Poster #457

Engineered tumor-infiltrating lymphocytes (TIL) with membrane-bound IL15 (mbIL15) and LIGHT (TNFSF14) generate significant antitumor efficacy in fibrotic tumor models

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Introduction

- Non-engineered TIL cell therapy, requiring concomitant administration of high-dose IL2, has demonstrated efficacy in melanoma, but with substantial toxicity[1,2]
- To develop IL2-independent TIL to treat immunologically hot tumors, cytoTIL15™ cells are engineered to express regulatable membrane-bound interleukin 15 (mbIL15) using an FDA-approved small-molecule drug, acetazolamide (ACZ), which binds to the drug-responsive-domain (DRD) carbonic anhydrase 2 (CA2; **Figure 1**), driving pharmacologically regulatable mbIL15-mediated expansion and persistence without the need for exogenous IL2 administration (OBX-115: NCT05470283, NCT06060613)
- To address immunologically cold tumors, another proinflammatory cytokine, LIGHT, was added to the cytoTIL15 backbone. LIGHT, a tumor necrosis factor family member (TNFSF14), interacts with lymphotoxin beta receptor (LTβR) and herpes virus entry mediator (HVEM) found on various tumor microenvironment (TME) cell types, such as cancer-associated fibroblasts (CAF; **Figure 2**)
- Preclinically, intratumoral LIGHT expression via viral or cell therapy leads to tertiary lymphoid structure (TLS) formation[3] and vascular normalization,[4] both associated with better outcomes[5]
- Previously, we showed co-regulated expression of mbIL15 and LIGHT increased tumor cell killing by autologous TIL and efficacy in the B16-F10 syngeneic model[6]
- Herein, efficacy of mbIL15- and LIGHT-engineered TIL was tested in fibroblast-rich patient-derived human xenograft (PDX) and syngeneic tumor models

Figure 1. cytoDRiVE technology

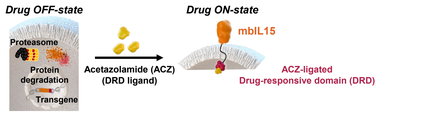

Figure 1. The cytoDRiVE platform uses a drug-responsive domain (DRD) to enable an FDA-approved small-molecule ligand to regulate expression of a protein fused to the DRD. In the absence of the cognate DRD ligand (drug OFF-state), the fusion protein is degraded by the proteasome. In the presence of the ligand (drug ON-state), the DRD is stabilized and the protein is expressed. In the case of cytoTIL15 and cytoTIL15-LIGHT cells, a DRD derived from carbonic anhydrase 2 (CA2) is used to regulate both mbIL15 and LIGHT via delivery of acetazolamide (ACZ).

Figure 2. Rationale for engineering TIL with mbIL15 and LIGHT

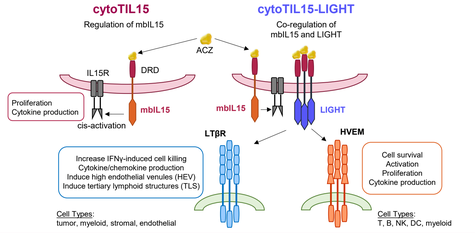

Figure 3. cytoTIL15-LIGHT cells show enhanced cytotoxicity against autologous stromal-rich CRC tumor spheroids

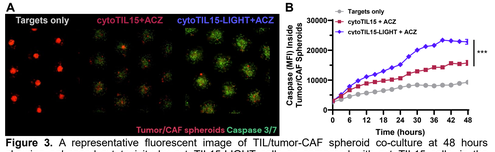

Figure 3. A representative fluorescent image of TIL/tumor-CAF spheroid co-culture at 48 hours showing enhanced cytotoxicity by cytoTIL15-LIGHT cells as compared with cytoTIL15 cells, in the presence of ACZ, as indicated by caspase 3/7 staining (green; **A**). Cytotoxicity quantification of CRC cytoTIL15 cells versus cytoTIL15-LIGHT cells against autologous tumor/CAF spheroids over the 48-hour co-culture **(B)**. Statistical tests: one-way ANOVA, cytoTIL15+ACZ v cytoTIL15-LIGHT+ACZ, ***p value<0.005. *Previously shown at AACR 2024.*[6]

Results

Figure 4. CRC PDX model generation and timeline

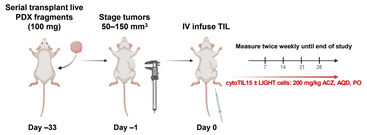

Figure 4. A CRC lung metastasis was excised from a patient and ~100 mg tumor fragment was implanted subcutaneously within 24 hours of surgery (Passage 0 [P0]). Two NSG female mice were implanted with similarly sized fragments that were allowed to grow for ~150 days. Serial passaging was performed until there was enough viable tumor tissue available to support a large-scale implantation for an efficacy study. Animals (n=8 per treatment group) were randomized on Day 32 following the large-scale PDX tumor implant, and TIL (cytoTIL15 or cytoTIL15-LIGHT cells) were infused IV the following day. Both treatment groups were dosed ACZ daily via oral gavage at 200 mg/kg from day of infusion (Day 0) until termination of the study.

Figure 5. Autologous cytoTIL15-LIGHT show similar persistence and enhanced activity in a CAF-rich CRC PDX model

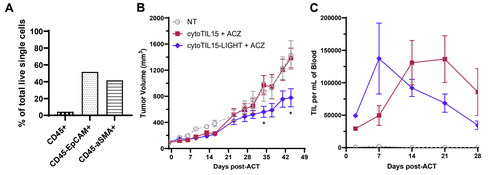

Figure 5. Expression levels of immune cell (CD45+), tumor (EpCAM+), and CAF (αSMA+) surface markers on P0 tumor tissue after growing in an NSG mouse for 150 days. PDX tumor was excised, homogenized, and analyzed by flow cytometry with anti-human CD45, anti-human EpCAM, and anti-human αSMA **(A)**. CRC PDX-bearing NSG mice were adoptively transferred with 25 × 10⁶ engineered autologous TIL exactly 33 days post-tumor implant **(B–C)**. Tumor measurements were collected twice a week via digital calipers **(B)** starting on the day before ACT and continuing for the duration of the study. Whole blood samples were collected weekly (n=4 per treatment group) via submandibular bleeding to quantitate TIL **(C)**. Statistical tests: Mann-Whitney, cytoTIL15-LIGHT v cytoTIL15, *p value<0.05; last two timepoints, cytoTIL15-LIGHT (n=8) v cytoTIL15 (n=7).

Figure 6. Utilizing transgenic CD8+ pmel TCR-T cells to investigate impact of LIGHT on the TME

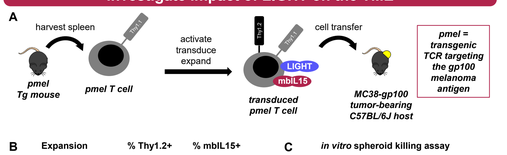

Figure 6. Splenocytes from transgenic mice expressing the gp100-specific pmel TCR were retrovirally transduced to express constitutive mbIL15 and LIGHT, or mbIL15 alone. After transduction, these CD8+ splenocytes were then expanded in the presence of murine IL2 for 6 days. The mouse colorectal cancer 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 **(A)**. Fold-increase in CD8+ pmel cells was monitored throughout the expansion; transduction efficiency and mbIL15 expression levels were determined by flow cytometry on day of ACT **(B)**. Cytotoxicity quantification of CRC cytoTIL15 cells versus cytoTIL15-LIGHT cells against autologous tumor/CAF or tumor-only spheroids over a 24-hour co-culture **(C)**.

Figure 7. LIGHT enhances the anti-tumor activity of mbIL15-expressing pmel T cells against large, fibrotic MC38-gp100 tumors

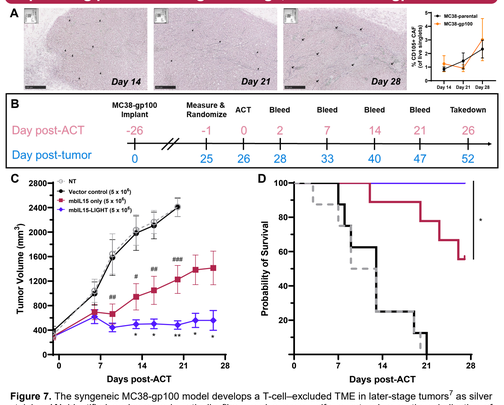

Figure 7. The syngeneic MC38-gp100 model develops a T-cell–excluded TME in later-stage tumors[7] as silver staining **(A)** identified an increase in reticulin fibers and a more uniform network over time, indicative of fibrosis **(B–D)** The mouse CRC cell line MC38 engineered to express gp100 was implanted subcutaneously in C57BL/6J mice. Animals (n=8 per treatment group) were randomized on Day 25 following the syngeneic tumor implant, which developed into large (~300 mm³ average) fibrotic tumors and pmel cells were infused IV the following day **(B)**. Tumor measurements were collected twice a week via digital calipers starting on the day before ACT and continuing for the duration of the study **(C)**, and overall survival was tracked based on tumor volume threshold **(D)**. Statistical tests: Mann-Whitney, mbIL15-LIGHT v mbIL15, *p value<0.05, **p value<0.01; mbIL15 v Vector control, #p value<0.05, ##p value<0.01, ###p value<0.005 **(C)**; log-rank (Mantel-Cox), mbIL15-LIGHT v mbIL15, *p value<0.05 **(D)**.

Figure 8. mbIL15- and LIGHT-expressing pmel T cells create a pro-inflammatory TME in MC38-gp100 tumors

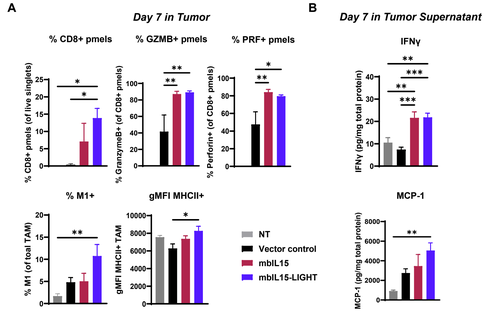

Figure 8. Fibrotic MC38-gp100 tumor-bearing mice were adoptively transferred with 5 × 10⁶ engineered CD8+ pmel cells 26 days post-tumor implant. Tumors were collected on Day 7 post-ACT and homogenized using GentleMACS with Mouse Tumor Dissociation Kit (Miltenyi Biotec) and TME phenotype was characterized using flow cytometry. Transduced pmel cells were identified as single live CD45+ CD3+ CD8+ Thy1.1+ Thy1.2+ cells; M1 phenotype of total TAM population were identified as single live CD45+ CD3- CD11b+ F4/80+ iNOS+ cells **(A)**. Tumor IFNγ and MCP-1 levels were determined using MSD (Mesocale Discovery) on supernatants collected during tumor homogenization **(B)**. Each bar represents the mean ± SEM from 5 individual tumors. Statistical tests: ordinary one-way ANOVA, all comparisons, *p value<0.05, **p value<0.01, ***p value<0.005.

Figure 9. mbIL15- and LIGHT-expressing pmel T cells induce B-cell aggregates and TLS-like structures in the fibrotic MC38-gp100 CRC syngeneic tumor model

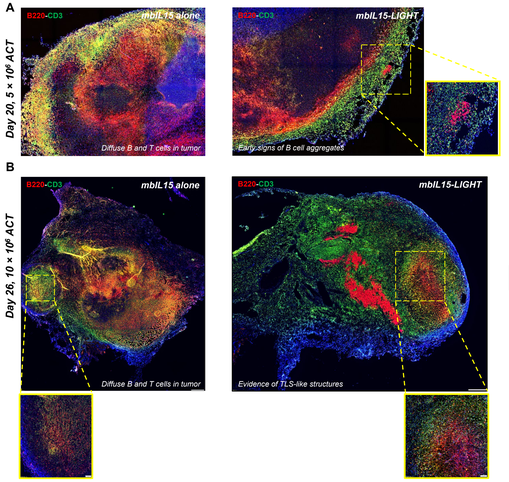

Figure 9. The mouse CRC cell line MC38 engineered to express gp100 was implanted subcutaneously in C57BL/6J mice. After 26 days, implants developed into large (~300 mm³ average) fibrotic tumors and mouse CD8+ pmel cells engineered with either mbIL15 alone or mbIL15-LIGHT were adoptively transferred into the tumor-bearing mice. Tumors were harvested at Day 20 **(A)** and Day 26 **(B)** post-ACT. After overnight incubation in 10% formalin, tumors were bathed in 30% sucrose solution for 3–5 days before being frozen in OCT cups. Fluorescent staining was performed on 10–12-µm thick cryosections using anti-B220 (B cells) and anti-CD3 (T cells) antibodies. *Images in (B) previously shown at AACR 2024.*[6]

Figure 10. LIGHT increases transferred pmel T cell activation while reducing endogenous tumor T cell exhaustion

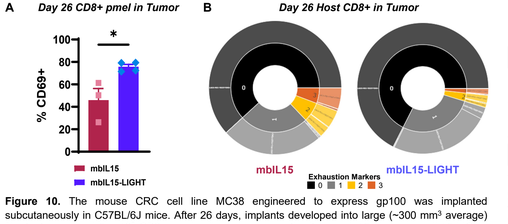

Figure 10. The mouse CRC cell line MC38 engineered to express gp100 was implanted subcutaneously in C57BL/6J mice. After 26 days, implants developed into large (~300 mm³ average) fibrotic tumors and 5 × 10⁶ CD8+ pmel cells engineered with either mbIL15 alone or mbIL15-LIGHT were adoptively transferred into the tumor-bearing mice. Tumors were collected on Day 26 post-ACT and homogenized using GentleMACS to characterize the TME using flow cytometry. CD69 expression of transduced pmel cells identified as single live CD45+ CD3+ CD8+ Thy1.1+ Thy1.2+ cells **(A)**. Pie charts represent the percentage of host CD8+ cells expressing 0, 1, 2, or 3 of the exhaustion markers (TIM3, LAG3, PD1, TIGIT) analyzed **(B)**. Host CD8+ cells identified as single live CD45+ CD3+ CD8+ Thy1.1- Thy1.2+ cells. Statistical tests: Unpaired t test, mbIL15-LIGHT v mbIL15, *p value<0.05 **(A)**; Chi-square test, mbIL15-LIGHT v mbIL15, p-value = 0.194 **(B)**.

Figure 11. LIGHT enhances epitope spreading to MC38-specific peptides

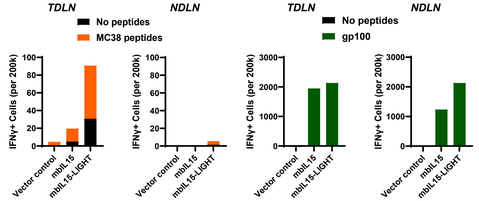

Figure 11. Tumor-draining (TDLN) and non-draining lymph nodes (NDLN) from opposite flank were collected on Day 20 post-ACT from mice described in **Figure 10**. To detect epitope spread, TDLN and NDLN were first homogenized by pushing through a 70-µm cell strainer using a syringe plunger. Single-cell suspensions were cultured in anti-mouse IFNγ Ab-coated ELISPOT plates (CTL) in presence of peptides relevant to MC38 tumor (Cpne1, Irgq, Reps1, Rp18 P15e, Zbtb40, Adpgk) or gp100 for 24 hours followed by the detection of IFNγ-producing cells. Data representative of the average of two technical replicates of n=5 pooled samples per treatment group.

Conclusions

- Adding LIGHT to mbIL15 in an autologous TIL product **yields greater anti-tumor activity** in a CAF-rich CRC PDX model
- Pmel cells expressing both mbIL15 and LIGHT **exhibited robust, durable tumor growth inhibition** and **provided a survival advantage** *in vivo* over cells expressing mbIL15 alone in a large, fibrotic syngeneic CRC tumor model
- Syngeneic mbIL15 and LIGHT co-expressing pmel cells **induce B-cell aggregates** and **TLS formation** in MC38 tumors *in vivo*
- LIGHT **induces spreading of the T-cell response** to MC38-specific epitopes in tumor-draining lymph nodes, corroborating its ability to promote priming of new endogenous T-cell immune responses
- These preclinical results suggest that engineering TIL with regulatable mbIL15 and LIGHT has the potential to enhance their efficacy against challenging-to-treat tumors with a fibrotic TME, **providing an opportunity to address unmet medical need**

References

1. Rohaan MW, et al. N Engl J Med 2022; 387(23):2113-2125.
2. Chesney J, et al. J Immunother Cancer 2022; 10(12):e005755.
3. Zhang N, et al. Mol Ther 2023; 31(9):2575-2590.
4. Ramachandran M, et al. Cancer Cell 2023; 41(6):1134-1151.
5. Sautès-Fridman C, et al. Nat Rev Cancer 2019; 19(6):307-325.
6. Koscso B, et al. Cancer Research 2024; 84(7_Supplement): Abstract LB065.
7. Shields NJ, et al. Front Immunol 2023; 14:1152035

Abbreviations

ACT, adoptive cell transfer; ACZ, acetazolamide; CAF, cancer-associated fibroblast; CRC, colorectal cancer; DRD, drug-responsive domain; HEV, high endothelial venule; IV, intravenous; LIGHT, homologous to Lymphotoxin, exhibits Inducible expression and competes with HSV Glycoprotein D to Herpesvirus entry mediator, a receptor expressed on T lymphocytes; mbIL15, membrane-bound IL15; NDLN, non-draining lymph node; NT, no transfer; P0, passage 0; PDX, patient-derived xenograft; TCR, T-Cell Receptor; TDLN, tumor-draining lymph node; TIL, tumor-infiltrating lymphocytes; TLS, tertiary lymphoid structure; TME, tumor microenvironment; TNFSF14, tumor necrosis factor (ligand) superfamily member 14.

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Disclosures

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