UB-VV100, A Novel Platform For In Vivo Engineering of Therapeutic Anti-CD19 CAR T Cells, Shows Effective T Cell Transduction, B Cell Depletion, And Tumor Control in a Humanized Murine Model

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* Disclosures: All authors hold equity with Umoja Biopharma

Abstract

- The emergence of chimeric antigen receptor (CAR) T cells as a therapeutic modality to treat refractory B-cell malignancies has inspired a wave of investment in cell-based immunotherapies.
- However, the field remains hindered by technical, logistical, consistency, cost, and efficacy limitations associated with autologous and allogeneic manufacturing.
- To overcome these challenges, Umoja is developing several integrated platforms, one of which is **VivoVec**, an **in vivo CAR engineering platform** which uses a unique vector envelope to facilitate transduction of T cells in vivo.
- VivoVec particles are pseudotyped with the Cocal glycoprotein, which has been shown to resist serum inactivation in humans. VivoVec particles also express a membrane-anchored anti-CD3 scFv that facilitates T cell activation and transduction.
- The payload includes a 2nd generation CAR that is co-expressed with a novel **rapamycin-activated cytokine receptor (RACR)** system designed to provide a selective growth signal to transduced T cells.
- Here we show proof-of-concept data utilizing **VivoVec** particles to deliver an anti-CD19 CAR along with the **RACR** system as a feasible treatment for B-cell malignancies (termed UB-VV100).
- This platform has the potential to increase the safety and availability of CAR T technology without the need for cell manufacturing or lymphodepletion.

UB-VV100 is designed to harnesses the body's own immune system to manufacture CD19 CAR T cells in vivo

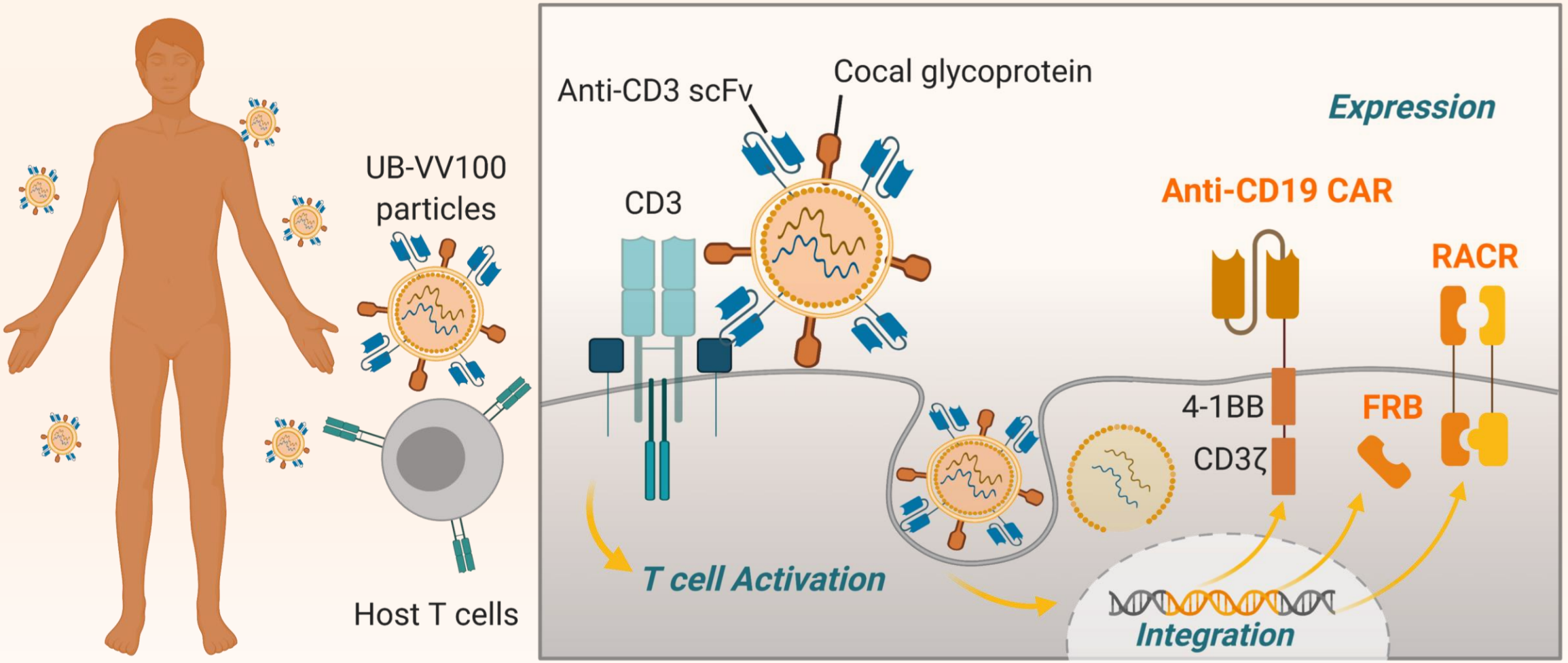


Figure 1. Umoja's UB-VV100 surface engineering engages human T cells via presentation of an anti-CD3 antibody fragment (scFv). CD3 binding promotes activation and enhances T cell transduction. Transduced T cells express the anti-CD19 CAR and Rapamycin Activated Cytokine Receptor (RACR) and FRB elements.

Rapamycin activated cytokine receptor (RACR) is designed to drive selection and expansion of in vivo transduced CAR T cells

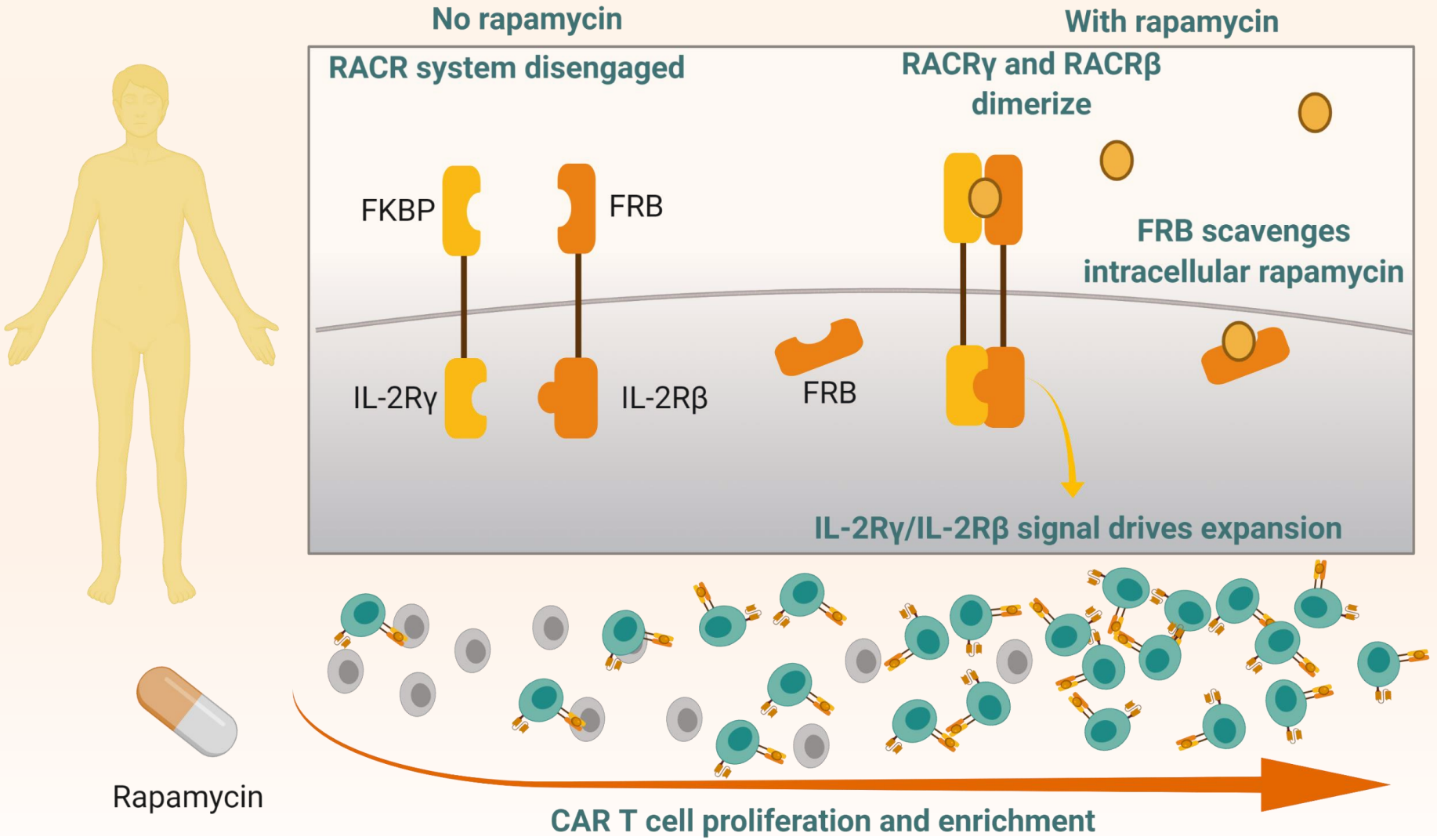


Figure 2. Rapamycin activates the RACR system which replicates IL-2/IL-15 cytokine signaling thus activating the STAT5 pathway for robust proliferation and survival. Naked intracellular FRB domain, a rapamycin-binding unit of mTOR, sequesters rapamycin thereby providing rapamycin resistance to transduced cells while non-transduced T and B cells are suppressed via mTOR inhibition.

Methodology for testing UB-VV100 transduction efficiency in vitro

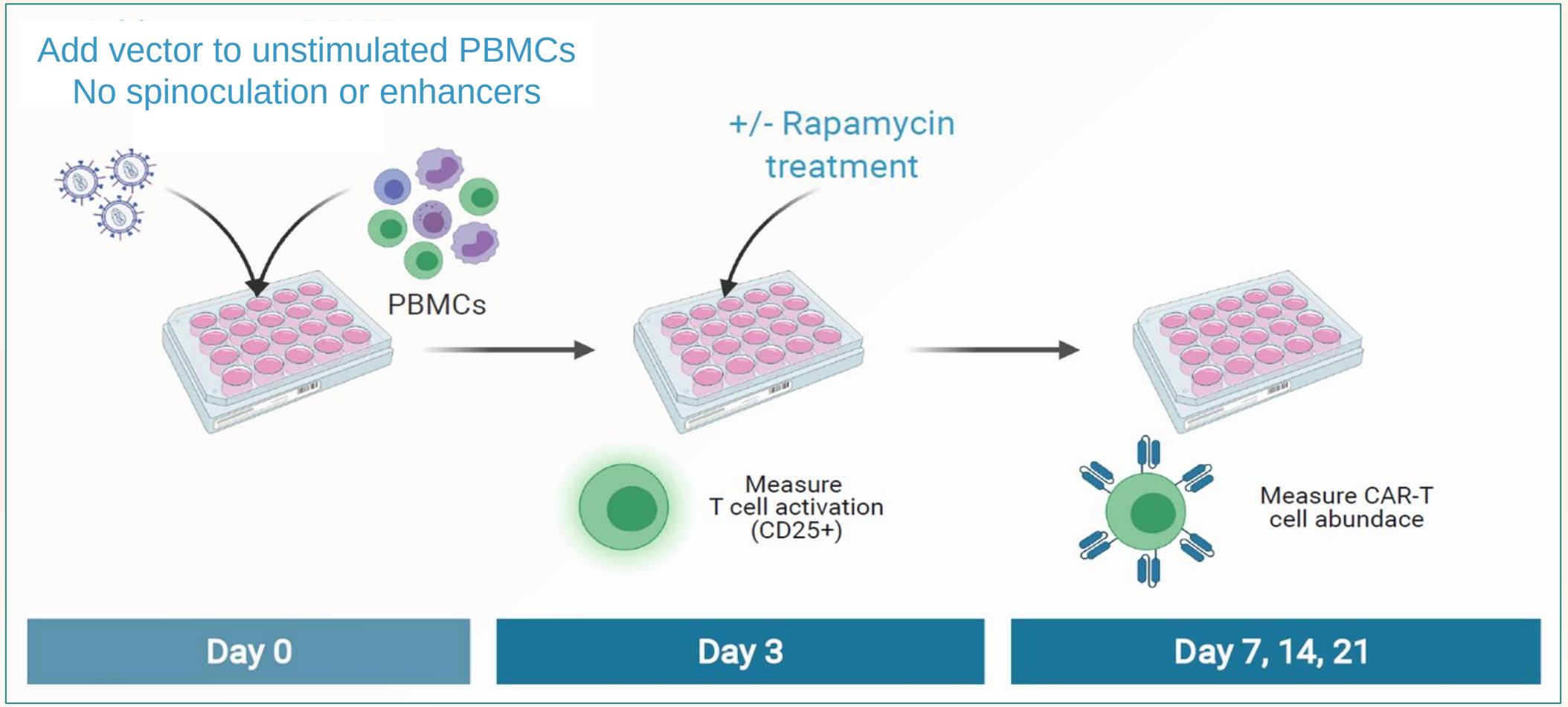


Figure 3. Vector particles are added directly to cultures of human peripheral blood mononuclear cells (PBMCs) in RPMI with 10% FBS and 50U/ml IL-2 without use of any additional enhancers. UB-VV100-dependent activation is assessed by flow cytometry 3 days later by measurement of activation markers (e.g. CD25 expression). Beginning day 3, some cultures are supplemented with 10nm rapamycin. T cell transduction is assessed beginning day 7 by flow cytometry for CAR expression using an anti-idiotype antibody.

Anti-CD3 + Cocal surface engineering facilitates activation and transduction of T cells

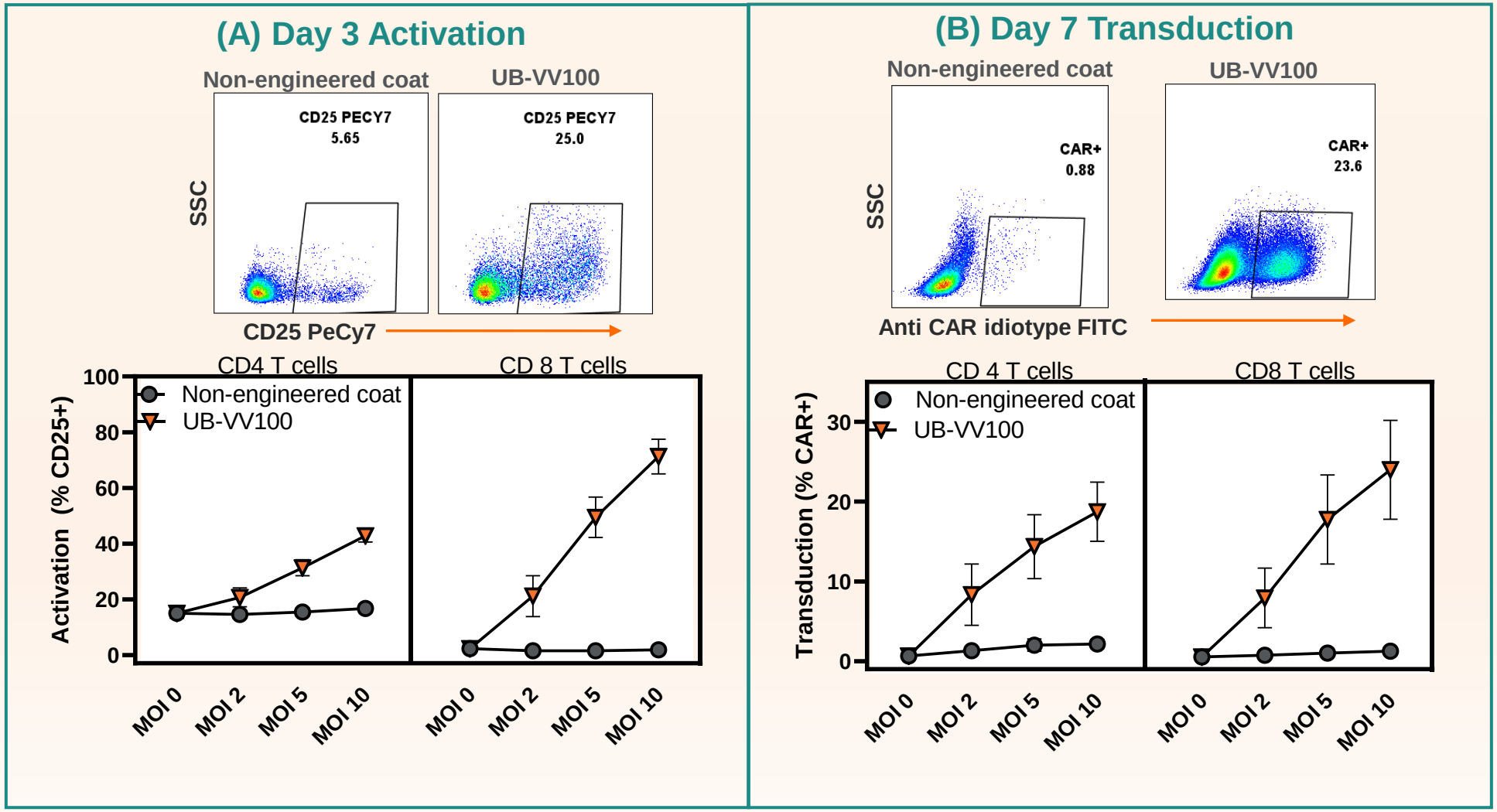
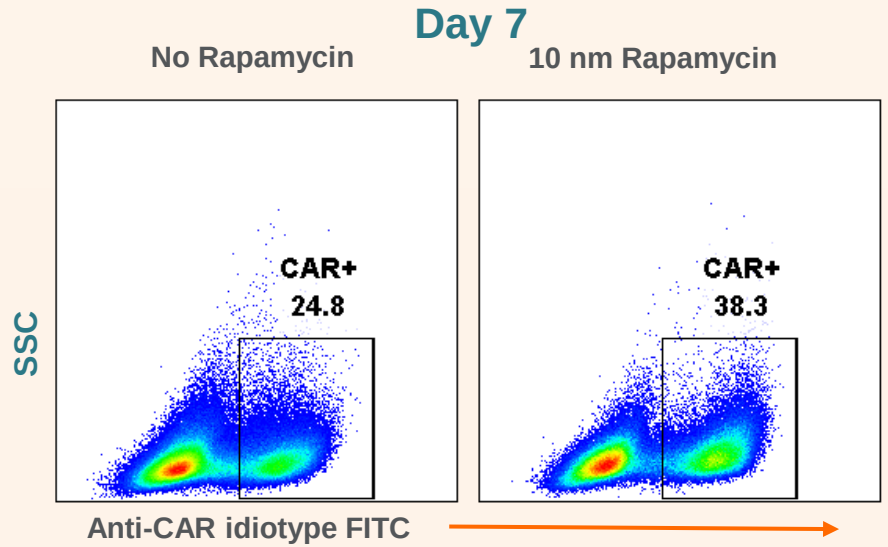
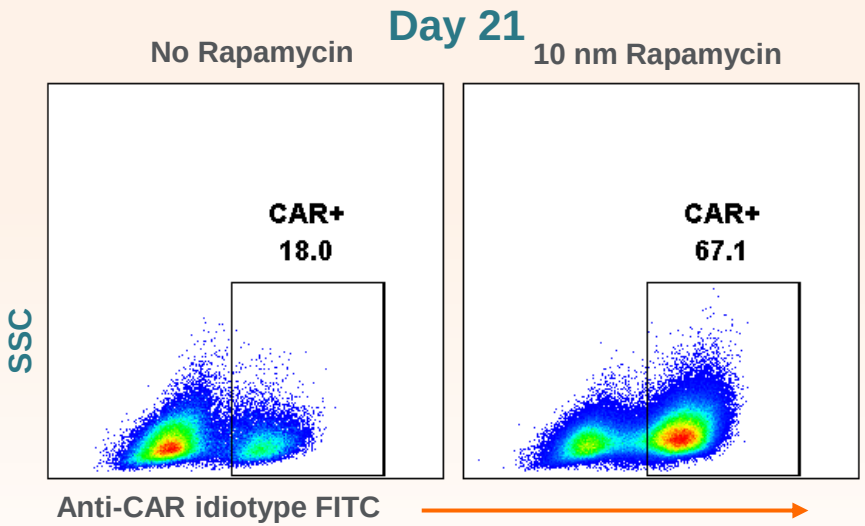
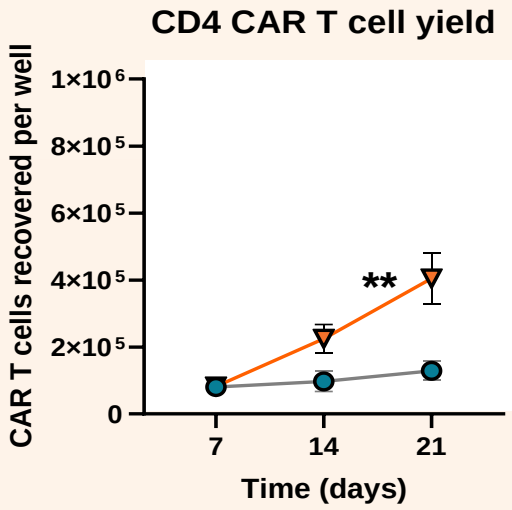


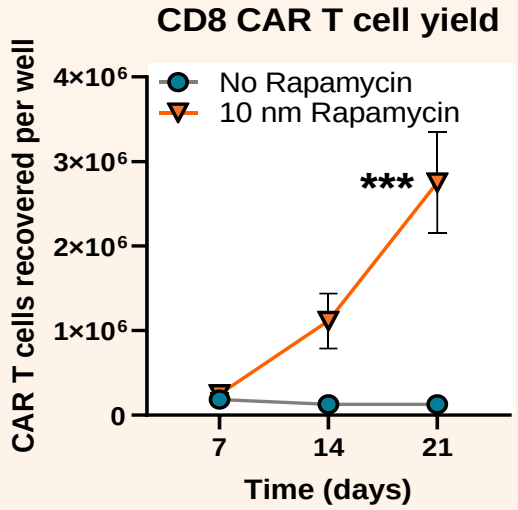
Figure 4. PBMCs from 3 donors were transduced with UB-VV100 or Cocal-pseudotyped lentivirus without anti-CD3 scFv. Activation was assessed after 3 days by flow cytometry for CD25 (A). Transduction was assessed after 7 days by flow cytometry for CAR expression (B). Plots showing data for CD25 are representative of all activation markers measured (i.e CD71 and CD69; data not shown). Plots are from a multiplicity of infection (MOI) of 5, gated on CD3+ live singlets. Summarized plots are combined data from 3 donors, error bars indicate ± 1 SEM.

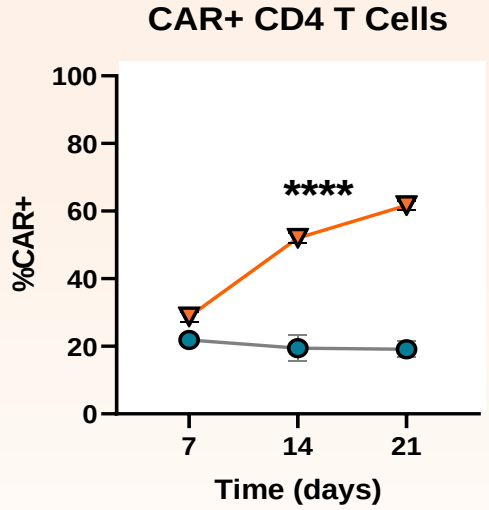
RACR engine drives enrichment and proliferation of CAR T cells in vitro











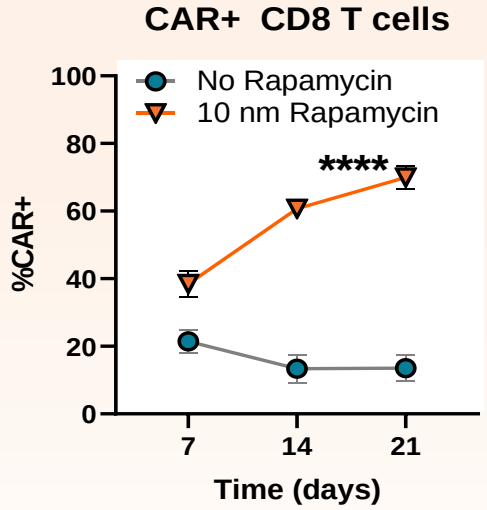
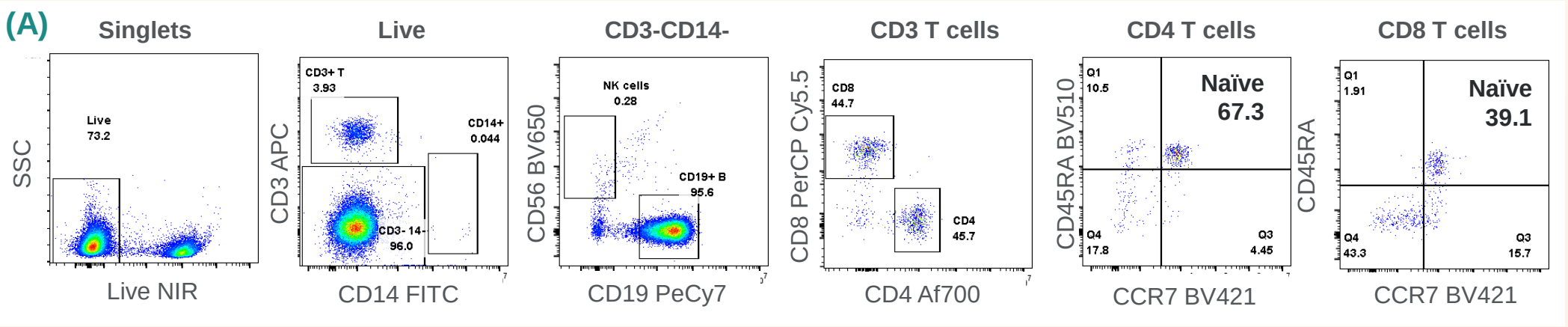


Figure 5. PBMCs were transduced with UB-VV100 at a multiplicity of infection of 10. Beginning on day 3, cells were split into separate cultures and treated with or without 10nM rapamycin. CAR T cell transduction and expansion were assessed by flow cytometry and cell enumeration using counting beads. Representative flow plots are gated on live CD3+ singlets. Summarized plots combine data from 3 donors, error bars indicate ± 1 SEM. **, ***, and *** indicate p values of <0.01, 0.001, and 0.0001, 2-way ANOVA multiple comparisons for rapamycin treatment over time.

UB-VV100 transduces T cells from a B-ALL patient with <4% T cells of total PBMCs

Patient sample composition at transduction



Patient sample composition 7 days post transduction

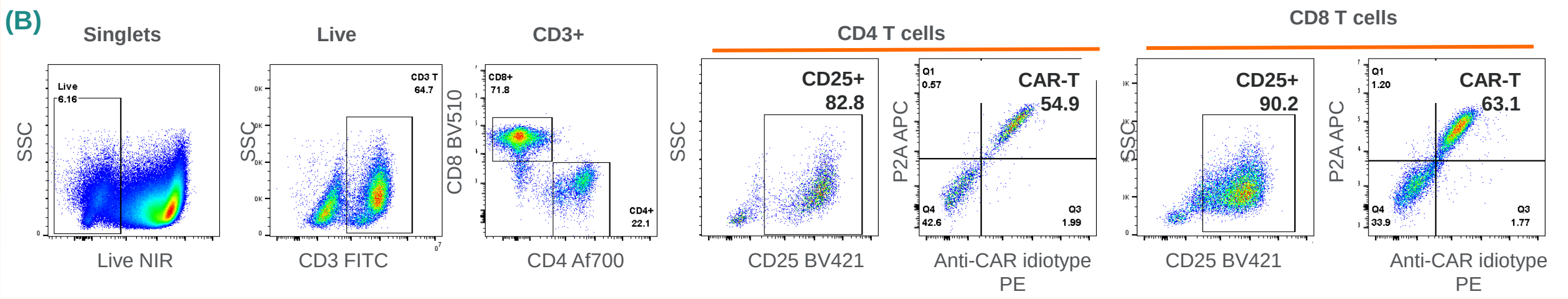
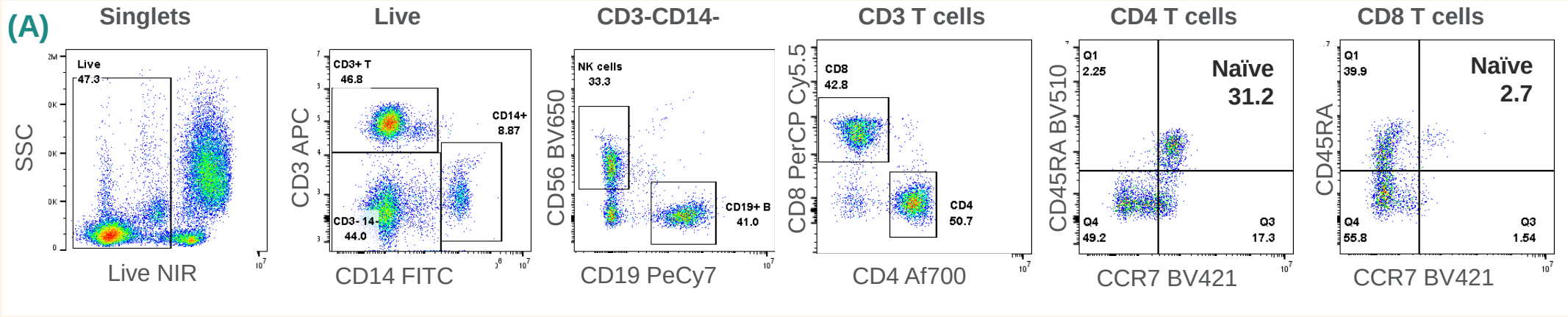


Figure 6. A B-ALL patient PBMC sample (male, 23 yo) was collected upon diagnosis and prior to initiating treatment. Hematology reports indicated the patient was in blast phase with 62% blasts in the blood, 95% blasts in the bone marrow, and a white blood cell count of 205.7×10^9 cells/L. T cells comprised <4% of total live PBMCs (A). Cells were transduced with two daily treatments of 2.5 UB-VV100 transducing units per live PBMC. T cell activation and transduction were assessed on day 7 by flow cytometry (B).

UB-VV100 transduces T cells from a 70 year-old DLBCL patient

Patient sample composition at transduction



Patient sample composition 7 days post transduction

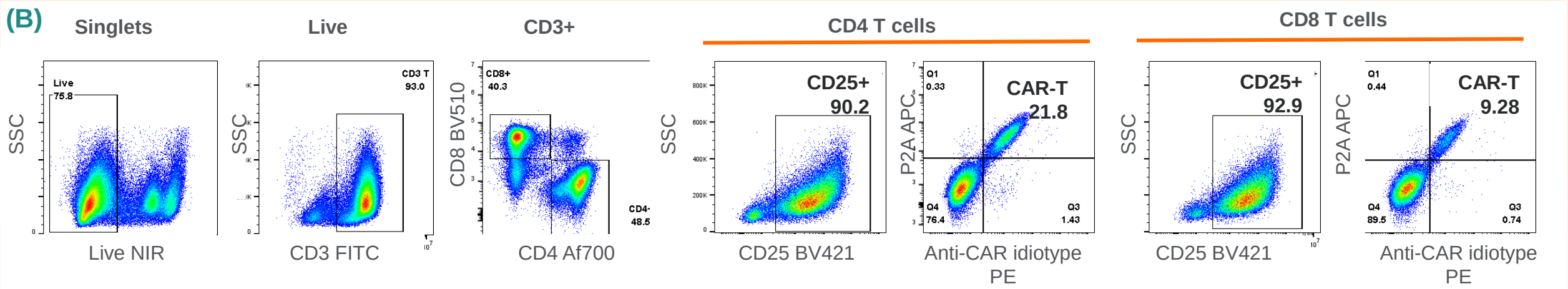


Figure 7. A DLBCL patient PBMC sample (male, 70 yo) was collected upon diagnosis and prior to initiating treatment. At the time of collection, white blood cell count was $11.3x10^9$ cells/L and viability was <50% (A). Cells were transduced with two daily treatments of 2.5 UB-VV100 transducing units per live PBMC. T cell activation and transduction were assessed on day 7 by flow cytometry (B).

UB-VV100-transduced CAR T cells display CD19-dependent cytotoxicity against Raji cells in vitro

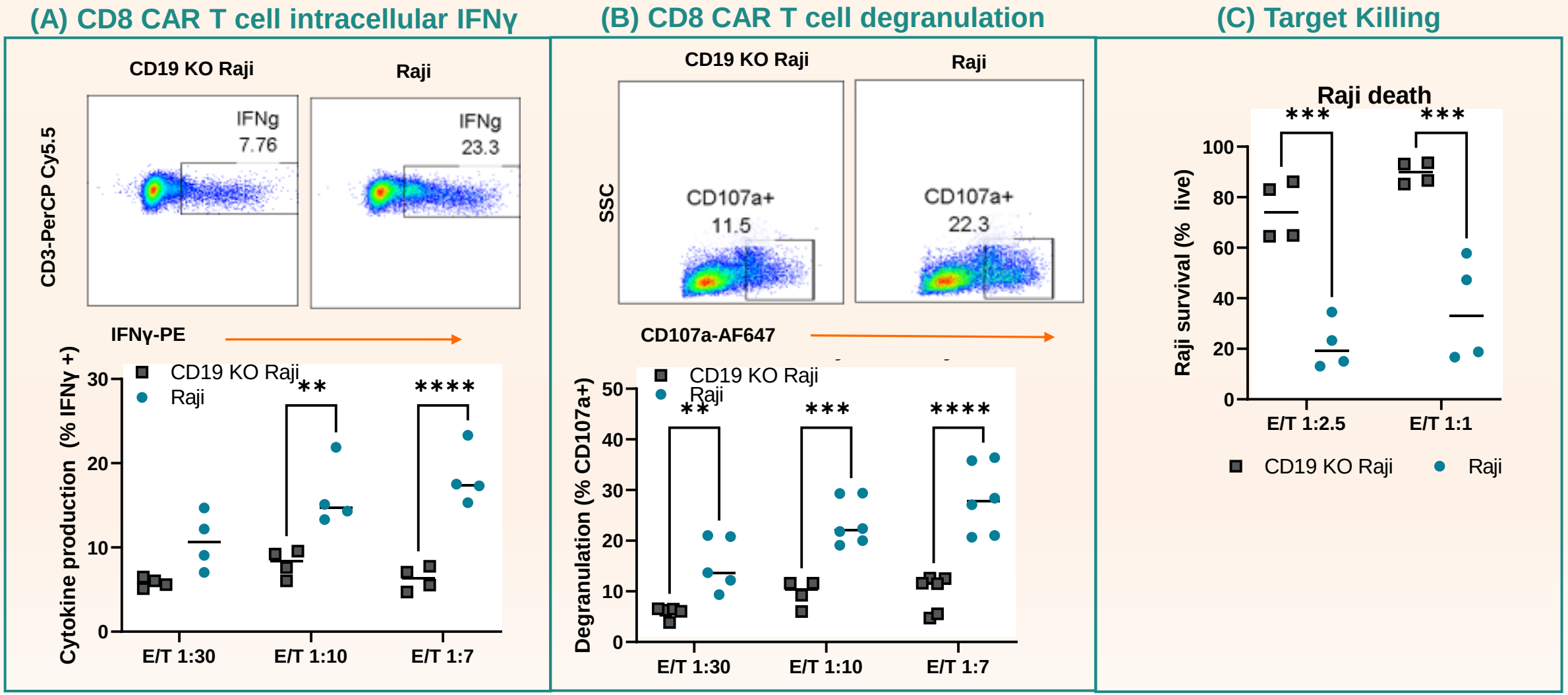
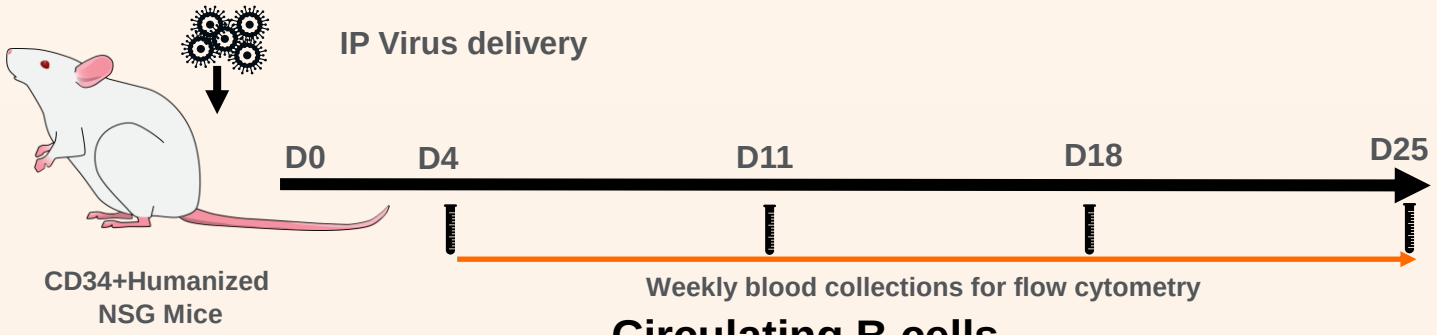


Figure 8. PBMCs from 2 donors transduced with UB-VV100 were co-cultured in duplicate with CD19-knock out (KO) or CD19-expressing Raji-GFP tumor cells for 5 hours with 2μM of Monensin, 5μg/mL of Brefeldin A, and 2μg/mL of CD107a Ab. E/T indicates ratio of CAR T cells to tumor target cells in the well. The cytotoxicity of CD8 T cells was assessed by intracellular staining of INFγ (A) and surface CD107a (B). (C) UB-VV100-transduced PBMCs were co-cultured with CD19-KO or CD19-expressing Raji-GFP tumor cells for 48 hours, and killing potency was assessed by (Viable Raji-GFP/ Total Raji-GFP). Representative flow plots are gated on CD8+CD3+ live singlets. **, ***, and *** indicate p values of <0.01, 0.001, and 0.001 by 2-way ANOVA multiple comparisons test.

UB-VV100 injection into CD34-humanized mice results in dose-dependent B cell depletion



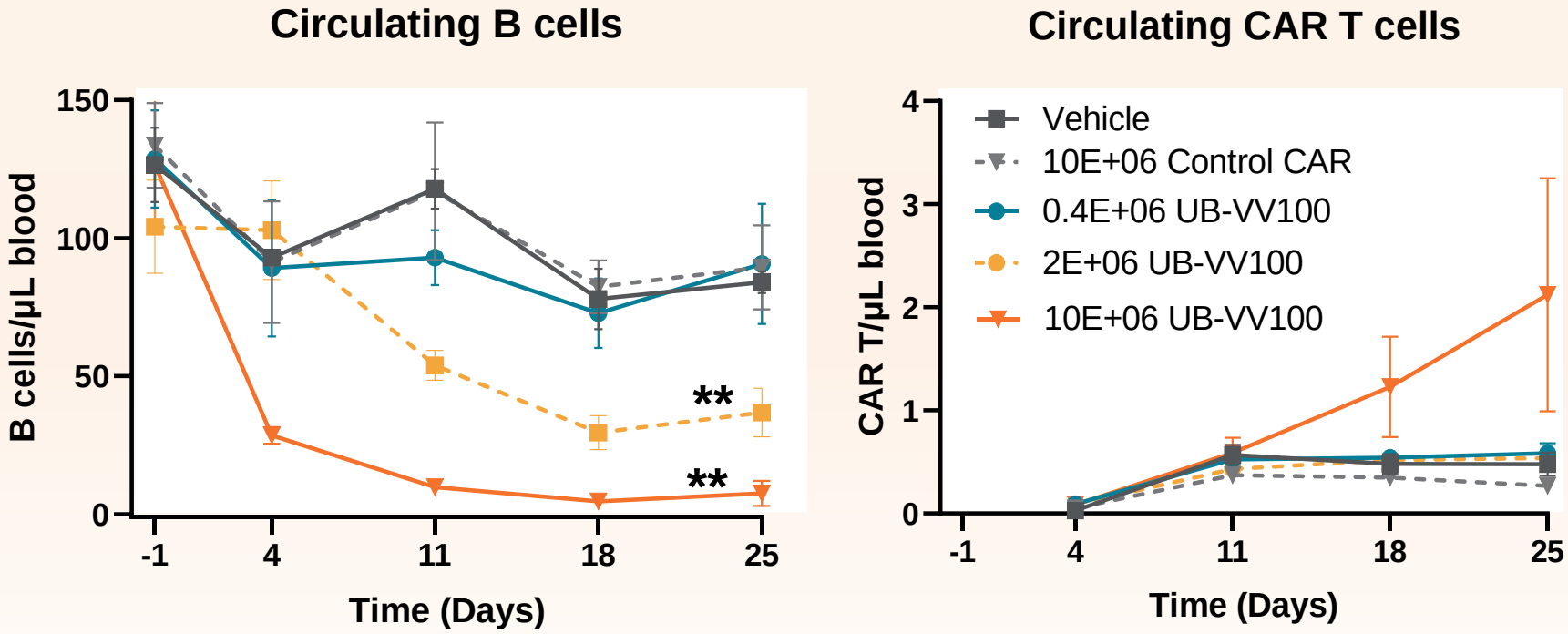
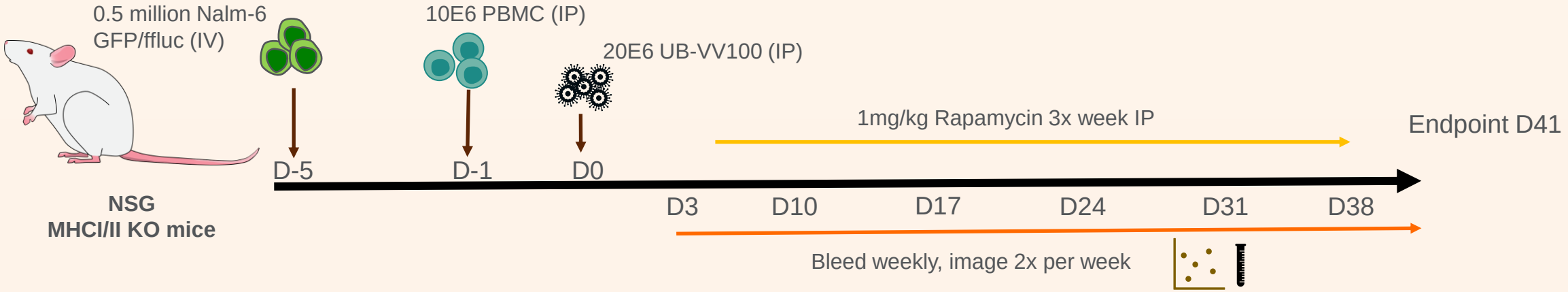
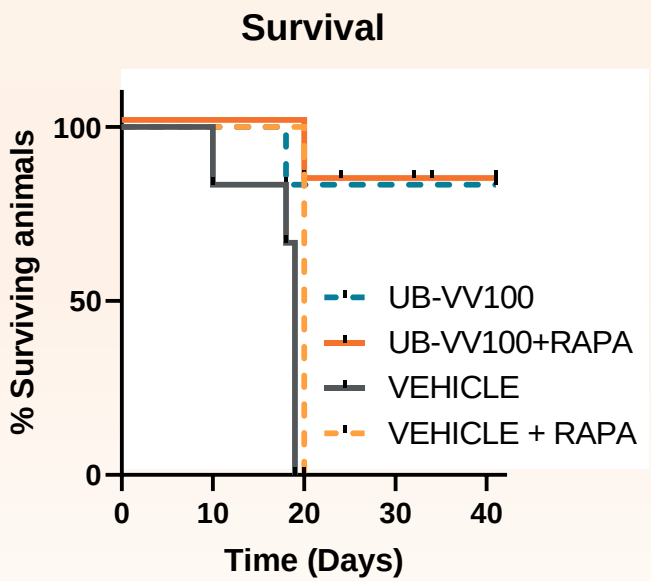
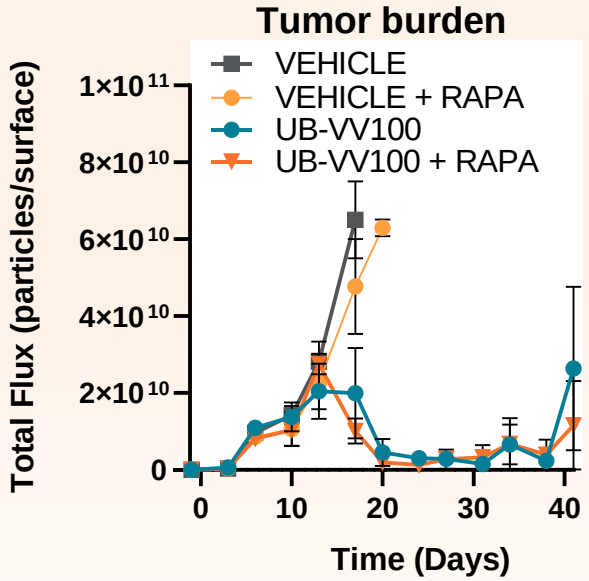


Figure 9. Female NSG mice 16 weeks post CD34+ HSC humanization (n=4 per groups) were treated via intraperitoneal (IP) injection with a vehicle control, 10E+06 transducing units (TU) of surface engineered vector encoding an irrelevant CAR, and 0.4E+06 TU, 2E+06 TU, or 10E+10 TU of UB-VV100. Depletion of endogenous B cells was used as a surrogate for CD19 CAR T cell activity. Circulating B cells and CAR T cells were assessed in weekly serial blood draws for flow cytometry. Error bars indicate ± 1 SEM. ** indicates p value of <0.01, 2-way ANOVA analysis for vector treatment.

UB-VV100 prolongs survival and slows tumor progression in a NALM6 systemic tumor model







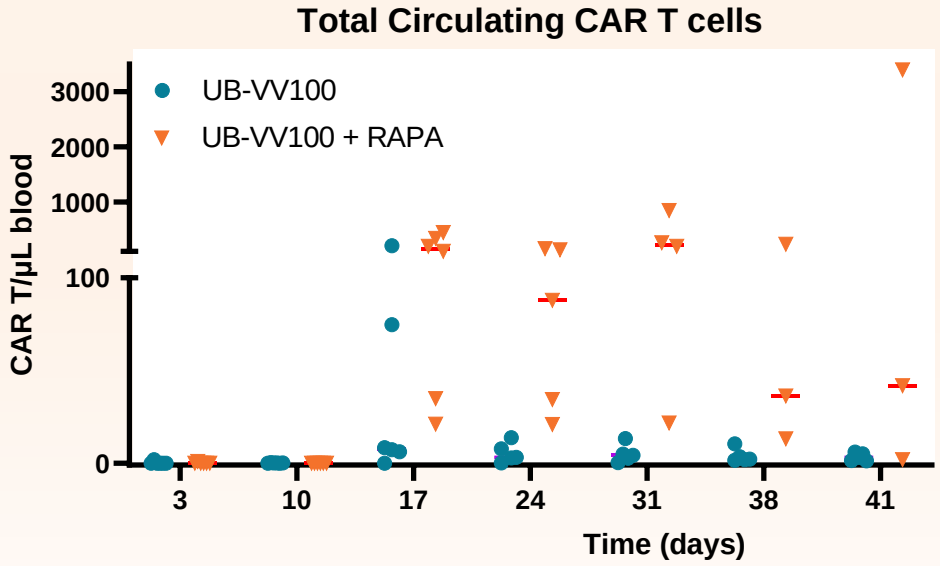


Figure 10. NSG mice lacking expression of MHC I and II (n=6 per group) were engrafted with 0.5+E06 NALM6 B-ALL tumor cells expressing green fluorescent protein and firefly luciferase (GFP:*ffluc)* via intravenous (IV) injection on study day -5. Mice were humanized on study day -1 with 10E+06 PBMCs and treated with 20E+06 transducing units (TU) of UB-VV100 via intraperitoneal (IP) injection on study day 0. Animals in indicated study groups were treated with 1 mg/kg rapamycin IP beginning study day 4 3x weekly. Animals were assessed for survival, tumor burden by in vivo bioluminescent imaging, and circulating CAR T cell populations in serial bleeds. Bars indicate +/- 1 standard error of the mean.

Conclusions

- UB-VV100 particles activate and transduce T cells from healthy human PBMCs in a surface-engineering dependent fashion
- UB-VV100 particles transduce T cells from the PBMCs of patients with B cell malignancies
- UB-VV100-transduced CAR T cells display CD19-specific anti-tumor activity in vitro
- UB-VV100 particle administration *in vivo* resulted in CAR T cell generation, expansion, and cytotoxicity against endogenous and malignant B cells in humanized mouse models
- Rapamycin drives expansion and selection for transduced CAR T cells in vitro and in vivo

IND-enabling pharmacology and toxicology studies have been initiated for UB-VV100

Acknowledgements

Umoja BIOPHARMA

Discovery

In Vitro Assay Development

Translational Research & Development

Vector Development

Process Development

Quality Assurance

Analytical Development

Clinical Development

Regulatory Affairs

Seattle Children's HOSPITAL · RESEARCH · FOUNDATION

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