**IMPROVED ANTI-TUMOR ACTIVITY OF NEXT-GENERATION TCR-ENGINEERED T CELLS THROUGH CD8 CO-EXPRESSION**


**Background** Successful targeting of solid tumors with TCR-engineered T cells (TCR-T) will require eliciting of antigen-specific, multi-dimensional, sustained anti-tumor immune response by infused T cells while overcoming the suppressive tumor microenvironment. First-generation TCR-T approaches have demonstrated clinical efficacy in some solid cancers. However, effective treatment across several solid tumor indications may require engineered T cells with enhanced anti-tumor activity. Here, we show pre-clinical data from one of the engineering approaches currently being developed for next-generation ACTengine® TCR-T product candidates. We evaluated the impact of co-expression of different CD8 co-receptors on functionality of CD4+ and CD8+ T cells genetically modified with an HLA class I-restricted TCR and determined the depth and durability of anti-tumor response in vitro.

**Methods** Here, we used a PRAME-specific TCR currently being tested in the ACTengine® IMA203 clinical trial. T cells expressing either the TCR alone or co-expressing the TCR and CD8α homodimer (TCR.CD8α) or CD8αβ heterodimer (TCR.CD8αβ) were characterized for transgene expression, antigen-recognition, and functional efficacy in vitro. Comprehensive evaluation of CD4+ T cells expressing TCR.CD8α or TCR.CD8αβ was performed focusing on cytotoxic potential and the breadth of cytokine response against target-positive tumor cell lines.

**Results** Introduction of CD8α or CD8αβ enabled detection of transgenic TCR on the surface of CD4+ T cells via HLA multimer-guided flow cytometry otherwise lacking in the TCR only transduced T cells. Co-expression of either form of CD8 co-receptor endowed CD4+ T cells with the ability to recognize and kill target positive tumor cells; however, genetic modification with TCR.CD8αβ led to more pronounced CD4+ T cell activation as compared to TCR.CD8α. Most distinct differences were observed in the breadth and magnitude of cytokine responses, less in cytotoxic activity against tumor cells. T cells expressing TCR.CD8αβ showed superior induction of Th1 cytokines e.g. IFNγ, TNFα, IL-2, GM-CSF in vitro upon antigen stimulation as compared to TCR.CD8α-T cells. Additionally, TCR.CD8αβ T cells demonstrated more efficient engagement with antigen-presenting cells and consequently, modulation of cytokine response than TCR.CD8α-T cells.

**Conclusions** Our findings illustrate that engaging CD4+ T cells via CD8 co-expression potentiates anti-tumor activity of HLA class I restricted TCR-T cells in vitro. The pleiotropic effects mediated by activated CD4+ T cells including acquired cytotoxicity may potentially improve outcomes in solid tumor patients when applied clinically. In addition, the differential functional profile of TCR-T cells co-expressing either CD8α or CD8αβ suggests that optimizing the type of co-receptor is relevant to maximize anti-tumor response.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.163