cytoTILs™ therapy is an IL2-independent, engineered TILs product which allows pharmaceutical control of membrane-bound IL5 (mIL5LS). We have previously shown that cytoTILs TILs demonstrate enhanced persistence and anti-tumor efficacy in a human allogeneic melanoma PDX model, utilizing the melanoma associated antigen, MART-1, as a model system based on conserved antigen reactivity. 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.

Rationale and Methods

cytoTILs™ therapy contains TILs engineered with mIl5 under the control of a carbonic-anhydrase-2 drug responsive domain, regulated by the ligand acetazolamide (ACZ). cytoTILs cells were generated from human melanomas through a proprietary rapid expansion process. Expanded TILs were phenotyped and assayed for in vitro polyfunctionality, 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 conserved melanoma-associated antigen (MMA) MART-1, in IULC approved animal studies. Tumors, spleen, bone marrow, and blood were harvested 14-21 days following adoptive cell transfer and assessed by flow cytometry. GeoMx® (NanoString®) digital spatial profiling, and single cell sequencing to characterize the TILs and the tumor microenvironment (TME).

cytoTIL15™ cells exhibit a distinct differential gene expression profile from unengineered TIL

Conclusions

➢ cytoTIL15™ therapy demonstrates potent anti-tumor cytotoxicity against allogeneic tumor targets in vitro and in vivo.

➢ Anti-tumor efficacy is associated with reactivity to the conserved melanoma associated antigen (MMA), MART-1, in this antigen-specific model.

➢ cytoTIL15™ cells expand and infiltrate into PDX tumors in vivo and produce cytokines (IFN and TNF) at the tumor site.

➢ Adoptive cell therapy (ACT) controls tumor growth in this allogeneic setting, and enriched MMA-reactive TILs demonstrate a favorable anti-tumor phenotype.

Specifically, the subpopulation of cytoTIL15™ cells reactive to tumor-associated antigen displayed increased expression of CD8+ T cells, which in melanoma patients has been associated with response to immune checkpoint blockade, as well as progression free survival (PFS) and overall survival (OS).

cytoTIL15™ cells showed a distinct profile of RNA expression consistent with their increased persistence and anti-tumor efficacy (e.g., IL2R, GNA1, CCL5, IFN, and TNFR1).