LB025

Spatiotemporally regulated expression of membrane-bound interleukin 12 (mbIL12) for armored adoptive cell therapy (ACT) shows strong antitumor activity in syngeneic solid tumor models without overt toxicity

Theresa Ross, Patricia Timpug, Sean G. Smith, Meghan Langley, Benjamin Primack, Violet Young, Dexue Sun, Dan Jun Li, Dhruv K. Sethi, Jan ter Meulen, Michelle Ols, Jeremy Tchaicha
Obsidian Therapeutics, Cambridge, MA, USA
TRoss@obsidiantx.com

Introduction

- ACTs armored with potent cytokines, such as IL12, have shown promising activity in solid tumors; however, clinical application has been hindered by systemic toxicities associated with secreted IL12 and only activation-dependent (nuclear factor of activated T cells [NFAT]-inducible) control[1]
- We have combined activation-dependent (spatial) control using NFAT response elements (NFAT-RE) with pharmacologic (temporal) regulation of membrane-bound IL12 (mbIL12) using the cytoDRiVE® platform **(Figure 1C)** to provide tight regulation of mbIL12 expression
- Previously we showed that B16-F10 tumor-bearing mice receiving pmel T cells expressing NFAT-inducible secreted IL12 (NFAT-sec-IL12) displayed significant body weight loss, unlike mice infused with pmel T cells expressing NFAT-inducible spatiotemporally regulated membrane-bound IL12 (NFAT-cytoDRiVE-mbIL12), while both groups showed tumor control **(Figure 2)**[2]
- Herein, we examined the spatiotemporal regulation of cytokines at the site of the tumor and changes in the tumor microenvironment (TME) of a syngeneic melanoma model, and the efficacy of NFAT-cytoDRiVE-mbIL12 in a syngeneic liver tumor model of colorectal cancer

Figure 1. NFAT-cytoDRiVE Technology

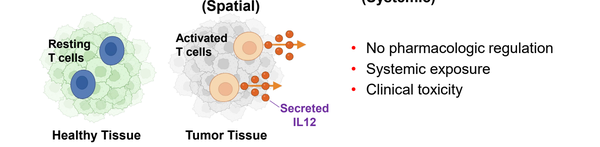

Figure 1. NFAT-RE provides activation-dependent spatial control of protein expression when the TCR is activated **(A).** The cytoDRiVE platform uses DRD tags that enable an FDA-approved small-molecule ligand to regulate expression of a protein fused to the DRD. In the absence of the cognate DRD ligand (Drug-OFF state), the fusion protein is degraded by the proteasome. In the presence of the ligand (Drug ON-state), there is temporal regulation and the protein is expressed. In the case of cytoDRiVE-mbIL12 cells, a DRD derived from human dihydrofolate reductase (hDHFR) is used to regulate mbIL12 via delivery of trimethoprim (TMP) **(B).** NFAT-cytoDRiVE-mbIL12 provides localized distribution through membrane tethering and both the activation-dependent spatial control of expression and pharmacological temporal regulation **(C)**.

Statistics

Statistics calculated in GraphPad Prism
*** p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001**

Phenotyping, ALT, and cytokines: Welch's T-Test
Bodyweight and tumor burden: Mann-Whitney Test
Survival: Log-Rank Mantel-Cox Test

Results

Figure 2. Utilizing syngeneic pmel system to investigate the enhanced safety of an IL12-armored ACT with NFAT + cytoDRiVE

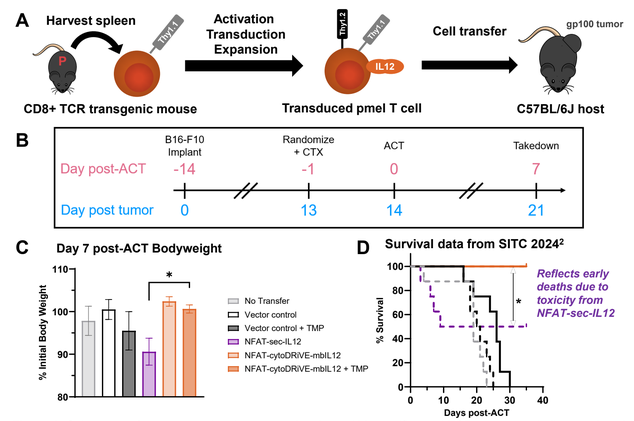

Figure 2. Splenocytes from transgenic mice expressing the gp100-specific pmel TCR were retrovirally transduced to express NFAT-sec-IL12 or NFAT-cytoDRiVE-mbIL12. After transduction, these CD8+ splenocytes were then expanded for 6 days. The mouse melanoma cell line, B16-F10, which express the gp100 melanoma antigen, served as a tumor-specific target for these adoptively transferred, engineered CD8+ pmel T cells **(A).** The syngeneic B16-F10 tumor cells were implanted subcutaneously in C57BL/6J mice. Animals (N=8 per treatment group) were randomized and lymphodepleted with Cytoxan at 200 mg/kg on Day 13. Mice were infused IV with 2.15 × 10^6 transduced engineered CD8+ pmel cells the following day. Treatment groups were dosed with either TMP or vehicle (PEG400) daily via oral gavage at 500 mg/kg from day of infusion (Day 0) until termination of the study. Blood was collected via submandibular bleed and plasma isolated on Day 7 post-ACT. Tumors and spleen were collected on Day 7 post-ACT **(B).** Bodyweight **(C)** and tumor measurements were collected twice a week via digital calipers starting on the day before ACT. In the same model, mice were randomized and lymphodepleted with Cytoxan at 200 mg/kg on Day 8 post tumor-implant. Mice were infused IV the following day with 10 × 10^6 engineered CD8+ pmel cells and survival was tracked based on tumor volume threshold (>2000 mm^3) and included endpoints of >20% bodyweight loss, being found moribund, or found dead **(D).**

Figure 3. NFAT + cytoDRiVE maintains localized control by reducing downstream systemic cytokines and ALT

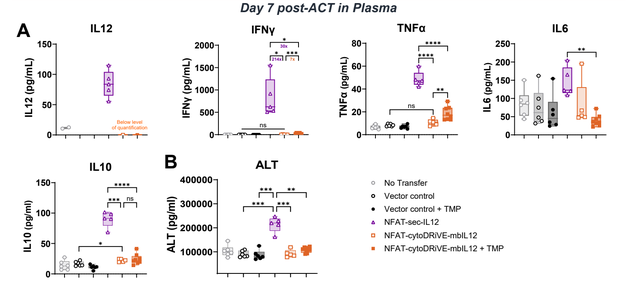

Figure 3. Systemic IL12, IFNγ, TNFα, IL6, and IL10 levels **(A)** were determined using MSD (Mesocale Discovery). ALT (alanine aminotransferase) level **(B)** was determined using a Mouse ALT SimpleStep ELISA kit on plasma isolated from whole blood on Day 7 post-ACT.

Figure 4. NFAT + cytoDRiVE demonstrates spatiotemporal regulation of cytokines localized to the tumor

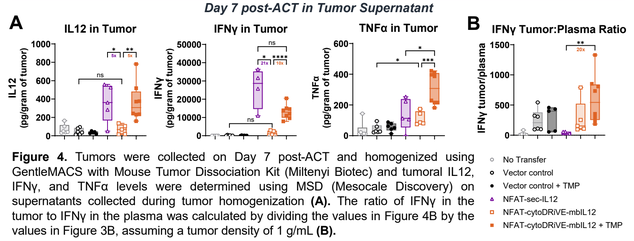

Figure 4. Tumors were collected on Day 7 post-ACT and homogenized using GentleMACS with Mouse Tumor Dissociation Kit (Miltenyi Biotec) and tumoral IL12, IFNγ, and TNFα levels were determined using MSD (Mesocale Discovery) on supernatants collected during tumor homogenization **(A).** The ratio of IFNγ in the tumor to IFNγ in the plasma was calculated by dividing the values in Figure 4B by the values in Figure 3B, assuming a tumor density of 1 g/mL **(B).**

Figure 5. NFAT + cytoDRiVE modulates proinflammatory cellular changes within the TME

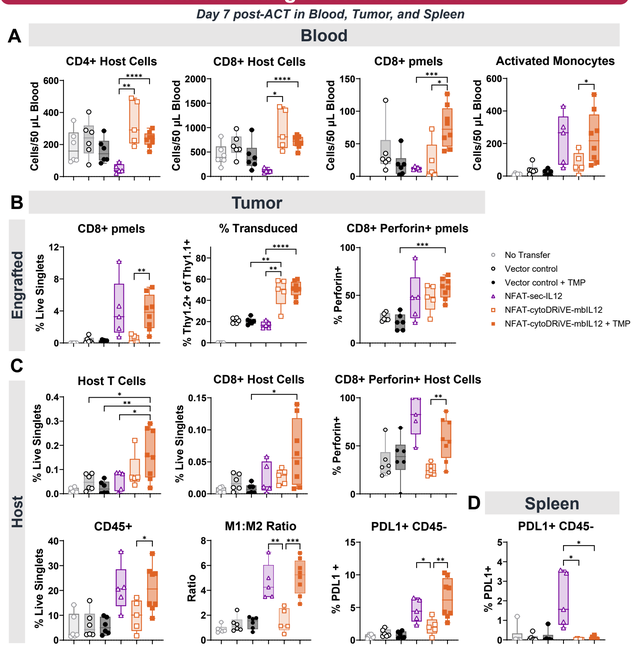

Figure 5. B16-F10 tumor-bearing mice were adoptively transferred with 2.15 × 10^6 transduced engineered CD8+ pmel cells 14 days post-tumor implant. Blood was collected on Day 7 post-ACT and flow cytometry was used to analyze cell populations. CountBright™ Absolute Counting Beads (Invitrogen) were used to normalize cell count values. In the blood, host CD4+ T cells were identified (CD45+CD3+Thy1.1-Thy1.2+CD4+), along with host CD8+ T cells (CD45+CD3+Thy1.1-Thy1.2+CD8+), CD8+ pmels (CD45+ CD3+CD8+Thy1.1+) and activated monocytes (CD45+CD11B+F4/80-Ly6C+CD86+) **(A)**. Tumors were collected and homogenized using GentleMACS with Mouse Tumor Dissociation Kit (Miltenyi Biotec). Engrafted CD8+ pmels were identified (as described above), as well as transduced engrafted CD8+ pmels (CD45+CD3+CD8+Thy1.1+Thy1.2+) and engrafted CD8+ perforin+ pmels (CD45+CD3+CD8+Thy1.1+ Perforin+) **(B)**. Host TME phenotype was characterized to ascertain host T cells (CD45+CD3+Thy1.1-Thy1.2+), host CD8+ cells (as described above), host CD8+ perforin+ cells (CD45+ CD3+Thy1.1-Thy1.2+CD8+perforin+), CD45+ cells (CD45+), M1 macrophages (CD45+CD11B+F4/80+ Ly6C-CD206-INOS+), M2 macrophages (CD45+CD11B+F4/80+Ly6C-CD206+INOS-) and PDL1+ CD45- cells (CD45-PDL1+) **(C)**. Spleens were processed and phenotype assessed as described above **(D)**.

Figure 6. Syngeneic pmel liver model of colorectal cancer without lymphodepletion

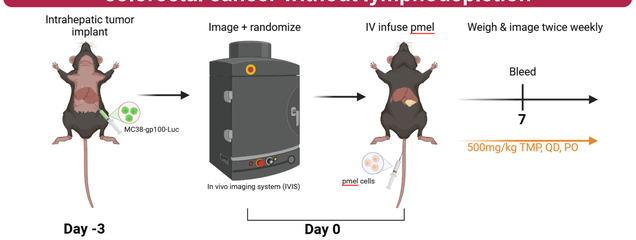

Figure 6. CD8+ splenocytes from transgenic mice expressing the gp100-specific pmel TCR were retrovirally transduced to express NFAT-sec-IL12 or NFAT-cytoDRiVE-mbIL12 as described in **Figure 2A**. The mouse colorectal cancer cell line MC38 was engineered to express the gp100 melanoma antigen to serve as a tumor-specific target for these adoptively transferred, engineered CD8+ pmel T cells, and luciferase (-Luc) to allow for *in vivo* live animal bioluminescent imaging. The syngeneic MC38-gp100-Luc tumor cells were implanted intrahepatically in C57BL/6J mice in a survival surgery. Animals were randomized on Day 3 post-tumor implant. Mice were infused IV the following day with 5 × 10^6 engineered CD8+ pmel cells. Treatment groups were dosed with either TMP at 500 mg/kg or vehicle (PEG400) daily via oral gavage from day of infusion (Day 0) until termination of the study. Animals were weighed and imaged for tumor burden twice weekly. Blood was collected via submandibular bleed and plasma isolated on Day 7 post-ACT.

Figure 7. NFAT + cytoDRiVE provides durable tumor control in a liver metastasis model of colon cancer without hepatotoxicity

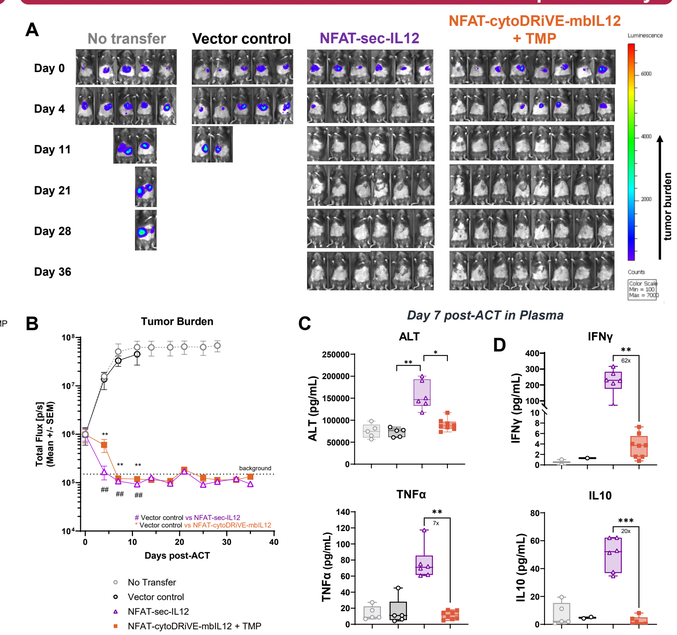

Figure 7. MC38-gp100-Luc liver tumor-bearing mice were adoptively transferred with 5 × 10^6 engineered CD8+ pmel cells 3 days post-tumor implant. Animals were measured twice weekly for bodyweight (not significant, not shown) and tumor burden via IVIS measured by total flux (p/s). Animals were removed from the study when they had two consecutive BLI measurements above 1 × 10^7 p/s **(A-B).** ALT was measured using a Mouse ALT SimpleStep ELISA kit **(C)** and systemic IFNγ, TNFα, and IL10 levels were determined using MSD (Mesocale Discovery) **(D)** from plasma isolated from whole blood on Day 7.

Conclusions

- mbIL12-engineered cells with **NFAT + cytoDRiVE regulation overcome systemic toxicity with tight control of cytokines and no bodyweight loss** compared to cells engineered with secreted IL12 under NFAT control only
- In a syngeneic model of melanoma, activation-dependent and cytoDRiVE-mediated pharmacologic regulation enables **spatiotemporal control of IL12 expression and downstream inflammatory cytokines in the tumor**
- Both T-cell activation (spatial) and DRD ligand (temporal) are required for a **controlled proinflammatory response localized within the TME**
- NFAT-cytoDRiVE-mbIL12 pmel cells **control a syngeneic liver model of colon cancer without the use of lymphodepletion** and with **no evidence of liver toxicity** or elevated systemic cytokines
- By overcoming systemic toxicity and localizing antitumor activity to the TME, this approach has the potential to **enable safe clinical application of this potent cytokine** in ACT

Abbreviations

ACT, adoptive cell therapy; ALT, alanine aminotransferase; BLI, bioluminescent imaging; CRC, colorectal cancer; CTX, Cytoxan; DRD, drug-responsive domain; hDHFR human dihydrofolate reductase; IFNγ, interferon gamma; IL6, interleukin 6; IL10, interleukin 10; IL12, interleukin 12; IV, intravenous; IVIS, in vivo imaging system; mbIL12, membrane-bound IL12; NFAT, nuclear factor of activated T cells; NFAT-RE, nuclear factor of activated T cells response elements; sec, secreted; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocytes; TME, tumor microenvironment; TMP, trimethoprim; TNFα, tumor necrosis factor-alpha.

References

1. Zhang L, et al. *Clin Cancer Res* 21.10(2015): 2278-2288.
2. Smith SG, et al. J ImmunoTher Cancer 2024;12(Suppl 2):A1-A1683 Abstract 463.

Acknowledgments

- Schematics were generated with biorender.io
- This study was funded by Obsidian Therapeutics, Inc. (Cambridge, MA USA)

Disclosures

- All authors report employment by Obsidian Therapeutics, Inc. (Cambridge, MA, USA)