SITC 38th Annual Meeting 2023
Abstract 348

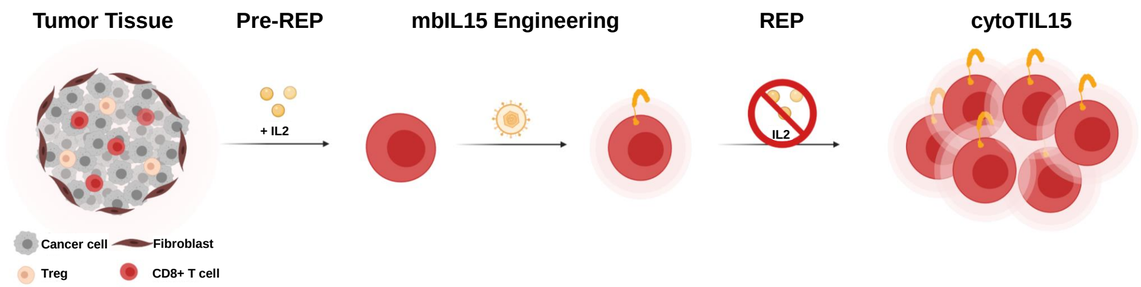
TIL engineered with membrane-bound IL15 (cytoTIL15™) are enriched for tumor-associated antigen reactivity and demonstrate pharmacologically tunable expansion and persistence in the presence of TAA

Rachel Burga, Alonso Villasmil Ocando, Arman Aksoy, Kyle Pedro, Gauri Kulkarni, Meghan Langley, Benjamin Primack, Theresa Ross, Violet Young, Jeremy Tchaicha, Jan ter Meulen, Michelle Ols
Obsidian Therapeutics, Inc. 1030 Massachusetts Avenue, Cambridge, MA 02138

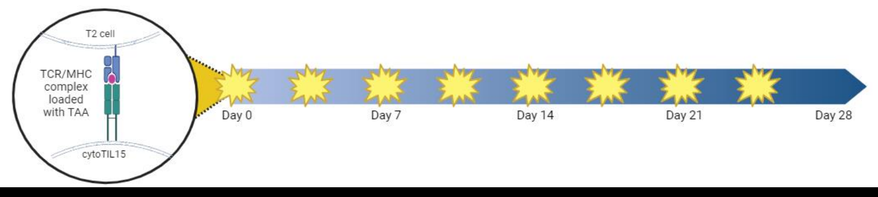
Background

cytoTIL15™ therapy is an investigational tumor-infiltrating lymphocyte (TIL) product engineered to express membrane-bound IL15 (mbIL15) under pharmacologic regulation by the FDA-approved small molecule acetazolamide (ACZ), avoiding the need for high-dose IL2 as used in unengineered TIL cell therapy. Previously, we have shown that ACZ regulates expansion and persistence of cytoTIL15 cells *in vitro* and *in vivo* in a dose-dependent fashion.[1,2] Here, we evaluate the kinetics of antigen-specific cytoTIL15 TIL expansion in the presence of conserved melanoma antigens in a chronic stimulation assay with ACZ. This preclinical model allows for an assessment of the potential functionality of this engineered TIL cell therapy in the context of clinically relevant antigen exposure.

Rationale and Methods



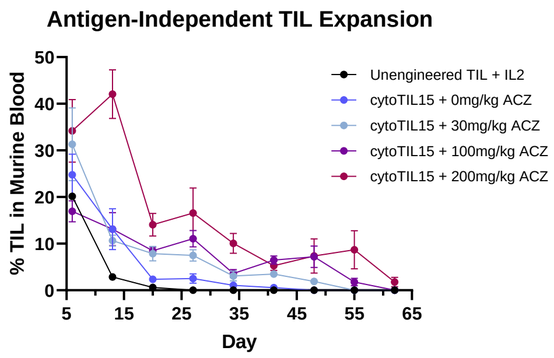
cytoTIL15 cells were engineered using TIL from donors with metastatic melanoma by viral vector transduction of mbIL15 under the control of a carbonic-anhydrase-2 (CA2) drug-responsive domain that is regulatable with ACZ. cytoTIL15 cells were expanded through a proprietary rapid expansion process including pre-REP and REP phases with mbIL21- and 4-1BBL–engineered feeder cells in the absence of IL2, as demonstrated above. In vitro, T-cell receptor-based functionality was assessed using peptide-loaded HLA-A*0201 T2 cells to present MART-1 or gp100 to HLA-matched TIL. cytoTIL15 cells treated with 0–25 µM ACZ were stimulated with antigen twice weekly, as depicted below, with routine assessment of mbIL15 expression, signaling, cell viability, phenotype, cytokine production, and gene expression profiling. As a positive control for these *in vitro* assays, unengineered TIL were treated similarly, supported for assay entirety by high-dose IL2.



Results

cytoTIL15 cells exhibit pharmacologically tunable persistence and expansion

In vivo Persistence



In vitro Expansion

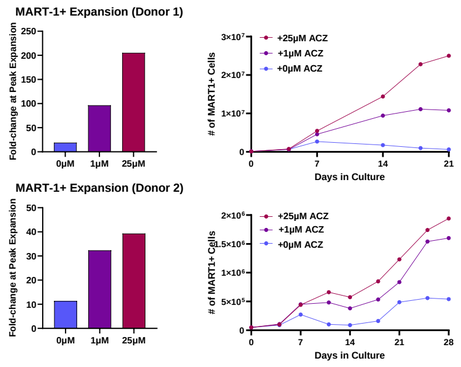


Figure 1: *Left:* cytoTIL15 cells were adoptively transferred into non–tumor-bearing NSG mice dosed with 0, 30, 100, or 200 mg/kg ACZ QD (or IL2 for the first 4 days) and tracked via flow cytometry on peripheral blood. Increased ACZ dosing correlates with enhanced cytoTIL15 persistence (mean AUC: 114, 199, 227, and 371 %TIL*day for 0, 30, 100, and 200 mg/kg ACZ QD, n=3 donors). *Right*: When stimulated with MART-1 as a model tumor-associated antigen (TAA), cytoTIL15 cells exhibit ACZ-dependent expansion of MART-1+ cells, expanding 19-, 96-, and 205-fold in response to 0, 1, and 25µM of ACZ, respectively for Donor 1 (top), and 11-, 32-, and 39-fold in response to 0, 1, and 25µM of ACZ for Donor 2 (bottom).

Tunable IL15 expression supports MART-1–specific expansion during repeated antigen exposure and drives polyfunctionality

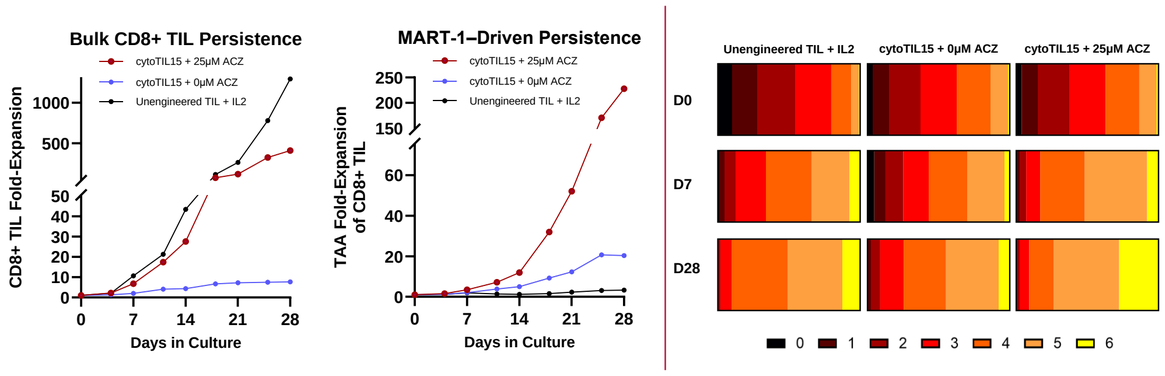
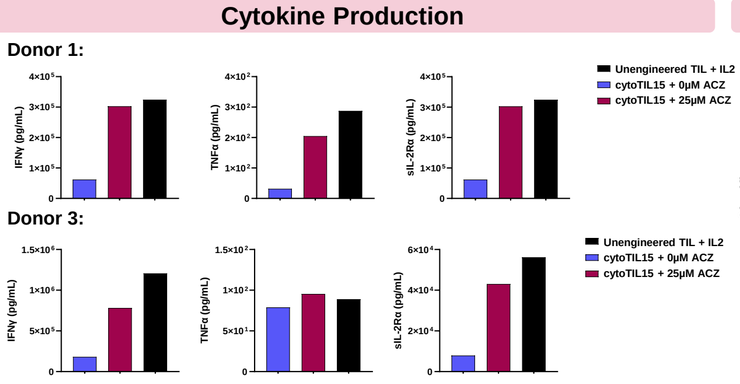


Figure 2: *Left:* Biweekly stimulation with antigen-pulsed T2 cells leads to IL15-dependent expansion of MART-1+ reactive TIL, which occurs to a lesser extent in unengineered TIL supplemented for 28 days with high-dose IL2 (data normalized to magnitude of antigen-independent expansion; repeated in n=3 with MART-1 or gp100 antigen). *Right:* These cytoTIL15 cells demonstrate robust polyfunctionality, as they exhibit IL15-dependent increases in production of effector cytokines following 28 days of repeat antigen exposure. Polyfunctionality is depicted as the frequency of cells expressing 1, 2, 3, 4, 5, or 6 of IFNγ, IL2Rα, TNFα, IL2, perforin, CD107a, and granzyme B (n=3).

Chronic antigen exposure drives an IL15-dependent effector phenotype and cytotoxic gene signature

Cytokine Production



Bulk RNAseq

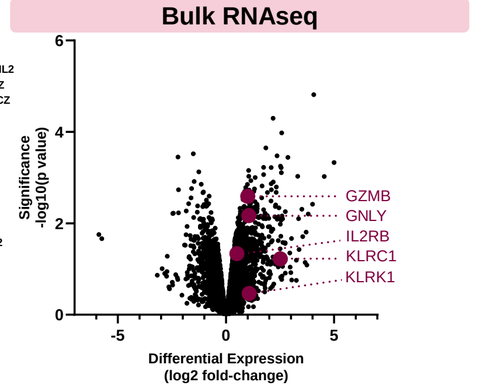
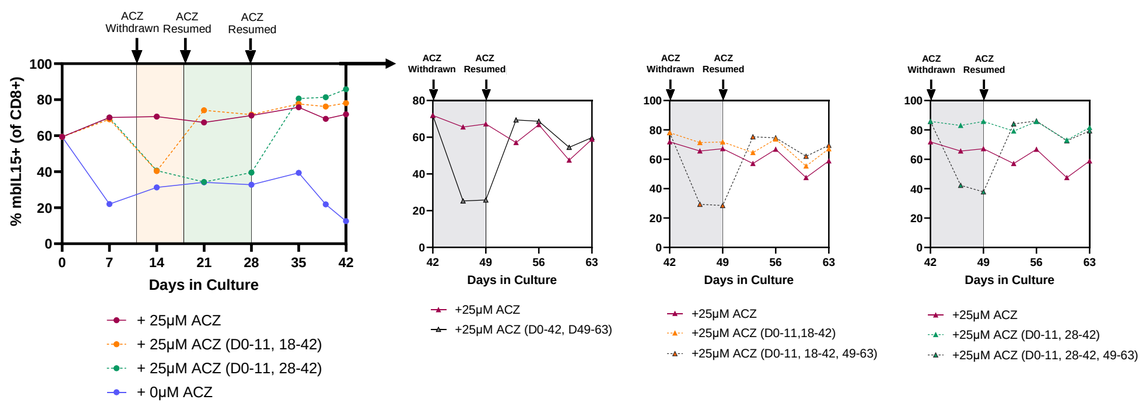


Figure 3: *Left:* TAA-reactive cytoTIL15 cells secrete soluble effector cytokines IFNγ, TNFα, and sIL-2Rα and exhibit enriched *KLRK1* gene expression (NKG2D; not shown) following repeated stimulation with either MART-1 or gp100 pulsed antigen-presenting cells, which occurs in an IL15/ACZ-dependent manner; Day 14 data; Unengineered cells receiving continuous IL2 not meant to mimic clinical regimens. *Right:* Bulk RNAseq transcriptomics reveal a differential gene-expression profile in cytoTIL15 cells driven by ACZ, underscored by an enrichment in genes associated with pro-effector function and a favorable TIL phenotype, such as *IL2RB*, *GNLY*, *GZMB*, *KLRK1* and *KLRC1*. Volcano plots comparing unengineered and 0µM ACZ vs. 25µM ACZ.

cytoTIL15 cells maintain ACZ-dependent regulation of IL15 expression and expansion in the presence of antigen long-term

Regulation of mbIL15 Expression



Regulation of cytoTIL15 Expansion

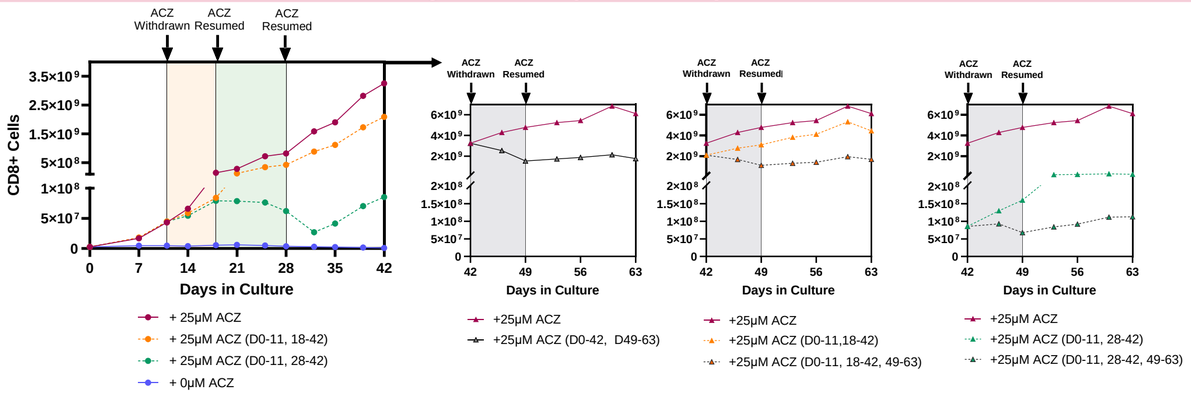


Figure 4: Despite multiple cycles of IL15 regulation, cytoTIL15 cells retain ACZ-dependent tunability of IL15 expression (*top*) and exhibit corresponding regulatability of CD8+ TIL persistence and expansion (*bottom*) with continuous antigenic stimulation, both of which are abrogated when ACZ is removed and return upon ACZ re-administration. Comparable regulation of MART-1+ cell expansion in cytoTIL15 with withdrawn and resumed ACZ dosing was observed (data not shown). ACZ was administered continuously or in incremental windows as described while TIL were maintained with repeated MART-1 antigen stimulation for 63 days.

Conclusions

- **cytoTIL15 cells demonstrated regulatable IL15-dependent functionality, as measured by enhanced persistence *in vivo***
- **IL15 engineering drives a tunable expansion of TAA-reactive TIL**
- **With repeated MART-1 or gp100 (data not shown) antigenic stimulation, cytoTIL15 cells enrich for MART-1 or gp100-reactive TIL**
- **cytoTIL15 cells demonstrated sustained polyfunctionality following 8 rounds of stimulation**
- **Persisting cytoTIL15 cells exhibited a robust effector profile**
- **cytoTIL15 cells remained responsive to ACZ-dependent regulation in the presence of antigen; this control was demonstrated through two cycles of withdrawal and re-exposure**

References and Abbreviations

1. Burga R, et al. Genetically engineered tumor-infiltrating lymphocytes (cytoTIL15) exhibit IL2-independent persistence and anti-tumor efficacy against melanoma in vivo. Presented at SITC 36th Annual Meeting 2021 (abstract 166).
2. Burga R, et al. Digital spatial profiling and antigen-dependent phenotypic analysis of IL15-engineered tumor-infiltrating lymphocytes (cytoTIL15 therapy) in an allogeneic melanoma PDX model. Presented at SITC 37th Annual Meeting 2022 (abstract 390).

ACZ, acetazolamide; AUC, area under the curve; D, day; IL2, interleukin 2; IL15, interleukin 15; mbIL15, membrane-bound IL15; MHC, major histocompatibility complex; QD, once a day; REP, rapid-expansion protocol; TAA, tumor-associated antigen; TIL, tumor-infiltrating lymphocytes; Treg, regulatory T cell.

Acknowledgments

The authors wish to acknowledge the Cooperative Human Tissue Network (CHTN) for their supply of human tissue.

Schematics generated from biorender.io.

This study was funded by Obsidian Therapeutics, Inc. (Cambridge, MA, USA).

Disclosures

RB, AVO, AA, GK, ML, BP, TR, VY, JT, JM, and MO report employment by Obsidian Therapeutics, Inc.

For questions, please contact Rachel Burga (rburga@obsidiantx.com).