Background We have previously demonstrated the successful generation of membrane-bound IL15 (mbIL15) engineered TIL (cytoTIL15™ therapy) from solid tumors, and acetazolamide (ACZ)-driven regulated expression of mbIL15 resulted in TIL persistence in an antigen-independent preclinical model (SITC 2021, 2022). Here, we evaluated the function of pharmacologically tunable mbIL15 in the setting of chronic antigen stimulation by melanoma tumor-associated antigens (TAAs), such as MART1.

Methods CytoTIL15 cells were manufactured from metastatic melanoma TIL donors by introducing mbIL15 under the pharmacological control of a carbonic-anhydrase-2 (CA2) drug responsive domain (DRD) via ACZ, the stabilizing ligand, and expanded through a proprietary rapid expansion process (REP). ACZ-dependent IL15 expression and downstream signaling were assessed. In vitro, we employed peptide-loaded HLA-A*0201 T2 cells to present MART-1 to TIL for evaluation of TCR-based functionality. CytoTIL15 cells treated with 0–25 μM ACZ were stimulated with antigen twice weekly over 28 days, with routine assessments of cell health, phenotype, cytokine production, and gene expression. In vivo, antigen-independent cytoTIL15 cell persistence in response to ACZ doses was evaluated after adoptive transfer of the TIL into immunodeficient NSG mice.

Results Compared to unengineered TIL, generation of cytoTIL15 therapy from melanoma-derived TIL led to an overall 2.3-fold enrichment of MART1-reactive TIL. CytoTIL15 cells exhibited ACZ-dependent expansion in response to repeat MART1 stimulation, with TIL reaching maximums of 2, 9, and 18-fold expansion for 0, 1, and 25 μM of ACZ, respectively. Chronic antigen exposure revealed an ACZ-driven IL15-dependent enrichment of >80% MART1-reactive TIL, and an increase in effector cytokine production and polyfunctionality (IFNγ, IL2Rα, TNFα, IL2, Perforin, CD107a, Granzyme B). CytoTIL15 cells driven by ACZ demonstrated maintenance of a functional cytotoxic signature, which was enriched in the antigen-reactive cell population. Despite repeated antigen-stimulation, withdrawal of ACZ reduced cytokine production and persistence of the MART1-enriched cytoTIL15 cell population in vitro. In vivo studies further underscored ACZ-dependent tunability of cytoTIL15 cells, as increased ACZ doses enhanced TIL persistence (AUC: 41, 111, and 306% TIL*day for 0, 30, and 200 mg/kg ACZ QD), and ACZ withdrawal after 8 days reduced TIL persistence by 1.7-fold.

Conclusions The expansion and persistence of tumor specific cytoTIL15 cells in the setting of chronic antigen exposure was regulatable by ACZ-dependent mbIL15 expression. This concept supports clinical evaluation of OBX-115 in the relapsed metastatic melanoma setting without concurrent IL-2 administration (NCT05470283).

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