IL15-engineered tumor infiltrating lymphocytes (cytoTIL15™) exhibit activity against autologous tumor cells from multiple solid tumor indications without IL2

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Abstract Presentation LB096

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

Background & Rationale

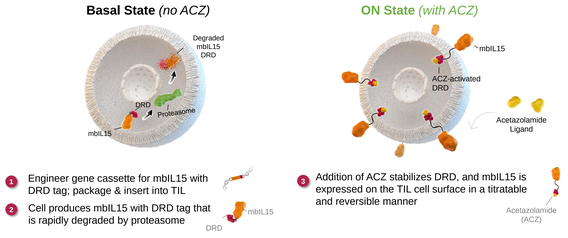
Investigational tumor infiltrating lymphocytes (TIL) therapy has shown promising results in the treatment of metastatic melanoma. However, TIL therapy has conventionally required co-administration of IL2, which is associated with side effects in patients. We previously showed that melanoma TIL engineered to express membrane-bound IL15 (mbIL15) under the control of a drug responsive domain (DRD) and the ligand acetazolamide (ACZ) (cytoTIL15™) can achieve IL2-independent expansion during manufacturing, antigen-independent persistence *in vitro* and anti-tumor activity *in vivo*[1]. In the current study, we extend the investigational cytoTIL15 cell therapy product candidate concept to indications beyond melanoma including non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC) and head and neck squamous cell carcinoma (HNSCC), tumor types which represent significant unmet medical needs, particularly in the post-checkpoint inhibitor refractory setting.

Methods

- cytoTIL15 cells derived from melanoma, HNSCC, NSCLC and TNBC; engineered to express mbIL15 in the presence of ACZ and were expanded using our proprietary rapid expansion protocol (REP). Un-engineered TIL were expanded in parallel with IL2.
- cytoTIL15 cells were phenotyped for CD8 positivity, mbIL15 expression, memory subtype and TCRVβ diversity by flow cytometry.
- To measure TIL polyfunctionality, expression of effector molecules in TIL was measured following stimulation with anti-CD3/CD28.
- *In vitro* antigen- and cytokine-independent survival of cytoTIL15 cells was measured from cultures that included ACZ.
- *In vivo*, cytoTIL15 cells were transferred into NSG mice and cytoTIL15 cell expansion and persistence in the peripheral blood was measured over time.
- To assess anti-tumor activity, cytoTIL15 cells were co-cultured with autologous patient-derived cell lines (PDc) or tumor digests, and cytotoxicity and IFNγ release into supernatant was measured.

Obsidian's cytoDRiVE® Platform & Application

What are cytoTIL15™ cells?



1. Engineer gene cassette for mbIL15 with DRD tag; package & insert into TIL
2. Cell produces mbIL15 with DRD tag that is rapidly degraded by proteasome
3. Addition of ACZ stabilizes DRD, and mbIL15 is expressed on the TIL cell surface in a titratable and reversible manner

DRD = Drug Responsive Domain | ACZ = acetazolamide | mb = membrane-bound

cytoTIL15 cell engineering

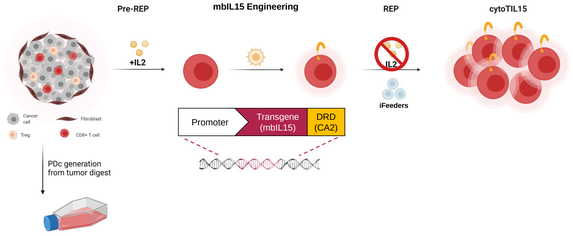


Figure 1. Schema of cytoTIL15 cell generation. *Left to Right*: The pre-REP expansion includes dissociation of the tumor followed by culture with IL2. Tumor digest is also utilized to generate PDc lines. Following pre-REP, extracted TILs are engineered with regulatable mbIL15 using viral vectors. Once transduced, these cytoTIL15 cells are initiated in a rapid expansion protocol (REP) which uses engineered feeder cells expressing 41BBL and IL21 (iFeeders) to provide co-stimulation and growth signals to cytoTIL15 cells, allowing expansion of mbIL15 expressing TILs. Uniquely, this REP process is entirely IL2-independent.

Robust expansion of TIL from multiple tumor indications in Pre-REP and REP processes

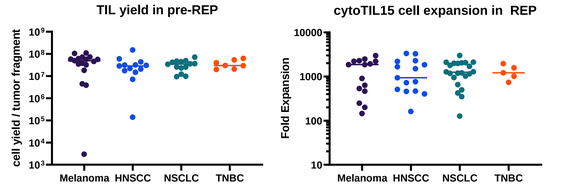


Figure 2. cytoTIL15 cells from NSCLC and TNBC achieve similar yields as melanoma and HNSCC in Pre-REP and REP. *Left:* Tumors from multiple tumor types were dissociated into tumor fragments and expanded in the presence of IL2 for 14-18 days. Cell yield is normalized to the number of tumor fragments used in the pre-REP process. *Right:* Pre-REP TILs derived from multiple indications were expanded in REP with feeder cells and ACZ, without IL2, for 14 days. Fold expansion of total cytoTIL15 cells was measured at the end of REP.

cytoTIL15 cells are predominantly CD8+, express mbIL15 and have an effector memory subtype

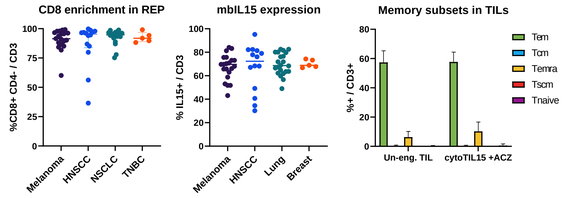


Figure 3. cytoTIL15 cells from NSCLC, TNBC, HNSCC and melanoma are predominantly CD8+, express mbIL15 and have an effector memory subtype. *Left and middle:* At the end of REP, un-engineered TIL and cytoTIL15 cells were measured by flow cytometry for T cell phenotype and expression of mbIL15. *Right:* Expression of CD45RO, CD45RA, CCR7, CD62L and CD95 were measured by flow cytometry and used to determine memory T cell subset in un-engineered TIL and cytoTIL15 cells (n=10 TIL donors) *Tem = CD45RO+CD45RA-CCR7-,CD62L-, Tcm = CD45RO+CD45RA-CCR7+CD62L+, Temra = CD45RO-CD45RA+CCR7-CD62L-, Tnaive = CD45RO-CD45RA+CCR7+CD62L+, Tscm = Tnaive+CD95+.*

In vitro antigen- and cytokine-free long-term survival and mbIL15 expression is dependent on ACZ

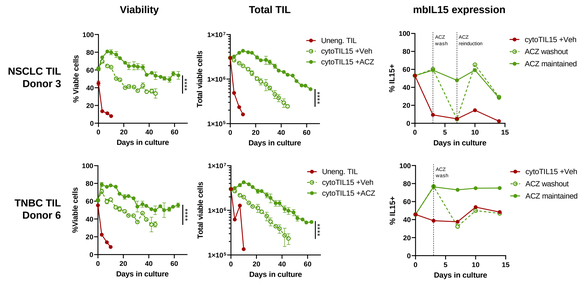
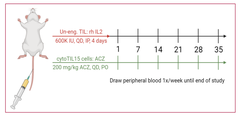


Figure 4. cytoTIL15 cells derived from NSCLC and TNBC persist in the absence of antigen or cytokine in an ACZ-dependent manner. 3x10^6 un-engineered TIL or cytoTIL15 cells were cultured in 24-well G-Rex plates for up to 63 days. Culture media was supplemented with either ACZ or vehicle and exchanged every 3-4 days. *Left, middle:* At each time point samples were taken for measurement of cell viability by acridine orange / propidium iodide stain and number of total viable cells. *Right:* To measure dependence of mbIL15 expression on ACZ, cytoTIL15 cells were cultured in media containing ACZ for three days, then washed and replaced by media without ACZ. MbIL15 expression was measured by flow cytometry over 14 days. *****p<0.0001.*

cytoTIL15 cells from NSCLC persist longer and expand more *in vivo* compared un-engineered TIL +IL2



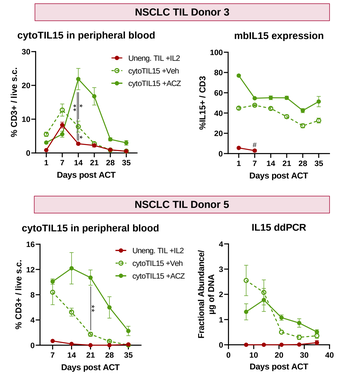


Figure 5. cytoTIL15 cells display improved persistence *in vivo* compared to un-engineered TIL without the need for IL2. Un-engineered TIL and cytoTIL15 cells derived from two different NSCLC donors were injected intravenously into n=5 NSG mice at 25e^6 cells per mouse. Mice dosed with un-engineered TIL received IL2 twice daily for the first 4 days following adoptive cell transfer, while mice dosed with cytoTIL15 cells were dosed with 200 mg/kg ACZ or vehicle daily. At weekly timepoints, submandibular blood was harvested and assessed for presence of TIL and mbIL15 expression by flow cytometry or ddPCR. *# = after day 7 timepoint, this condition remained below minimum event threshold. *p<0.05, **p<0.01*

cytoTIL15 cells maintain diverse TCR Vβ repertoire and are polyfunctional

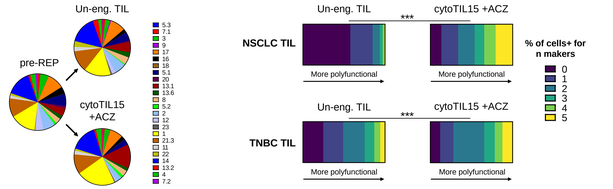
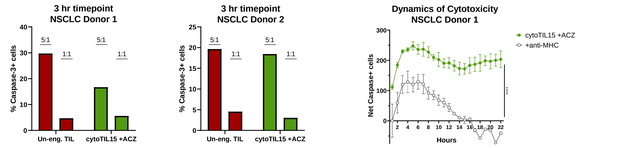


Figure 6. TCR Vβ diversity of cytoTIL15 cells are maintained through REP, and polyfunctionality is similar or increased compared to un-engineered TIL. *Left:* TIL from pre-REP and the end of REP were assessed using a flow cytometry-based assay to measure frequencies of TCR Vβ subfamilies. Representative donor shown. *Right:* Un-engineered TIL and cytoTIL15 cells were stimulated with anti-CD3/CD28 antibodies for 6 hours in the presence of the transport inhibitors brefeldin A and monensin. Intracellular staining of CD107a, IL2, IFNγ, TNFα, Perforin and Granzyme B was performed and measured by flow cytometry. Number of values refer to the number of effector molecules simultaneously expressed among single cells. ****Chi-square p<0.001.*

cytoTIL15 cells display cytotoxic activity against autologous PDc and secrete IFNγ in response to autologous tumor digests

Cytotoxicity Assays



IFNγ Release Assay

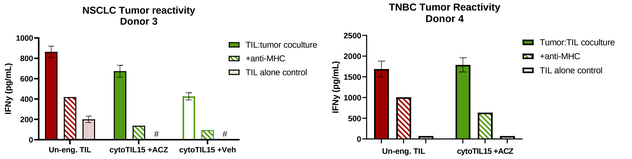


Figure 7. cytoTIL15 cells are cytotoxic and secrete IFNγ in response to coculture with autologous PDc lines and tumor digests. *Top, left:* Un-engineered TIL and cytoTIL15 cells supplemented with IL2 or ACZ, respectively, were cocultured with Cell Trace Far Red-labeled autologous PDc lines at 5:1 and 1:1 effector-to-target ratios. After 3 hours of coculture, cells were evaluated for expression of cleaved caspase-3 by flow cytometry. *Top, right:* cytoTIL15 cells and PDc were stained with caspase 3/7 green dye, and net caspase positive cells were measured over 22 hours using the Incucyte. *Bottom:* Un-engineered TIL and cytoTIL15 cells supplemented with IL2, ACZ or vehicle were cocultured with autologous tumor digests for 24 hours at a 5:1 E:T ratio. Anti-HLA-A,B,C antibody was added to cocultures as a control. At the end of 24 hours, culture supernatants were collected and assessed for IFNγ secretion by MSD. *# = below the lower limit of detection. ****p<0.0001.*

Conclusions

These data demonstrate:

- cytoTIL15 cells can be **generated from multiple solid tumor types** including melanoma, HNSCC, NSCLC and TNBC.
- Through engineering and expansion in REP, cytoTIL15 cells maintain **diversity of the TCR Vβ repertoire**, and are heavily enriched for **polyfunctional CD8 T cells** and mbIL15 expression.
- Unlike un-engineered TIL, cytoTIL15 cells driven by ACZ **persist without IL2** in *in vitro* and *in vivo* settings.
- cytoTIL15 cells maintain **reactivity and are cytotoxic to autologous tumor** digests and patient-derived cell lines

References:
1. *Burga et al. 2021. Journal of Immunotherapy of Cancer (2021).*

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For questions, please reach out to Kyle Pedro (kpedro@obsidiantx.com). Schematics generated from biorender.io

obsidiantx.com