DIGITAL SPATIAL PROFILING AND ANTIGEN-DEPENDENT PHENOTYPIC ANALYSIS OF IL15-ENGINEERED TUMOR-INFILTRATING LYMPHOCYTES (CYTOTIL15® THERAPY) IN AN ALLOGENEIC MELANOMA PDX MODEL


Background CytoTIL15® therapy is an IL2-independent, engineered TIL product which allows pharmacological control of membrane-bound IL15 (mbIL15). We have previously shown that cytoTIL15® TILs demonstrate enhanced persistence and anti-tumor efficacy in a human allogeneic melanoma PDX model. Here we use digital spatial profiling and single cell sequencing to characterize the RNA expression profile and phenotypic markers of tumor infiltrating immune cells as well as tumor cells in this model and compare the results to unengineered, IL2-dependent TIL.

Methods cytoTIL15® therapy contains TILs engineered with mbIL15 under the control of a carbonic-anhydrase-2 drug responsive domain, regulated by the ligand acetazolamide (ACZ). cytoTIL15® cells were generated from human melanomas through a proprietary rapid expansion process. Expanded TILs were phenotyped and assayed for in vitro polyfunctional-ity, cytotoxicity, and frequency of tumor-associated antigen-specific TCR. In vivo phenotype and anti-tumor functionality was examined through adoptive transfer of TILs into NSG mice bearing subcutaneous, HLA-matched, patient-derived-xenograft (PDX) tumors expressing melanoma-associated antigen MART-1, in IACUC approved animal studies. Tumors, spleen, bone marrow, and blood were harvested 14-21 days following adoptive cell transfer and assessed by flow cytometry, GeoMx digital spatial profiling, and single cell sequencing for characterization of TIL and the tumor microenvironment (TME).

Results cytoTIL15® cells demonstrated enrichment and reactivity for melanoma antigen-specific TCRs, while maintaining TCRβ diversity. Fifteen days post-ACT, tumors from animals treated with cytoTIL15® cells exhibited significantly (p=0.0175) higher frequency (3.4-fold) of TILs, in which MART-1 tetramer positive cells demonstrated increased T-cell factor 1 (TCF-1) and CD69 expression, and secreted significantly greater amounts of IFNγ and TNFα cytokines into the TME, compared to unengineered TILs with IL2. In addition, cytoTIL15® TILs had a distinct differential gene expression profile, demonstrating an increase in effector genes such as IL2RB, GZMB, GNLY and CCL5 and reduction in exhaustion-related genes such as EOMES. cytoTIL15® cells accumulating in the bone marrow exhibited a lower frequency of CD39+ terminally differentiated CD8+ T cells, while maintaining higher levels of memory phenotype makers.

Conclusions In this allogeneic melanoma PDX model, cytoTIL15® cells showed a distinct profile of RNA expression and phenotypic markers, consistent with their increased persistence and anti-tumor efficacy. Interestingly, the subpopulation of cytoTIL15® cells reactive to tumor-associated antigen MART-1 displayed increased expression of TCF-1, which in melanoma patients has been associated with responses to immune checkpoint blockade, in addition to progression-free and overall survival.

Ethics Approval All animal studies were IACUC approved.