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Tumor-infiltrating lymphocytes (TIL) engineered with regulatable membrane-bound IL15 (mbIL15) and LIGHT (TNFSF14) show enhanced efficacy in fibroblast-containing cold tumors

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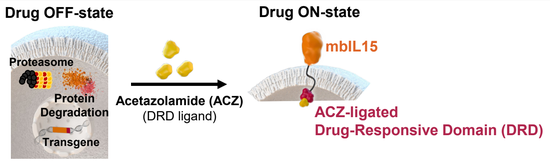
Introduction

- Adoptive cellular therapies have encountered challenges in solid tumors due in part to the immunosuppressive tumor microenvironment (TME)
- We have developed cytoTIL15™ (OBX-115 - NCT05470283), TIL engineered to express regulatable membrane bound (mb) IL15 using the cytoDRiVE® platform, which allows for TIL expansion, persistence, and anti-tumor efficacy under control of the FDA-approved small-molecule ligand, acetazolamide (ACZ), which eliminates the need for co-administration of IL2 in a clinical setting
- LIGHT, a tumor necrosis factor family member, interacts with lymphotoxin beta receptor (LTbR) and herpes virus entry mediator (HVEM) found on various TME cell types, including stromal cells such as cancer associated fibroblasts (CAF)
- In preclinical studies, LIGHT expression within a tumor has been linked to the formation of tertiary lymphoid structures and vascular normalization,[1] both associated with better clinical outcomes[2]
- We hypothesized that engineering TIL with regulatable mbIL15 and LIGHT expression (cytoTIL15-LIGHT™ cells) could enhance their efficacy in fibroblast-containing cold tumors by modifying the TME.

cytoDRiVE Technology



The cytoDRiVE platform uses drug-responsive domain (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), 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 1. Rationale for TIL engineering

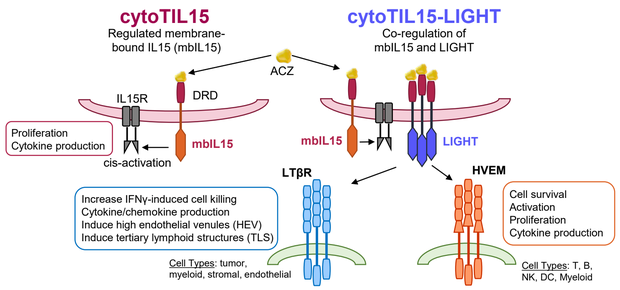
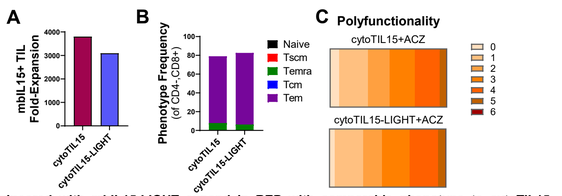


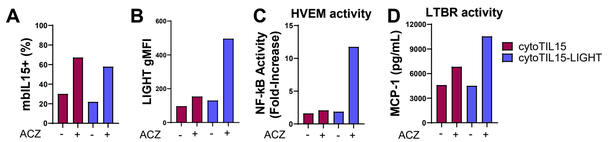
Figure 2. cytoTIL15-LIGHT cells expand in REP with phenotype comparable to cytoTIL15 cells



TIL engineered with mbIL15-LIGHT expand in REP with comparable phenotype to cytoTIL15 cells. TIL isolated from a colorectal carcinoma (CRC) donor were transduced with retroviral vectors and expanded using a proprietary REP process including ACZ. **A**. Fold-expansion of transduced TIL at the end of REP over initial day. **B**. TIL memory phenotype was analyzed using flow cytometry at the end of REP, CD3+ cells were identified as effector memory Tem (CD45RO+CD45RA-CCR7-CD62L-), central memory Tcm (CD45RO+CD45RA-CCR7+CD62L+), terminally differentiated effector memory Temra (CD45RO-CD45RA+CCR7-CD62L-), naïve Tnaive (CD45RO-CD45RA+CCR7+CD62L+), and stem cell memory TSCM (CD95+ naïve T cells). **C**. TIL polyfunctionality was analyzed using flow cytometry at the end of REP following 6-hour activation with anti-CD3 and anti-CD28. The charts show percentage of TIL expressing 0–6 of the functionality markers CD107a, IFNγ, TNFα, IL2, perforin, granzyme B. Data shown are representative of 4 donors.

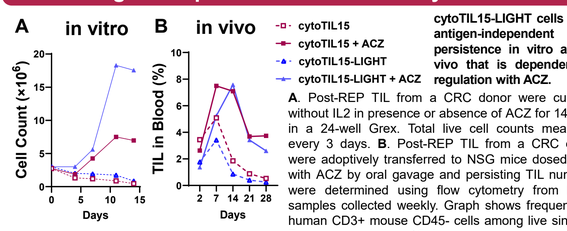
Results

Figure 3. cytoTIL15-LIGHT cells show regulated expression and function in vitro



mbIL15 and LIGHT expression on cytoTIL15-LIGHT cells is regulated by ACZ. A-B. Post-REP TIL from a CRC donor cultured with or without ACZ for 24 hours; cell surface LIGHT and mbIL15 analyzed using flow cytometry. **C**. Post-REP TIL from a CRC donor incubated with or without ACZ for 48 hours followed by 3 hours co-culture with Jurkat-HVEM-NF-kappaB reporter cells (BPS Bioscience); HVEM activation by LIGHT detected by measuring luciferase activity. **D**. Post-REP TIL rested for 48 hours after thaw followed by co-culture with HUVEC cells (ATCC) for 24 hours in presence or absence of ACZ; MCP-1 levels determined in supernatants using MSD. Data representative of 4 donors.

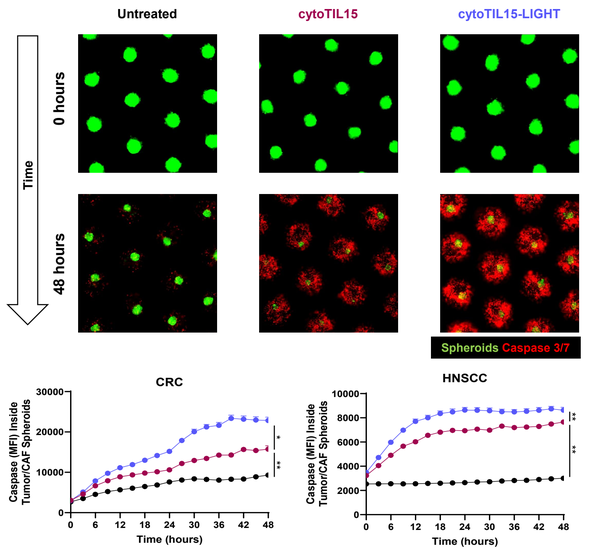
Figure 4. cytoTIL15-LIGHT cells show regulated persistence similar to cytoTIL15



cytoTIL15-LIGHT cells show antigen-independent persistence in vitro and in vivo that is dependent on regulation with ACZ.

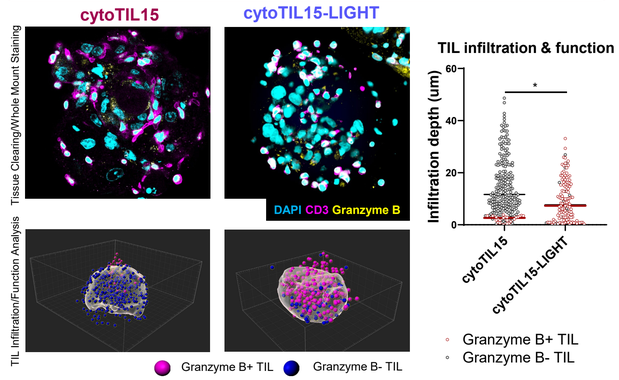
A. Post-REP TIL from a CRC donor were cultured without IL2 in presence or absence of ACZ for 14 days in a 24-well Grex. Total live cell counts measured every 3 days. **B**. Post-REP TIL from a CRC donor were adoptively transferred to NSG mice dosed daily with ACZ by oral gavage and persisting TIL numbers were determined using flow cytometry from blood samples collected weekly. Graph shows frequency of human CD3+ mouse CD45- cells among live singlets. Data representative of 2 donors.

Figure 5. cytoTIL15-LIGHT cells show enhanced cytotoxicity against autologous fibrotic tumor spheroids



cytoTIL15-LIGHT cells show enhanced cytotoxicity against autologous fibroblast-rich, non-melanoma spheroid models. (*Top*) Representative fluorescence images of TIL/tumor-CAF spheroid co-culture at 0 and 48 hours showing enhanced cytotoxicity by cytoTIL15-LIGHT cells as compared with cytoTIL15 cells, as indicated by caspase 3/7 staining (red); (*Bottom*) Quantification of cytotoxicity of CRC and head and neck squamous cell carcinoma (HNSCC) cytoTIL15 cells versus cytoTIL15-LIGHT cells against autologous tumor/CAF spheroids. Statistical tests: ANOVA: *p value<0.05, **p value<0.005.

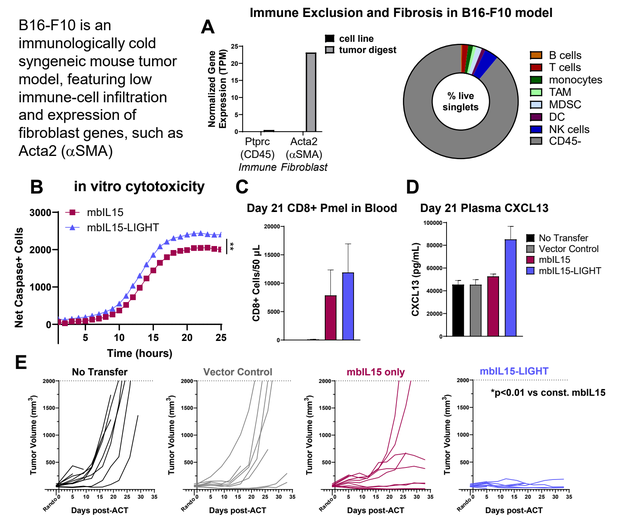
Figure 6. cytoTIL15-LIGHT cells show enhanced function in fibrotic tumor spheroids



cytoTIL15-LIGHT cells show enhanced function in CAF-containing tumor spheroids. (*Top*) Representative fluorescence images x-y plane of tissue-cleared, whole-mount staining of TIL/tumor-CAF spheroid for CD3/granzyme B post 24-hour co-culture; yellow arrows mark granzyme B+ CD3+ TIL. (*Middle*) Computational representation of tissue-cleared, whole-mount staining of CD3+ Granzyme B+ T cells (Granzyme B+ TIL) and CD3+ Granzyme B- T cells (Granzyme B- TIL). (*Bottom*) Quantification of spatial distribution of Granzyme B+ and Granzyme B- TIL within the tumor spheroids or tumor/CAF hybrid spheroids in co-culture conditions with cytoTIL15 and cytoTIL15-LIGHT cells. Statistical tests: ANOVA: *p value<0.005

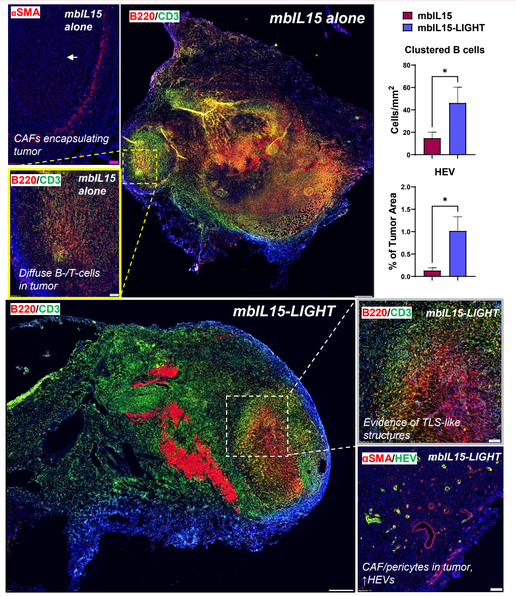
Figure 7. mbIL15- and LIGHT-expressing CD8+ Pmel cells control tumor growth in the cold B16-F10 model

B16-F10 is an immunologically cold syngeneic mouse tumor model, featuring low immune-cell infiltration and expression of fibroblast genes, such as Acta2 (αSMA)



mbIL15 and LIGHT expression on CD8+ Pmel cells increases cytotoxicity to B16-F10 in vitro and tumor growth control in vivo. A. Expression levels of immune cell marker CD45 (Ptprc) and fibrosis marker alpha smooth muscle actin (Acta2) in RNA-sequencing dataset from B16-F10 cell line and tumor digest (left). B16-F10 tumors were homogenized using Gentlemacs instrument and immune cells populations were analyzed using flow cytometry (right). **B-E**. CD8+ cells from spleens from Pmel mice (transgenic for a gp100 specific T cell receptor) were engineered with mouse retroviral vectors and expanded in presence of mouse IL2 for 3 days. **B**. CD8+ Pmel cells were co-cultured with B16-F10 cells pre-treated with IFNγ for 24 hours in presence of Caspase-3/8 fluorescent dye and imaged every hour using Incucyte system. **C-E**. B16-F10 bearing C57Bl/6J mice were adoptively transferred with 10^6 engineered CD8+ Pmel cells 9 days post-tumor implant and 1 day post-lymphodepletion using cyclophosphamide. Whole blood and plasma collected at Day 21 post-ACT to analyze (**C**) CD8+ Pmel cell numbers and (**D**) CXCL13 levels, respectively. Tumor measurements were performed twice a week as shown on growth curves (**E**). Statistical tests: ANOVA: *p value<0.01, **p value<0.005.

Figure 8. mbIL15- and LIGHT-expressing CD8+ Pmel cells induce HEV and TLS in a syngeneic mouse CRC model



mbIL15-LIGHT induces TLS formation in fibrotic mouse CRC model MC38 in vivo. The mouse CRC cell line MC38 engineered to express gp100 were implanted s.c. in C57Bl/6J mice. After 24 days implants developed into large (300 mm^3) fibrotic tumors and mouse Pmel cells engineered with mbIL15 alone and mbIL15-LIGHT were adoptively transferred to the tumor bearing mice. Tumors were harvested at Day 29 post-ACT and after overnight incubation in 10% formalin, were bathed in 30% sucrose solution for 3 days before being frozen in OCT cups. Fluorescent staining was performed on 12-µm thick cryosections using anti-B220 (B cells) and anti-CD3 antibody (T cells) or with anti-alpha smooth muscle actin (α-SMA) antibody (CAF or pericytes) and PNAd/MECA-79 antibody (high endothelial venules [HEV]). For quantification, B cells enumerated based on clustering and normalized to total tumor area. HEV areas were quantified based on PNAd/MECA-79 staining and morphology criteria. Statistical tests: Mann-Whitney U test, *p-value<0.05.

Conclusions

- These data demonstrate a novel application of cytoDRiVE technology to **coregulate expression of two cytokines using a single DRD**
- cytoTIL15-LIGHT cells have similar phenotype and persistence as cytoTIL15 cells, and both mbIL15 and LIGHT expression are regulatable with ACZ
- mbIL15- and LIGHT-expressing cells demonstrate enhanced cytotoxicity and function to **control growth of fibrotic/cold tumors** (data from human TIL and murine Pmel cells)
- Syngeneic mbIL15- and LIGHT-expressing Pmel cells **induce HEV and TLS formation in tumors** in vivo
- These preclinical results suggest that TIL engineered with regulatable mbIL15 and LIGHT using the cytoDRiVE platform have the **potential to address the high unmet clinical need in cold tumors with suppressive TME**, which are currently not amenable to adoptive cell therapy

Abbreviations

ACT, adoptive cell transfer; CAF, cancer-associated fibroblasts; CRC, colorectal cancer; DRD, drug-responsive domain; HEV, high endothelial venule; HNSCC, head and neck squamous cell carcinoma; 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; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocytes; TLS, tertiary lymphoid structure; TME, tumor microenvironment; TNFSF14, tumor necrosis factor (ligand) superfamily member 14.

References

1. Ramachandran M, et al. *Cancer Cell* 2023; 41:1134–1151 e1110.
2. Sautes-Fridman C, et al. *Nat Rev Cancer* 2019; 19:307–325.

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Disclosures

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