Allogeneic, IL-2-independent tumor-infiltrating lymphocytes expressing membrane-bound IL-15 (cytoTIL15™) eradicate tumors in a melanoma PDX model through recognition of shared tumor antigens

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OBSIDIAN THERAPEUTICS

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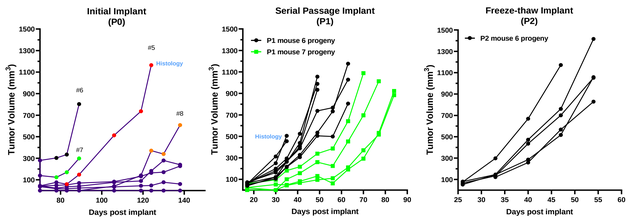
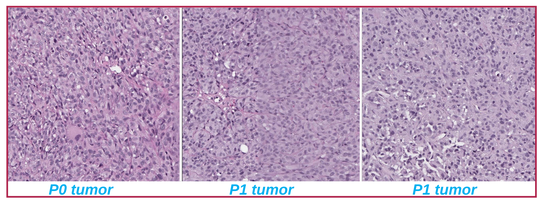
Background & Rationale

Adoptive cell therapy with tumor-infiltrating lymphocytes (TILs) has demonstrated promise in clinical trials for patients with solid tumors. Currently, TIL therapy requires IL-2 administration to support TIL expansion and survival, but this cytokine is associated with T cell exhaustion and can result in severe toxicities that limit patient eligibility.[1] To this end, we genetically engineered TILs to express membrane-bound IL-15 (mbIL15) under the control of Obsidian's cytoDRiVE® technology (cytoTIL15™), which allows regulation of protein expression via a drug-responsive domain (DRD) upon acetazolamide (ACZ) administration. IL-15 is a preferred cytokine over IL-2 to mediate TIL activation and expansion because it does not result in CD8 T cell exhaustion or stimulation of regulatory CD4 T cells but supports homeostatic proliferation of memory T cells. We have previously demonstrated IL-2-independent, 3-6-fold increased cytoTIL15 cell persistence in an antigen-independent, *in vivo* setting relative to unengineered TIL therapy (uTIL) with IL-2[2]. Since generating autologous tumor/TIL-matched pairs poses multiple challenges, we developed an allogeneic, HLA-matched, patient-derived xenograft (PDX) model which allows antigen-specific comparison of cytoTIL15 anti-tumor efficacy across multiple donors.

Primary melanoma PDX model developed to assess TIL efficacy

- Melanoma biopsied & implanted into NSG mice within 24 hours
  - ✓ Initial tumor take
  - ✓ Serially passage, freeze-thaw and cryobank
  - ✓ Confirm epithelial histology





✓ PDX Growth Kinetics Study

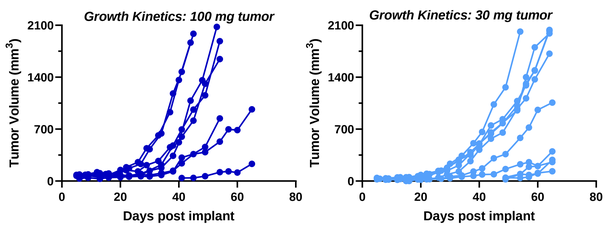


Figure 1. Schema of PDX model generation. *Top to Bottom*: A primary melanoma was excised from the patient and implanted as a tumor fragment within 24 hour of surgery. Eight NSG female mice were implanted with similarly sized fragments that were allowed to grow for ~130 days. Serial passaging, freeze-thaw growth and tumor histology were assessed. Once established, a growth kinetics study was performed pinpointing the time to a reasonable starting tumor volume as well as assessing tumor take. Ten female NSG mice were serially implanted with warm 30mg or 100mg tumors and tracked for tumor growth over a two-month period.

PDX tumor expresses conserved melanoma antigens

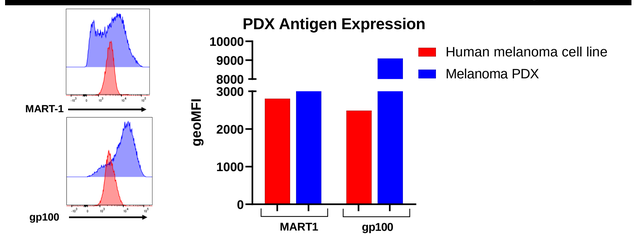


Figure 2. Expression of melanoma associated antigens (MAAs). *Left to Right*: A cryopreserved PDX tumor sample was digested with Miltenyi GenteMACS reagent to generate a single cell suspension. This single cell suspension and A375 human melanoma cells were fixed, permeabilized, and stained with antibodies for conserved MAAs MART-1 and gp100 expression as assessed by flow cytometry.

HLA-matched TIL donors demonstrate reactivity to PDX tumor

- ✓ HLA-A*02 selected melanoma TIL donors
- ✓ TIL/PDX reactivity assay (IFNγ levels)
- ✓ MART1 & gp100 TCR expression

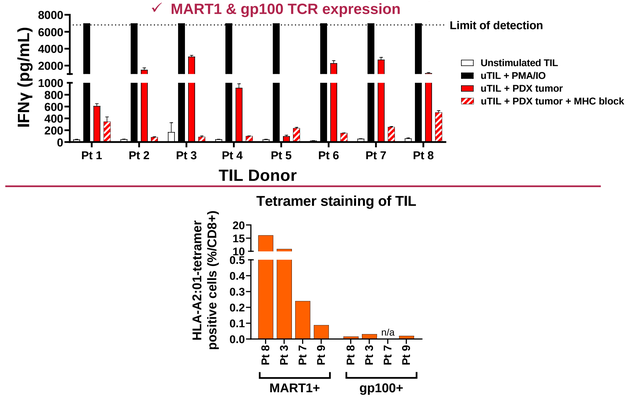


Figure 3. HLA-A*02 melanoma TIL donors elicit MHC-I-dependent cytotoxicity *in vitro*. *Top*: uTIL + IL2 were cryopreserved at the end of REP. Cryopreserved uTIL were thawed and rested in cytokine-free conditions overnight. A cryopreserved PDX tumor sample was digested into a single cell suspension. After the overnight rest, TILs were co-cultured at 1:1 effector:target ratio with PDX tumor single cell suspension, with and without 80ug/mL HLA ABC blocking reagent. Co-cultures of effectors and targets were supplemented with 10 IU/mL IL2. After 24 hours, supernatant was assessed for IFNγ content by MSD. *Bottom:* uTIL were assessed for TCR recognizing shared melanoma antigens (MART1 and gp100) by tetramer-specific flow cytometry.

Cells engineered with cytoDRiVE® technology to enable regulation of fusion proteins via drug responsive domains

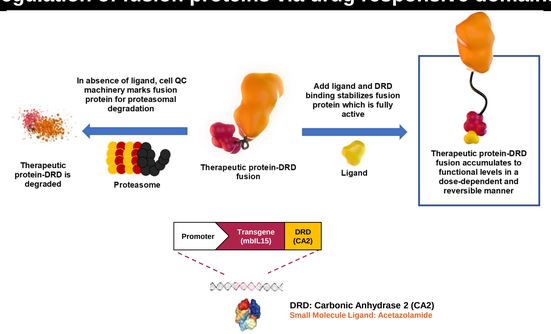
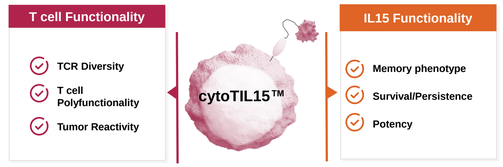


Figure 4. Schema of Obsidian's cytoDRiVE® platform: Obsidian's cytoDRiVE® platform includes small human protein sequences called drug responsive domains (DRD)s that enable regulated expression of a fused target protein under control of FDA-approved, ideally orally bioavailable small molecule ligands. cytoTIL15 cells contain TILs engineered with mbIL15 under the control of a carbonic-anhydrase-2 (CA2) DRD, controlled by the ligand acetazolamide (ACZ), which is a weak diuretic.

cytoTIL15 cells maintain T cell functionality without IL2



Allogeneic, HLA-matched cytoTIL15 cells significantly inhibit PDX tumor growth in a MART1-dependent manner

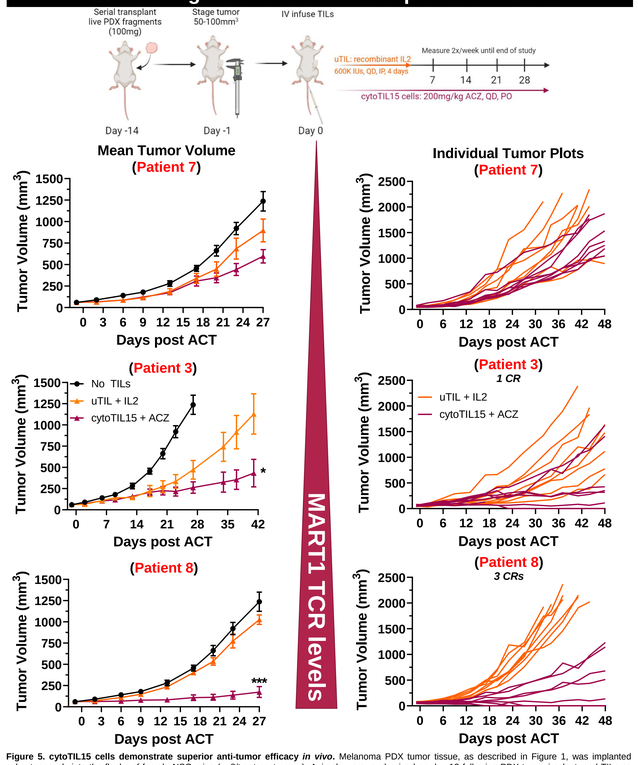


Figure 5. cytoTIL15 cells demonstrate superior anti-tumor efficacy *in vivo*. Melanoma PDX tumor tissue, as described in Figure 1, was implanted subcutaneously into the flanks of female NSG mice (n=8/treatment group). Animals were randomized on day 13 following PDX tumor implant, and TILs as described in Figure 3, were infused day 14 following PDX tumor implant. cytoTIL15 cells (mice dosed with 200mg/kg ACZ, PO daily) demonstrated significantly enhanced anti-tumor efficacy as compared to uTIL + IL2 (600,000 IU IL-2, QD, IP for 4 days). Anti-tumor efficacy was associated with increased frequency of MART1-reactive TILs, as shown in Figure 3. (*p<0.05; ***p<0.001 – Mann Whitney Test)

Allogeneic, HLA-matched PDX pharmacodynamics study with cytoTIL15 cells

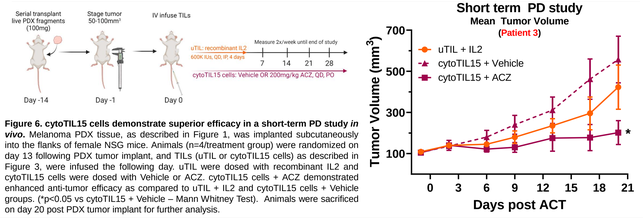


Figure 6. cytoTIL15 cells demonstrate superior efficacy in a short-term PD study *in vivo*. Melanoma PDX tissue, as described in Figure 1, was implanted subcutaneously into the flanks of female NSG mice. Animals (n=4/treatment group) were randomized on day 13 following PDX tumor implant, and TILs (uTIL or cytoTIL15 cells) as described in Figure 3, were infused the following day. uTIL were dosed with recombinant IL2 and cytoTIL15 cells were dosed with Vehicle or ACZ. cytoTIL15 cells + ACZ demonstrated enhanced anti-tumor efficacy as compared to uTIL + IL2 and cytoTIL15 cells + Vehicle groups. (*p<0.05 vs cytoTIL15 + Vehicle – Mann Whitney Test). Animals were sacrificed on day 20 post PDX tumor implant for further analysis.

cytoTIL15 cells + ACZ show significantly increased persistence in blood, tumor, bone marrow and spleen

- ✓ ACZ-mediated upregulation of mbIL15 significantly increases:
  - cytoTIL15 cell frequency in blood, lymphoid organs and intratumorally
  - Pro-inflammatory cytokines intratumorally

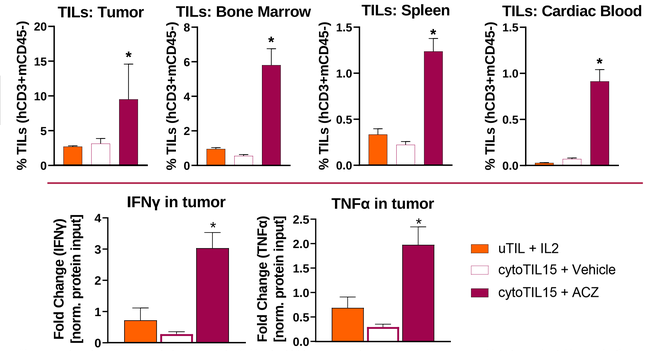


Figure 7. cytoTIL15 cells demonstrate improved trafficking to PDX tumor, persistence in lymphoid organs, and intratumoral cytokine production. From the study described in Figure 6, PDX tumors were excised for analysis on Day 20 post ACT. *Top:* TILs were quantitated in homogenized tumor tissue, bone marrow, spleen and whole blood by flow cytometry stained with anti-human CD3 and negative for anti-mouse CD45. *Bottom:* Cytokines (IFNγ and TNFα) were measured by MSD normalized to tumor homogenate protein levels. (*p<0.05 vs cytoTIL15 + Vehicle and/or uTIL + IL2 – Mann Whitney Test)

cytoTIL15 cells + ACZ significantly (8-10-fold) increased tumor infiltration

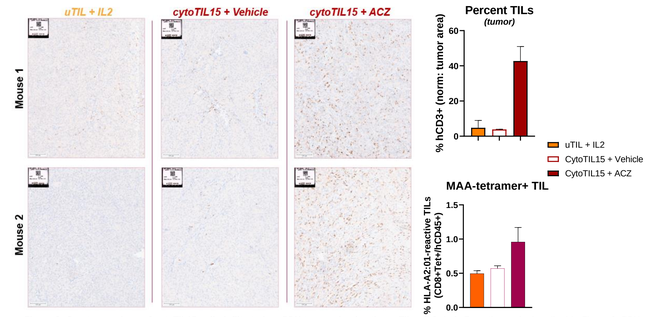


Figure 8. Greater numbers of cytoTIL15 cells infiltrate into PDX tumors *in vivo* than uTIL + IL2. *Left:* From the study described in Figure 6, PDX tumors were excised for analysis on Day 20 post ACT. Formalin-fixed paraffin embedded blocks were generated and sectioned. Unstained slides were probed with an anti-human CD3 antibody to mark the intratumoral TILs. *Top-right:* Total TIL were quantitated in each tumor with HALO® Image analysis software. *Bottom-right:* MART-1 tetramer reactive TIL are enriched in tumor

Conclusions

In a first report of its kind, these data demonstrate the feasibility of comparing multiple TIL donors in a standardized, allogeneic, HLA-matched PDX tumor efficacy model rather than evaluating each in the traditional autologous format. Evaluation of three donors in the model showed:

- Significantly **greater anti-tumor activity** of ACZ-regulated cytoTIL15 cells compared to uTIL plus IL2, including complete responses.
- Significantly **increased persistence** of ACZ-regulated cytoTIL15 cells compared to uTIL plus IL2.
- Significantly (8-10-fold) **increased tumor infiltration** of regulated cytoTIL15 cells, including MART-1 specific TIL.
- Significantly **increased intratumoral and plasma levels of TNFα and IFNγ** in mice treated with ACZ-regulated cytoTIL15 cells.

Taken together, the striking ability of cytoTIL15 cells to control or eradicate melanoma tumor outgrowth, in the absence of exogenous IL-2, highlights the clinical potential of cytoTIL15 cells as a novel TIL product with enhanced safety and efficacy for patients with melanomas and other solid tumors. Furthermore, these data support the utility of an allogeneic PDX model for comparative evaluation of tumor-antigen specific TIL reactivity across different patients.

References

1. Yang JC. Toxicities associated with adoptive T-cell transfer for Cancer. *Cancer J.* 2015.
2. Burga R. et al Genetically engineered tumor-infiltrating lymphocytes (cytoTIL15) exhibit IL-2-independent persistence and anti-tumor efficacy against melanoma in vivo. *SITC 36th annual meeting 2021.*

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