

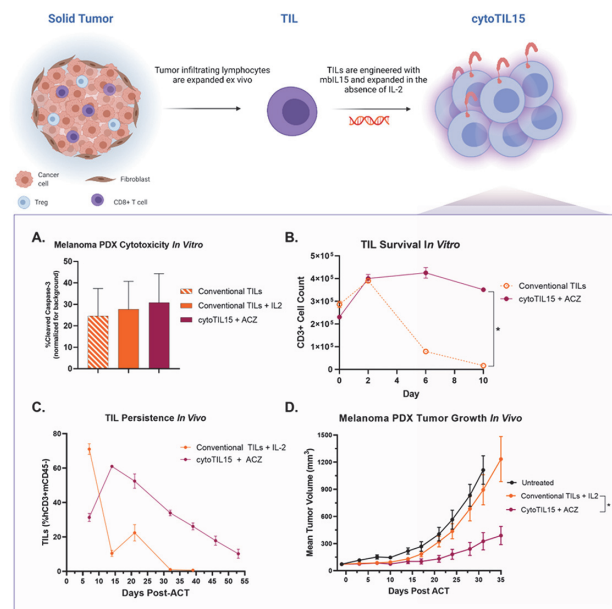
GENETICALLY ENGINEERED TUMOR-INFILTRATING LYMPHOCYTES (CYTOTIL15) EXHIBIT IL-2-INDEPENDENT PERSISTENCE AND ANTI-TUMOR EFFICACY AGAINST MELANOMA IN VIVO

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Background Adoptive cell therapy with tumor-infiltrating lymphocytes (TILs) has demonstrated tremendous promise in clinical trials for patients with solid or metastatic tumors.¹ However, current TIL therapy requires systemic administration of IL-2 to promote TIL survival, and IL-2-associated toxicities greatly limit patient eligibility and reduce the long-term clinical benefit of TIL therapy.^{2,3} Unlike IL-2, which promotes T cell exhaustion, IL-15 maintains antigen-independent TIL persistence through homeostatic proliferation and supports CD8+ T cell anti-tumor activity without stimulating regulatory T cells. We designed genetically engineered TILs to express a regulated form of membrane-bound IL-15 (mbIL15) for tunable long-term persistence, leading to enhanced efficacy and safety for the treatment of patients with solid tumors.

Methods 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, bioavailable small molecule ligands. cytoTIL15 contains TILs engineered with mbIL15 under the control of a carbonic-anhydrase-2 DRD, controlled by the ligand acetazolamide (ACZ). After isolation from tumors, TILs were transduced and expanded in vitro through a proprietary TIL expansion process. cytoTIL15 were immunophenotyped and assessed for in vitro antigen-independent survival and co-cultured with tumor cells to assess polyfunctionality and cytotoxicity. In vivo TIL persistence and anti-tumor efficacy was evaluated through adoptive transfer of TILs into immunodeficient NSG mice, either naïve or implanted with subcutaneous patient-derived-xenograft (PDX) tumors.

Results cytoTIL15 and conventional IL2-dependent TILs isolated from melanoma tumor samples expanded to clinically relevant numbers over 14 days. Throughout expansion, cytoTIL15 were enriched for CD8+ T cells and acquired enhanced memory-like characteristics, while maintaining diverse TCRVβ sub-family representation. cytoTIL15 demonstrated enhanced potency over conventional TILs, as measured by increased polyfunctionality and cytotoxicity against tumor and PDX lines in vitro (figure 1A). In a 10-day antigen-independent in vitro assay, cytoTIL15 persisted at greater frequencies than conventional TILs in the absence of IL-2 (figure 1B; *p<0.05). cytoTIL15 adoptively transferred into naïve NSG mice demonstrated ACZ-dependent long-term persistence without antigen or exogenous IL-2, whereas conventional TILs were undetectable >30 days following adoptive cell transfer (figure 1C). Importantly, cytoTIL15 achieved significant tumor control in a human PDX model (figure 1D), which correlated with increased TIL accumulation in secondary lymphoid organs.



Abstract 166 Figure 1 cytoTIL15 demonstrate superior persistence. cytoTIL15 is an engineered TIL product expressing regulatable mbIL15. (A) cytoTIL15 demonstrate enhanced in vitro cytotoxicity after co-culture with melanoma tumor lines (representative data from 3 TIL donors). (B) cytoTIL15 have improved persistence in antigen- and IL2- independent culture conditions in vitro compared to conventional TILs cultured in the absence of IL-2 as well as (C) in vivo compared to conventional TILs supplemented with IL-2, when engrafted into NSG mice (in vitro: representative data from 1 TIL donor, performed in >3 replicate donors, in vivo: n=5/group, representative of 1 TIL donor, performed in >3 replicate donors). (D) cytoTIL15 (with 200mg/kg ACZ PO QD) demonstrate enhanced anti-tumor efficacy in a xenograft melanoma model as compared to conventional TILs (with 50000 IU IL-2 q8h BID, IP for 5 days) (n=8/group, representative of 1 TIL donor, performed in >2 replicate donors; ACT = adoptive cell transfer).

Conclusions Taken together, the superior persistence and potency of cytoTIL15 in the complete absence of IL-2 highlights the clinical potential of cytoTIL15 as a novel TIL product with enhanced safety and efficacy for patients with melanomas, and other solid tumors.

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