cytoTIL15: A novel TIL therapy for melanoma with superior potency and enhanced persistence without IL2 to improve safety and efficacy and expand patient eligibility

OBSIDIAN THERAPEUTICS

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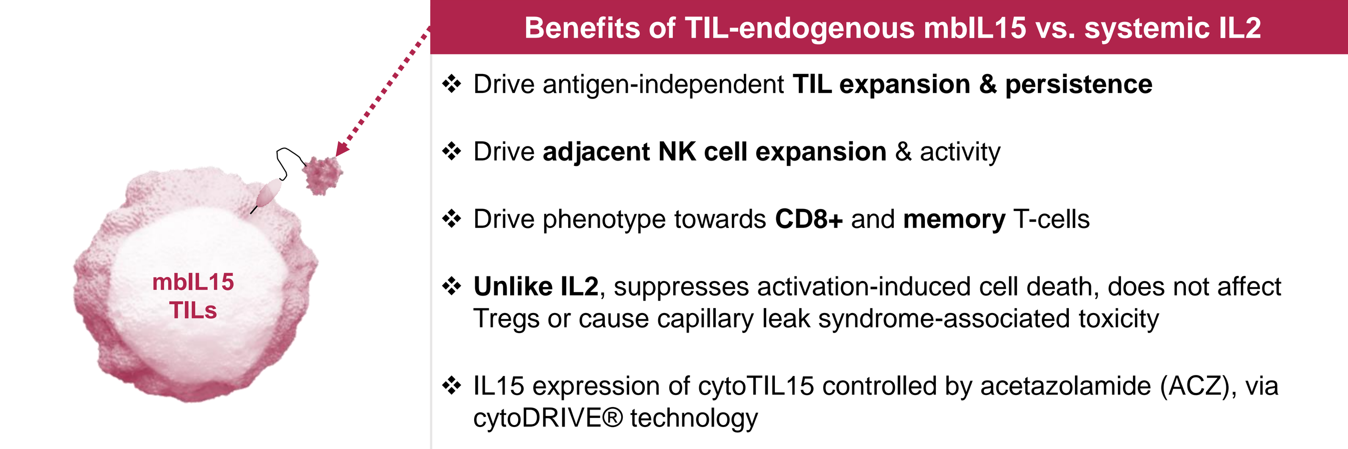
Abstract

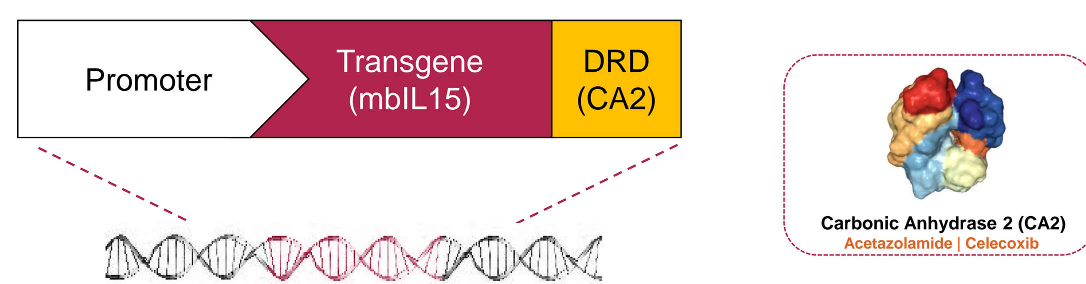
- *Background*: Tumor-infiltrating lymphocyte (TIL) therapy is at the cusp of approval for heavily pretreated patients with solid tumor malignancies. TIL therapy currently requires IL2 for *in vivo* maintenance of TILs, significantly limiting its application due to patient safety and eligibility hurdles. cytoTIL15 is a TIL product engineered with regulatable membrane-bound IL15 (mbIL15) designed via our cytoDRiVE® platform technology. Arming TILs with endogenous mbIL15 has several advantages over systemic IL2. Unlike IL2, IL15 does not increase immunosuppressive regulatory T cells and drives T cell differentiation towards memory phenotypes associated with long-term persistence.
- *Methods*: cytoTIL15 uses Obsidian's cytoDRiVE® platform technology and consists of a carbonic anhydrase 2 (CA2) derived drug responsive domain that enables regulated expression of mbIL15 under control of acetazolamide (ACZ), an FDA-approved orally bioavailable small molecule ligand. We use a proprietary process for high efficiency transduction of TILs with regulatable mbIL15 and expansion without IL2, after which TILs can be cryopreserved or used fresh in downstream *in vitro* and *in vivo* assays.
- *Results*: Our process achieved robust expression of ACZ-regulatable mbIL15 on TILs that expand in the absence of IL2 to levels required for clinical manufacturing. Upon administration in NSG mice, cytoTIL15 exhibited significantly higher expansion and persistence compared to conventional TILs treated with clinically analogous IL2 dosing. cytoTIL15 have a CD8+ effector T cell biased immunophenotypic profile distinct from conventional TILs, while maintaining a diverse TCR Vβ diversity and tumor reactivity with robust IFNγ production. In addition, cytoTIL15 demonstrated significantly higher tumor cytotoxicity and polyfunctionality *in vitro* in the absence of exogenous IL2 in comparison to conventional TILs, indicative of superior potency.
- *Conclusions*: cytoTIL15 is a more potent and persistent TIL product that does not require infusion of IL2, thereby enhancing the safety and durable efficacy of TIL therapy for patients with metastatic melanoma and other solid tumor malignancies.

Background and Product Concept

***Product*: An engineered TIL product that expresses a regulatable mbIL15, making it a more potent and efficacious therapeutic compared to conventional TILs + IL2**



Obsidian's cytoDRiVE® technology:



Fusion of a carbonic anhydrase (CA2) drug responsive domain (DRD) to the target protein (mbIL15) enables regulated expression of mbIL15 under control of acetazolamide (ACZ), an FDA-approved orally bioavailable small molecule ligand

Process: IL-2 independent expansion of cytoTIL15 in a novel manufacturing process using engineered feeders

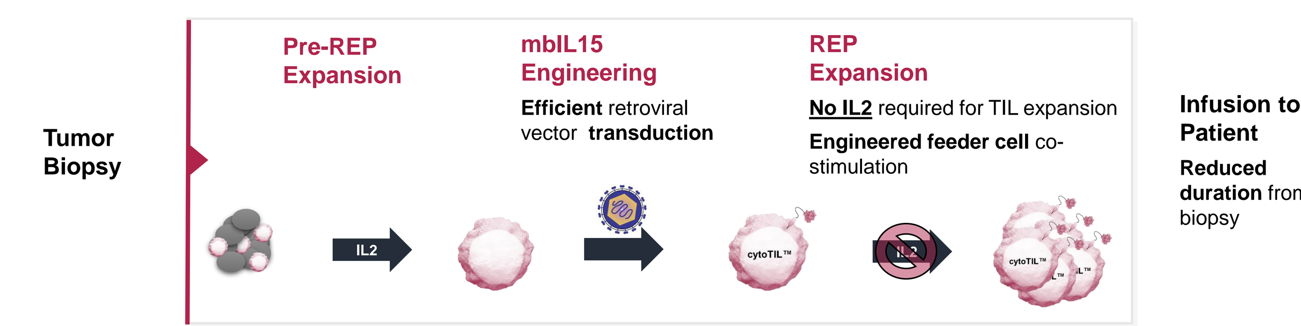


Figure 1. Visual of our high-level process and its advantages. *Left to Right*: The pre-REP expansion includes manual dissociation of the tumor followed by culture with IL2. In our novel Rapid Expansion Process (REP), we engineer Pre-REP TILs with regulatable mbIL15 using viral vectors and achieve over 50-60% transduction in a broad range of donors at our site. Moreover, we eliminate the need for IL2 completely during REP expansion & use engineered feeder cells to provide co-stimulation and growth signals to cytoTIL15, allowing proliferation of mbIL15 expressing TILs. Finally, we expect our product to be superior as we expect comparable or lower duration of production and higher potency compared to conventional TILs.

Engineering: Successful transduction & IL15 expression

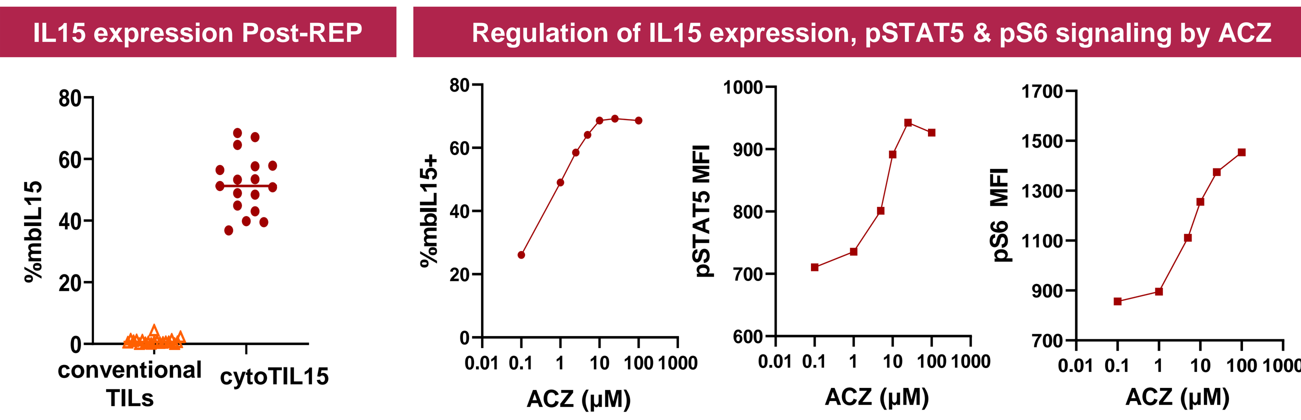


Figure 2. Expression and downstream signaling of engineered membrane-bound IL15 (mbIL15) in cytoTIL15 is regulated by ACZ in a dose-dependent fashion. A. cytoTIL15 and un-engineered TILs (conventional TILs) were expanded in REP with feeder cells +/- IL-2 for 14-days, after which cells were immediately assessed for expression of mbIL15 by flow cytometry. **B-D.** Cryopreserved cytoTIL15 were thawed and rested in ACZ-free culture media for 24 hours, then cultured overnight in increasing concentrations of ACZ. The next day cytoTIL15 were analyzed for (B.) IL-15 expression and phosphorylation of the signaling proteins (C.) STAT5 and (D.) S6 using a phospho-flow cytometry-based assay. Geometric mean fluorescent intensity of the phosphorylated proteins was calculated using the analysis software, FlowJo.

Phenotype: cytoTIL15 have a favorable cytotoxic T cell phenotype

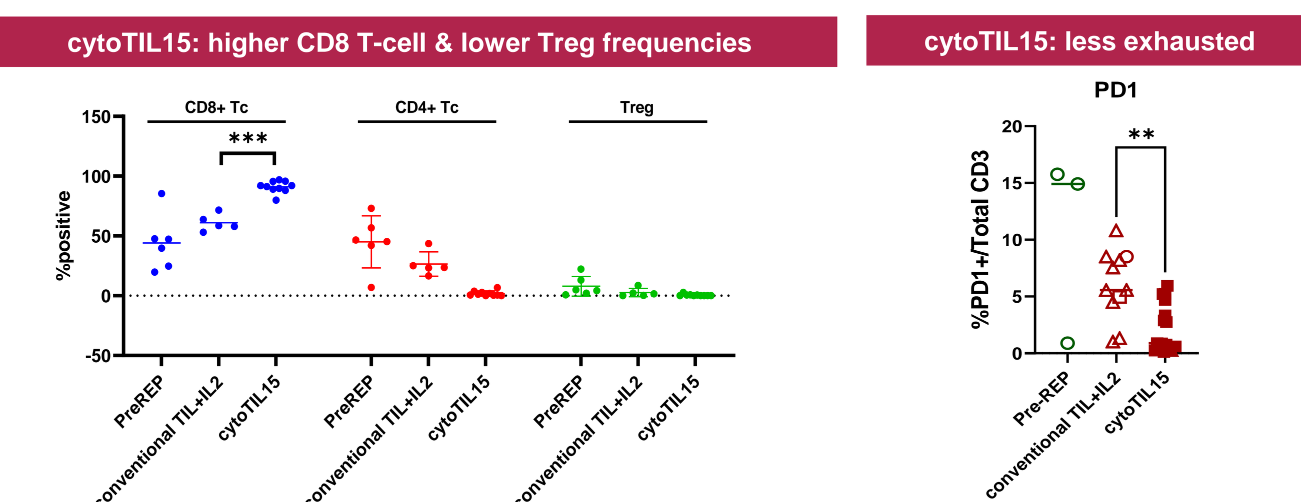


Figure 3. cytoTIL15 have a favorable cytotoxic phenotype with decreased PD1 expression as compared to conventional TILs. *Left:* TILs were assessed at the end of Pre-REP and REP (from engineered cytoTIL15 as well as un-engineered conventional TIL processes) for populations of CD3+ CD8+ T cells, CD3+ CD4+ T cells, and CD3+CD4+CD25+FoxP3+ regulatory T cells (Tregs) by flow cytometry. *Right:* Likewise, PD1 expression was quantified in CD3+ TILs at both the Pre-REP and end of REP time points by flow cytometry. *Statistical analyses: unpaired t-test *p<0.05, **p<0.005, ***p<0.0005*

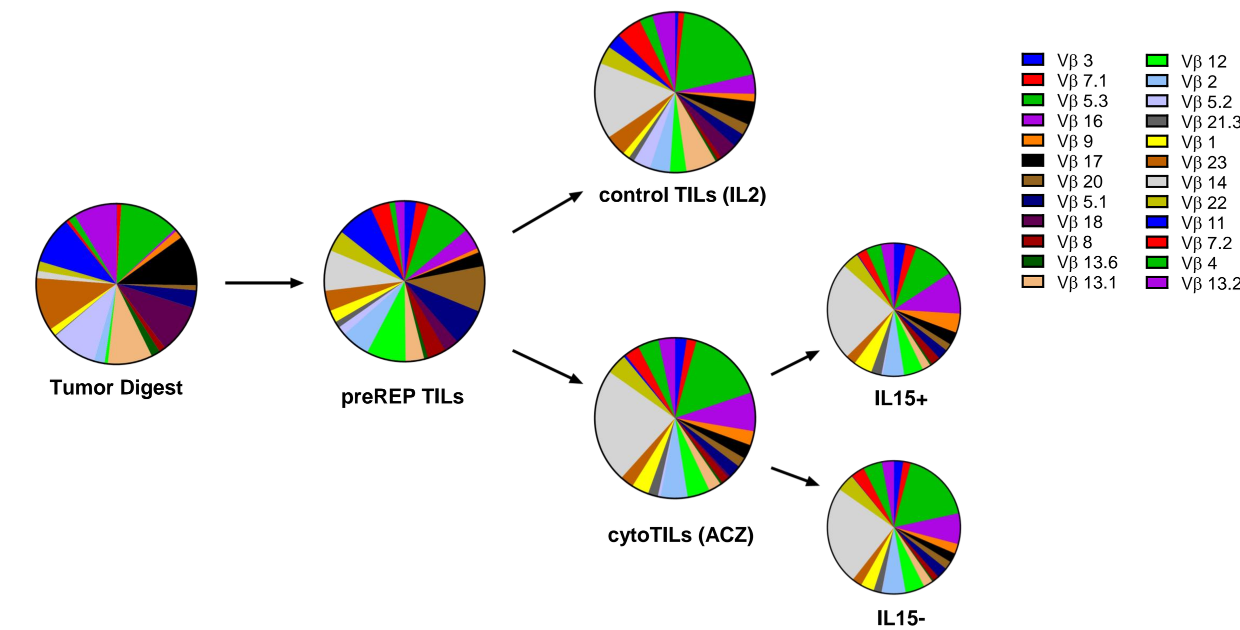


Figure 4. mbIL15 engineering preserves TIL TCRVβ diversity. TILs (CD3+ cells) were assessed from tumor digest, at the end of Pre-REP, and REP time points (from engineered cytoTIL15 as well as un-engineered conventional TIL processes) using a flow cytometry-based multiparametric assay to evaluate frequencies of TCR Vβ sub-families. Evaluation of 24 different TCR Vβ sub-families (covering 70% normal human Vβ repertoire) revealed maintenance of a diverse TCR Vβ repertoire through the manufacturing process (Pre-REP and REP) as well as within both the mbIL15 positive and mbIL15 negative compartments of engineered cytoTIL15.

Potency: cytoTIL15 demonstrate superior cytotoxicity & polyfunctionality than conventional TILs + IL2

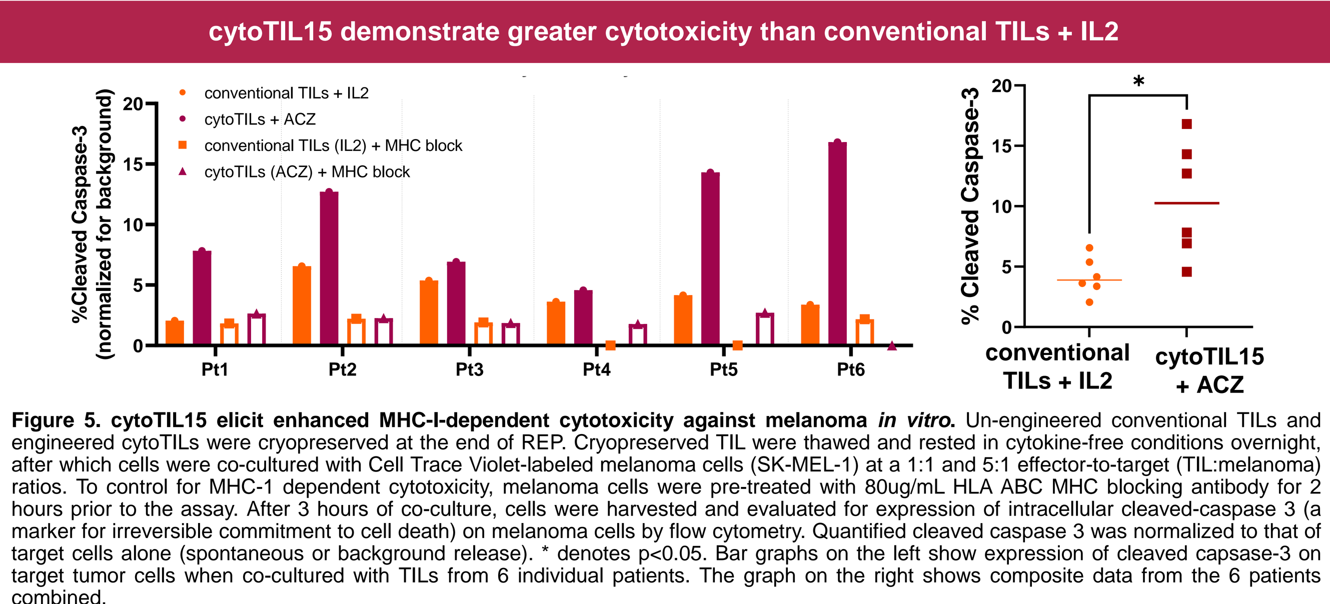


Figure 5. cytoTIL15 elicit enhanced MHC-I-dependent cytotoxicity against melanoma *in vitro*. Un-engineered conventional TILs and engineered cytoTILs were cryopreserved at the end of REP. Cryopreserved TIL were thawed and rested in cytokine-free conditions overnight, after which cells were co-cultured with Cell Trace Violet-labeled melanoma cells (SK-MEL-1) at a 1:1 and 5:1 effector-to-target (TIL:melanoma) ratios. To control for MHC-1 dependent cytotoxicity, melanoma cells were pre-treated with 80ug/mL HLA ABC MHC blocking antibody for 2 hours prior to the assay. After 3 hours of co-culture, cells were harvested and evaluated for expression of intracellular cleaved-caspase 3 (a marker for irreversible commitment to cell death) on melanoma cells by flow cytometry. Quantified cleaved caspase 3 was normalized to that of target cells alone (spontaneous or background release). * denotes p<0.05. Bar graphs on the left show expression of cleaved capsase-3 on target tumor cells when co-cultured with TILs from 6 individual patients. The graph on the right shows composite data from the 6 patients combined.

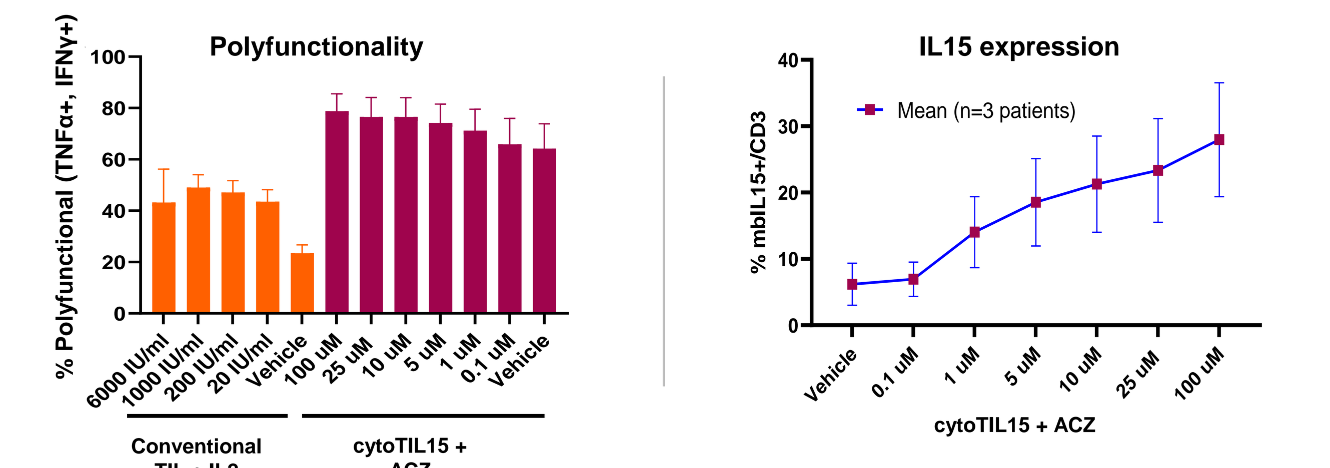


Figure 6. cytoTIL15 are more polyfunctional than conventional TILs. Post-REP TILs from 3 TIL donors were rested for 24 hours in cytokine or ACZ-free culture media. Next, conventional TILs were cultured with different concentrations of IL-2 and cytoTIL15 were cultured overnight with different ACZ concentrations as marked. The next day, TILs were stimulated with PMA + ionomycin for 6 hours in the presence of protein transport inhibitors, then assayed by flow cytometry for the frequencies of mbIL-15 positive (*Right*) and dual expression of the effector molecules TNFα and IFNγ (*Left*) (defined as polyfunctionality in this assay) within total CD3+ TILs (n=3 TIL donors). Graphs show mean values of 3 TILs donors ± SEM.

Persistence: cytoTIL15 demonstrate improved persistence *in vivo* compared to conventional TILs + IL2

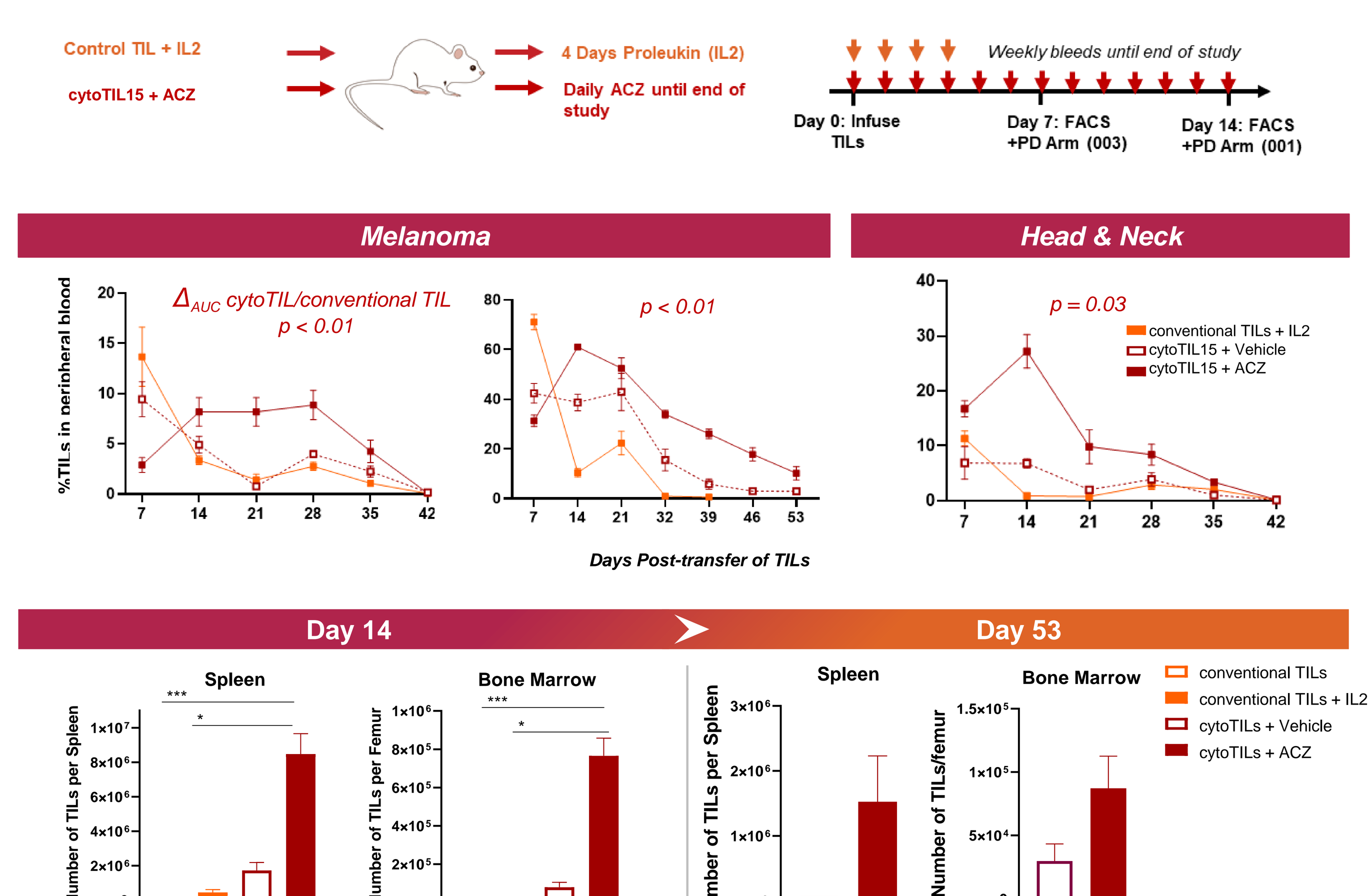


Figure 7. cytoTIL15 demonstrate improved persistence *in vivo*. Un-engineered conventional TILs and cytoTIL15 generated from three different donors (2 melanoma & 1 head & neck) were injected intravenously in NSG (NOD-SCID-γc-/-) at $10e^6$ TIL/mouse. Mice dosed with conventional TILs received 50000IU IL-2 BID q8h for the first 4 days post-adoptive cell transfer, while mice dosed with cytoTIL15 received 200mg/kg ACZ or vehicle every day post-adoptive cell transfer. *Top:* At weekly intervals, submandibular blood was collected, processed to lyse red blood cells, and resultant single cell suspension was stained with murine CD45 and human CD3 antibodies. Percent TILs (human CD3+ murine CD45- cells) were quantified via flow cytometry at each time point, with cytoTIL15 demonstrating a peak in expansion at Day 14, followed by continued persistence through day 53 post-transfer (n=5 mice/group). *Bottom:* Cohorts of 4-5 mice were sacrificed at 14 and 53-days post-transfer, and spleen and bone marrow harvested, processed into single cell suspension, and stained with murine CD45 and human CD3 antibodies. Absolute number of TILs (percent human CD3+ murine CD45- cells x total cell count per tissue) were quantified via flow cytometry. cytoTIL15 demonstrated a significantly higher persistence in splenic and bone marrow samples both at Day 14 and Day 53 post-transfer.

Conclusions

- **cytoTIL15** is an engineered TIL product that expresses a **regulatable mbIL15** controlled by the DRD ligand, Acetazolamide, in a dose-dependent fashion.
- cytoTIL15 displays a favorable **cytotoxic CD8+ T cell phenotype** while maintaining TCR Vβ diversity during manufacturing.
- cytoTIL15 exhibits superior ***in vitro* anti-tumor cytotoxicity** as well as **polyfunctionality,** compared to conventional TILs + IL2.
- *In vivo*, cytoTIL15 demonstrates greater antigen-independent **expansion and persistence** compared to conventional TILs treated with IL2.

Acknowledgements: The authors wish to acknowledge the Cooperative Human Tissue Network (CHTN) for their supply of human tumor tissue, and the MD Anderson Cancer Center for technical support.

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