Armoring tumor infiltrating lymphocytes (TIL) with regulatable, membrane bound IL15 for increased persistence and potency, without the use of concomitant IL2

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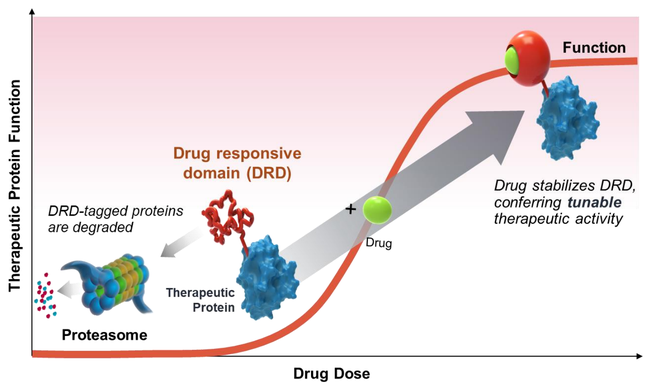
[1] Background

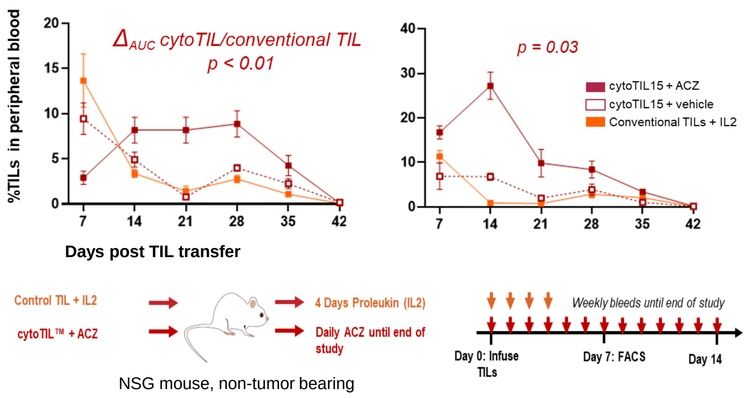
Solid tumors can be effectively treated with *ex vivo* expanded tumor infiltrating lymphocytes (TIL). However, the clinical use of TIL therapy is currently limited by the toxicity of concomitantly administered IL2, the reduced efficacy observed outside of melanoma and limited persistence of the cells. To this end, we have developed cytoTIL15™ as an IL2-free TIL, where activity of engineered membrane-bound IL15 (mbIL15) is regulated via a degron system ("drug responsive domain", DRD) that is pharmacologically controlled with an FDA-approved small molecule drug (cytoDRIVE® technology).

cytoDRiVE Technology

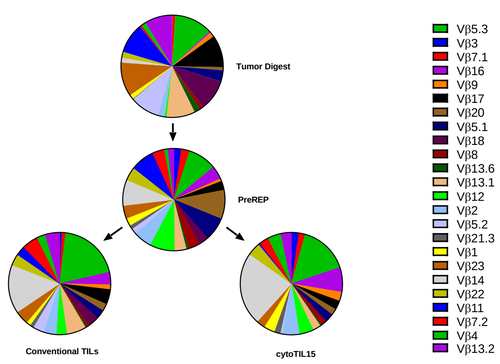


CytoTIL15 cells are manufactured using feeder cells constitutively expressing IL21 and 4-1BBL, which results in a distinct product enriched in CD8 T-cells, very low in CD4 T-cells, and with lower expression of PDL1 compared to standard TIL manufactured on PBMC with IL2. mbIL15 expression can be controlled in the patient with the diuretic Acetazolamide (Diamox®) and a DRD derived from carbonic anhydrase 2.

[2] cytoTIL15 persist *in vivo* at 3-6x greater area under the curve (AUC) than conventional TIL + IL2

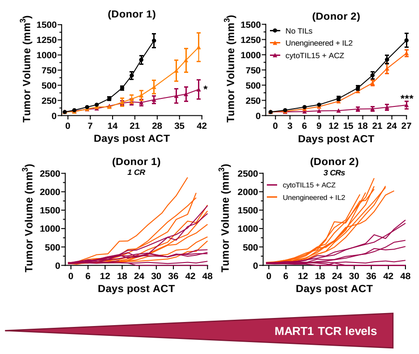


[3] cytoTIL15 cells maintain TCR Vβ representation



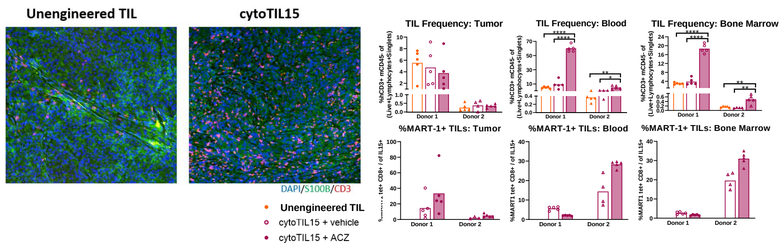
Flow cytometry for 24 TCR Vβ families (~70% of total TCR repertoire) in fresh post-REP. Data pooled from 3 donors

[4] cytoTIL15 therapy controls allogeneic, HLA-matched melanoma PDX tumors in a MART-1-dependent manner



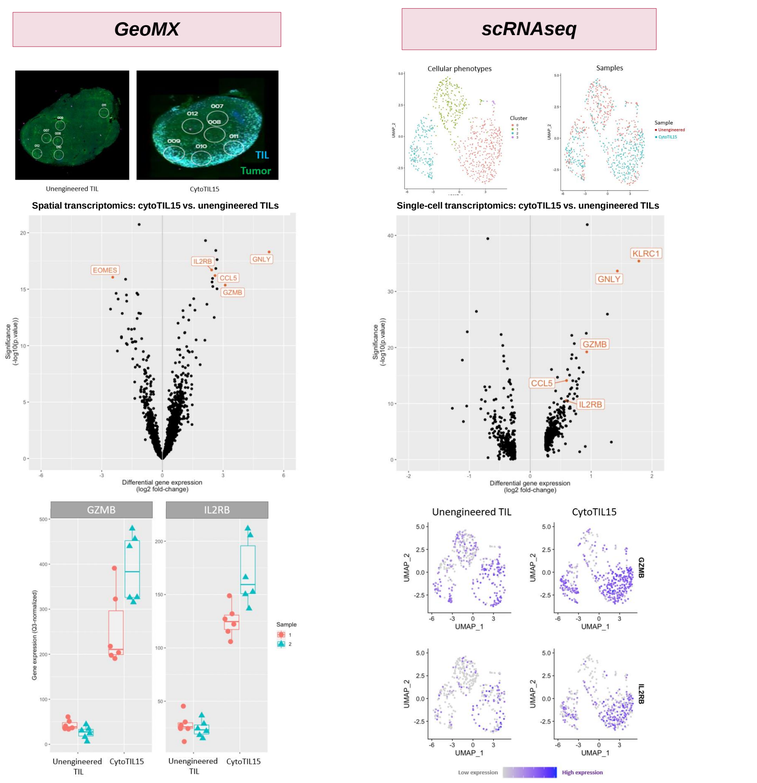
cytoTIL15 therapy demonstrates superior tumor control *in vivo*, in an HLA-matched melanoma PDX model. *Top:* MART1+ melanoma tumor tissue was excised from the donor and implanted subcutaneously into female NSG mice. Serial passaging was performed in vivo to establish a PDX model, and animals were randomized on day-13 following PDX implant. On Day 14 post-PDX implant, cytoTIL15 cells and unengineered TILs (expanded in REP with feeder cells +/- IL2 or ACZ for 14-days) from two HLA-matched and MART-1-reactive donors were adoptively transferred fresh intravenously. cytoTIL15 cells (animals dosed with 200 mg/kg ACZ PO daily), demonstrated improved anti-tumor efficacy as compared to unengineered TILs (supported with 600,000 IU IL2 QD IP for 4 days). *Bottom:* Individual spider plots for each animal on study reveals that anti-tumor efficacy was associated with increased frequency of MART-1-reactive TILs, as described in Figure 2. (n=8/arm; *p<0.05, ***p<0.001).

[5] cytoTIL15 cells infiltrate into tumors, circulate and accumulate in bone marrow, and are enriched for MART-1 TCR



cytoTIL15 demonstrate enhanced infiltration and accumulation. *Left*: Subcutaneous PDX tumors were harvested 20-days following ACT with unengineered TILs (+IL2) or cytoTIL15 therapy. Tumors were formalin-fixed and paraffin-embedded, and immunofluorescence was performed to identify the TILs (CD3+, red color) infiltrating the tissue. *Right Top*: 14-days following ACT, PDX tumors, cardiac blood, and bone marrow were harvested, processed into single cell suspension, and stained and assessed via flow cytometry. Staining for the fraction of cells positive for anti-human CD3 and negative for anti-mouse CD45 revealed significant tumor infiltration by all TILs, and improved trafficking and accumulation into blood and bone marrow by cytoTIL15 therapy + ACZ. *Right Bottom*: Flow cytometry was also used to evaluate the fraction of transduced TILs (IL15+) staining positive with MART-1 TCR tetramer; MAA-reactive TILs enriched within all compartments, supporting the correlation of superior MAA-reactive TIL infiltration and improved anti-tumor efficacy. (n=5/arm; *p<0.05, **p<0.01, ****p<0.0001).

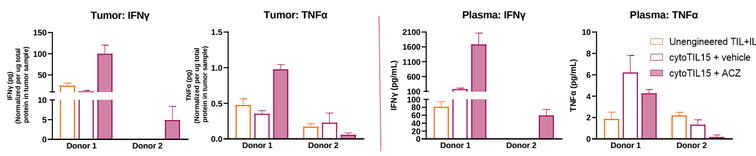
[6] cytoTIL15 cells show enrichment in genes associated with enhanced infiltration and anti-tumor effector function



cytoTIL15 demonstrates enrichment in genes associated with enhanced infiltration and anti-tumor effector function. *Top:* Representative microscopy of digital spatial profiling revealing region of interest (ROI) selection for TIL (blue) and tumor (green) regions for PDX tumors treated with unengineered TILs or cytoTIL15 therapy. Similarly, representative unsupervised clustering of unengineered TIL- and cytoTIL15 therapy-treated tumors. *Middle*: Spatial and single-cell transcriptomics reveal a differential gene expression profile in cytoTIL15 cells vs. unengineered TILs, underscored by an enrichment in genes associated with pro-effector function and a favorable TIL phenotype such as IL2RB, GNLY, CCL5, GZMB, and KLRC1, and a decrease in exhaustion-associated genes such as EOMES. *Bottom*: GeoMX and scRNAseq reveal a significant ($p < 0.05$) enrichment in GZMB and IL2RB occurring in infiltrating cytoTIL15 cells but not unengineered cells, which is associated with the distinct cluster profiles of each cell treatment.

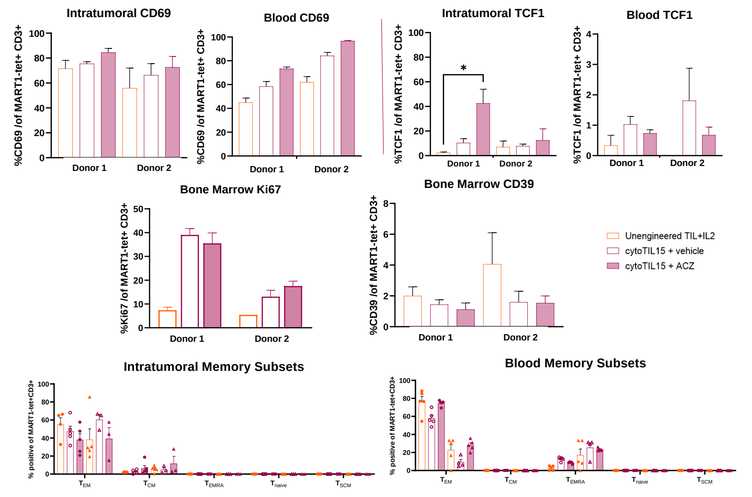
[7] Tumor-infiltrating and peripheral cytoTIL15 cells demonstrate enhanced stem-like anti-tumor phenotype

cytoTIL15 cells produce potent effector cytokines



cytoTIL15 cells demonstrate improved intratumoral and circulating cytokine production. Cytokines (IFNγ and TNFα) were measured from excised PDX tumor suspension lysate (left) or from plasma obtained from cardiac blood (right) following ACT with unengineered or cytoTIL15 cells.

MART1-specific cytoTIL15 cells have an activated, stem-like, memory phenotype



Infiltrating and circulating MAA-specific cytoTIL15 cells have an activated, stem-like phenotype and exhibit memory formation. *Top*: Infiltrating and circulating TILs are highly activated, expressing robust levels of CD69 (left), and cytoTIL15 cells demonstrate increased expression of transcription factor TCF1, which is associated with an acquisition of stem-like potential and in melanoma patients correlates with favorable therapeutic outcomes and responses to immunotherapy (right). *Middle:* Further supporting this distinct phenotype, cytoTIL15 cells accumulating in bone marrow niches have higher levels of Ki67, a nuclear protein associated with increased proliferative capacity, and have decreased levels of CD39 expression, which is associated with T cell terminal differentiation. *Bottom*: Intratumoral and circulating TILs have a distinct profile of T cell memory subsets, with an increase in central memory cells identified in infiltrating TILs, in contrast to the largely effector memory and TEMRA populations that were present in the TILs at the end of REP and are reflected in the blood and bone marrow distributions. *p<0.05

[8] Conclusions

- cytoTIL15 administered without IL2 show superior *in vivo* persistence compared to IL2 dependent TIL
- cytoTIL15 therapy demonstrates superior tumor control in allogeneic, HLA-matched melanoma PDX model
- cytoTIL15 cells expand and infiltrate into PDX tumors and produce IFNγ and TNFα
- Anti-tumor efficacy of cytoTIL15 in allogeneic PDX model is associated with reactivity to conserved melanoma associated antigen MART-1
- Anti-MART-1 cytoTIL15 display increased expression of TCF-1, which in melanoma patients has been associated with responses to immune checkpoint blockade, as well as progression free survival (PFS) and overall survival (OS)
- cytoTIL15 cells show a distinct profile of RNA expression consistent with their increased persistence and anti-tumor efficacy (*e.g.,* IL2RB, GNLY, CCL5, GZMB, and KLRC1)
- The University of Texas MD Anderson Cancer Center is currently enrolling patients in a Phase 1 clinical trial of OBX-115, which is being evaluated in patients with metastatic melanoma (NCT05470283).