Poster #463

Antigen-responsive promoters coupled with cytoDRiVE® technology provides tight spatiotemporal regulation for tumor-infiltrating lymphocytes (TIL) expressing membrane-bound IL12 (mbIL12)

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Introduction

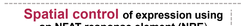
Interleukin 12 (IL12) is a promising candidate for armoring adoptive cell therapies (ACT); however, safety concerns have limited its utility

- IL12 is a potent cytokine known to remodel immunosuppressive tumor microenvironments and promote antitumor immunity through diverse innate and adaptive immune mechanisms[1]
- Activation-induced control of secreted IL12 production in tumor-infiltrating lymphocytes (TIL) was not sufficient to prevent toxicities in a clinical trial[2]
  - Secreted IL12 was driven by NFAT response elements driving an IL2 promoter
  - Trial was halted due to unpredictable toxicities despite a 63% objective response rate (including 1 complete response) with doses greater than 0.3 × 10^9 cells (~10–100× lower cell doses than standard TIL cell therapy)
- **Thus, additional layers of regulation are needed to armor TIL with IL12**

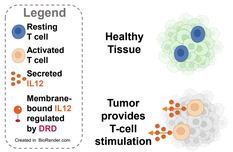
Spatiotemporal regulation of IL12 by combining activation-dependent expression of membrane-bound IL12 (spatial) with cytoDRiVE (temporal)

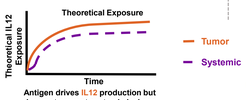
The cytoDRiVE platform can be used to regulate protein expression, acting as a titratable rheostat for on-demand, temporal regulation

Spatial control of expression using an NFAT-response element (NRE) (concept used clinically)



Spatial control of **expression** (NRE) but **NOT distribution** and **NO temporal** control

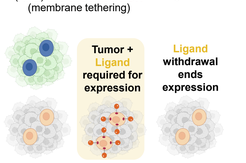


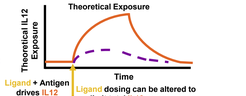


Spatiotemporal control by combining the cytoDRiVE® platform with novel activation-dependent promoters

Spatial control of **expression** (NRE) and **distribution** (membrane tethering)

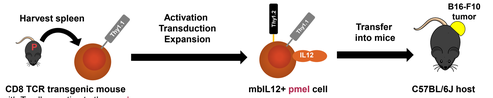
Temporal regulation (DRD)





NFAT: nuclear factor of activated T cells – binds to NREs and drives cytokine expression upon T-cell activation
ARRE1: antigen receptor response element 1 – NFAT binding site in the IL2 minimal promoter; published NRE[2]

pmel model for testing IL12-engineered T cells in immune-competent hosts



Statistics

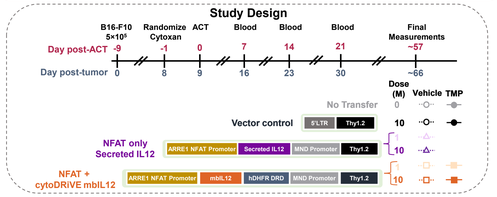
Statistics calculated in GraphPad Prism
Phenotyping and MSD: Welches T-Test
Tumor volume and BW comparisons: Mann-Whitney Test
Survival: Log-Rank Mantel-Cox Test
* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Gating

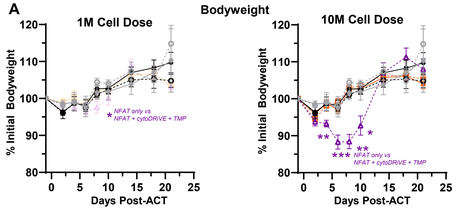
Host T cells: mCD45+ CD3+Thy1.2+Thy1.1-
Transduced pmel cells: mCD45+ CD3+Thy1.1+Thy1.2+
Human T cells: mCD45-hCD3+

Spatiotemporally regulated mbIL12 controls efficacy with no signs of toxicity in syngeneic melanoma

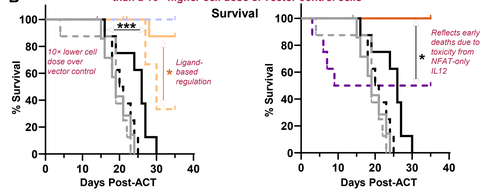
Figure 1. cytoDRiVE platform + T-cell activation dependence enables DRD ligand–regulated efficacy without safety signals



NFAT + cytoDRiVE mbIL12-engineered cells did not cause weight loss even at high cell doses; NFAT-only control did reduce weight, leading to early animal death



mbIL12-engineered cells were more effective at extending survival than a 10× higher cell dose of vector control cells



NFAT + cytoDRiVE mbIL12-engineered cells showed ligand-regulated tumor suppression

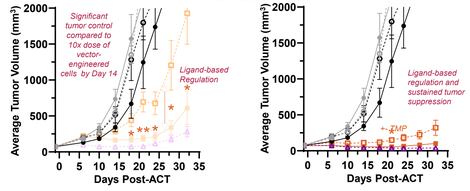
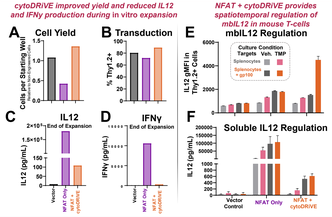


Figure 2. cytoDRiVE + T-cell activation dependence enables TMP-regulated efficacy of mbIL12 expressing pmel-reactive T cells against syngeneic B16-F10 tumors without safety signals. The syngeneic B16-F10 model was implanted subcutaneously in C57BL/6J mice. Animals (n=8 per treatment group) were randomized and lymphodepleted with Cytoxan (200 mg/kg) on Day 8 post-tumor implant. Cells were infused intravenously the following day. No transfer, vector control, and NFAT + cytoDRIVE groups were dosed with either TMP daily via oral gavage at 500 mg/kg or vehicle (PEG400) from the day of infusion. Bodyweight (**A**) and tumor measurements (**C**) were collected twice a week. Blood (100 µL via submandibular bleed) was collected on Day 7, 14, and 21 post-ACT. Survival was tracked based on tumor volume threshold (2000 mm³) and included endpoints of >20% bodyweight loss, being found moribund, or found dead (**B**).

Figure 2. T-cell activation and the presence of DRD ligand are both required for IL12 expression *in vitro* (mouse T-cells)

cytoDRIVE improved yield and reduced IL12 and IFNγ production during in vitro expansion



NFAT + cytoDRiVE provides spatiotemporal regulation of mbIL12 in mouse T-cells

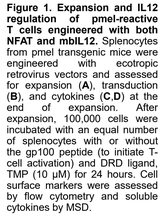
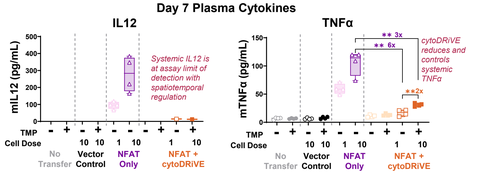


Figure 1. Expansion and IL12 regulation of pmel-reactive T cells engineered with both NFAT and mbIL12. Splenocytes from pmel transgenic mice were engineered with ecotropic retrovirus vectors and assessed for expansion (**A**), transduction (**B**), and cytokines (**C**,**D**) at the end of expansion. After expansion, 100,000 cells were incubated with an equal number of splenocytes with or without the gp100 peptide (to initiate T-cell activation) and DRD ligand, TMP (10 µM) for 24 hours. Cell surface markers were assessed by flow cytometry and soluble cytokines by MSD.

Figure 3. NFAT + cytoDRiVE controls downstream systemic cytokines



Spatiotemporal regulation reduces systemic IFNγ by orders of magnitude over NFAT-only control

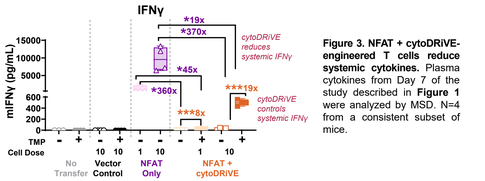


Figure 3. NFAT + cytoDRiVE-engineered T cells reduce systemic cytokines. Plasma cytokines from Day 7 of the study described in **Figure 1** were analyzed by MSD. N=4 from a consistent subset of mice.

Figure 4. NFAT + cytoDRiVE controls T-cell infiltration into tumors

T cells engineered with spatiotemporally regulated IL12 infiltrate tumors in a TMP-regulated manner and drive more robust host–T-cell infiltration than NFAT control alone

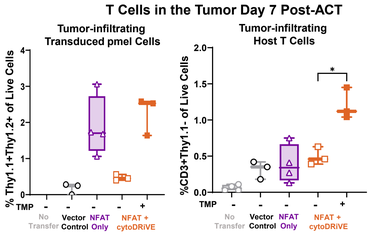
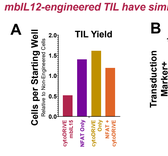


Figure 4. IL12 increases infiltration of T cells into B16 tumors. In a standalone study, B16 tumors were established for 14 days before ACT with 4.5 million pmel cells and dosed with TMP or vehicle as described in **Figure 2**. On Day 7 post-ACT, tumors were digested and assessed for immune cell infiltration using flow cytometry.

Spatiotemporally regulated mbIL12 in human TIL

Figure 5. Human TIL engineered with mbIL12 have enhanced reactivity *in vitro*

mbIL12-engineered TIL have similar yield as non-engineered TIL



mbIL12-engineered TIL have increased tumor reactivity

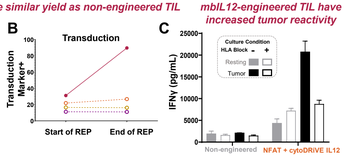
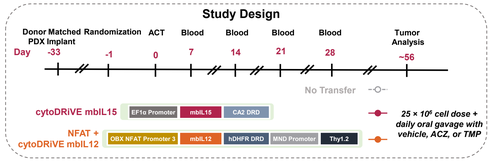
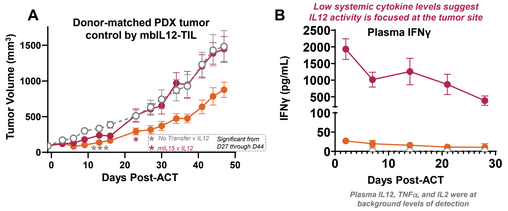


Figure 5. mbIL12-engineered TIL expand in REP and are more reactive to tumor digest than non-engineered TIL. Human TIL were isolated from tumor fragments through pre-REP and engineered with lentivirus as a part of REP. All TIL not engineered with mbIL15 received soluble cytokines to support cell growth as reflected in the cell yield (**A**) while mbIL15-engineered TIL enriched during the REP (**B**). Cryopreserved TIL (500,000) were thawed and incubated with 10 µM TMP and freshly digested donor-matched PDX cells (50,000) with or without HLA blockade for 24 hours before measuring IFNγ (MSD).

Figure 7. mbIL12 TIL demonstrate efficacy against an autologous colorectal cancer lung metastases PDX tumor *in vivo* with low levels of TIL and IFNγ in the periphery





mbIL12-engineered TIL persist in the tumor nearly 2 months post-ACT without growth-factor support, despite rapidly leaving circulation

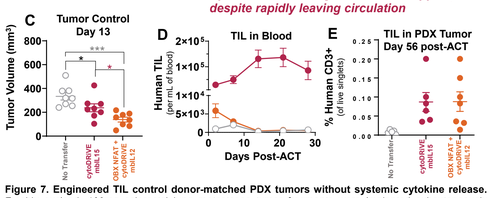


Figure 7. Engineered TIL control donor-matched PDX tumors without systemic cytokine release. Freshly excised, 100mg colorectal lung metastases tumor fragments were implanted subcutaneously within 24 hours of surgery into two female NSG mice and allowed to grow for ~150 days (passage 0). Serial passaging continued until there was enough tumor available to support a large-scale implantation (passage 2). Animals were randomized 32 days after tumor implant and TIL as described in **Figure 5** transferred i.v. the next day (day 0). Tumor volume was measured twice weekly (**A**, **C**). Mice were bled weekly and assessed for TIL persistence (**D**, flow cytometry) and cytokines (**B**, MSD). The study was ended on Day 56 post-ACT, the tumors digested, and TIL infiltration assessed (**E**). (n=8, $25×10^6$ cell dose; ACZ [200 mg/kg, mbIL15 group] or TMP [500 mg/kg, mbIL12 group] via daily oral gavage).

Figure 6. Combining novel antigen-responsive promoters with cytoDRiVE enables spatiotemporal regulation of IL12 in human TIL

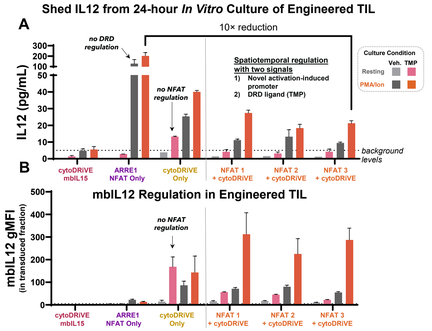


Figure 6. Spatiotemporal regulation of soluble IL12 and mbIL12 in TIL. Cryopreserved TIL as described in **Figure 5** were thawed and plated at 100,000 transduced cells per well with or without activation with PMA and 10 µM TMP for 24 hours before assessing (**A**) soluble cytokine (MSD) and mbIL12 levels (**B**) in the transduced fraction (flow cytometry).

Conclusions

- TCR activation-dependent transcriptional control was successfully combined with the cytoDRiVE platform to enable **spatiotemporal regulation of IL12** *in vitro* and *in vivo*
  - NFAT + cytoDRiVE mbIL12-engineered pmel cells **controlled syngeneic mouse melanoma tumors** in a **DRD ligand–regulated fashion** with **no signs of overt toxicity** even at high cell doses
  - Spatiotemporal regulation of mbIL12-engineered cells enabled **tight control of systemic IFNγ** within levels that were orders of magnitude less than cells engineered with secreted IL12 under NFAT control only
- **Spatiotemporal regulation** was demonstrated with 3 novel TCR signal-responsive promoters in combination with cytoDRiVE in **TIL**
  - NFAT + cytoDRiVE mbIL12-engineered TIL showed **enhanced tumor reactivity** *in vitro* and **tumor control** *in vivo* against autologous PDX tumors with minimal systemic cytokine release
- Combining pharmacologic regulation with activation-induced promoters could unlock the therapeutic window of IL12-armored cell therapies with the potential for **increased efficacy against solid tumors**

Abbreviations

ACT, adoptive cell therapy; ACZ, acetazolamide; CA2, carbonic anhydrase; DRD, drug-responsive domain; hDHFR, human dihydrofolate reductase; HLA, human lymphocyte antigen; interferon gamma, IFNγ; IL2, interleukin 2; IL12, interleukin 12; i.v. intravenously; mbIL12, membrane-bound IL12; NFAT, nuclear factor of activated T cells; NRE, NFAT response element; PDX, patient-derived xenograft; REP, rapid expansion protocol; sec, secreted; TIL, tumor-infiltrating lymphocytes; TMP, trimethoprim; TNFα, tumor necrosis factor alpha.

References

1. Nguyen KG, et al. *Front Immunol* 11(2020): 575597.
2. Zhang L, et al. *Clin Cancer Res* 21.10(2015): 2278-2288.

Acknowledgments

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