context of additional antigen-targeting TumorTags
- See more exciting work from Umoja at Posters **#366 (Samantha O'Hara)** and **#1230 (Chris Nicolai)**

For questions about this poster, please contact:
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Poster made with BioRender

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