372 TAC-T CELLS PERSIST AND REMAIN FUNCTIONAL DURING AND AFTER REPEATED TUMOR EXPOSURE *IN VITRO* AND *IN VIVO*

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Background T cell antigen coupler (TAC) is a chimeric receptor that redirects T cells (TAC-T) towards surface-expressed tumor antigens to create safe and durable anti-cancer immune responses. The TAC receptor activates T cells by co-opting the endogenous T cell receptor machinery via a CD3ϵ-specific binding motif and a cytoplasmic co-receptor tail. TAC01-HER2, a first-in-class TAC-T product targeting HER2 (ERBB2), has entered a phase I/II clinical trial. Here, we show that TAC-T cells retain their cytotoxicity capacity during and after repeated tumor challenges *in vitro* and *in vivo*.

Methods The robustness of anti-tumor T cell responses were assessed *in vitro* in a recursive killing assay by repeatedly exposing HER2-specific TAC-T cells to HER2-expressing tumor cells for 11 successive rounds (39 days). T cells were characterized by flow cytometry to correlate T cell phenotypes with anti-tumor activity. *In vivo*, ongoing tumor control established by a single infusion of TAC-T cells was assessed in a tumor rechallenge experiment. MHC I/II-deficient NSG mice were engrafted subcutaneously with HER2+ tumor cells and rechallenged with the same tumor cell line 28 days later. TAC-T cells were isolated from mice at various time points for phenotypic and functional characterization.

Results TAC-T products controlled tumor cell growth through 11 rounds of tumor cell challenge *in vitro*. Signs of reduced functionality were observed at round 11, which coincided with the emergence of a dysfunctional phenotype. During *in vivo* tumor rechallenge experiments, a single infusion of TAC-T cells led to complete clearance of the solid tumor xenograft and protected mice from a second tumor challenge 28 days after adoptive T cell transfer. TAC-T cells recovered from tumor sites at various time points exhibited phenotypic markers of activation, whereas TAC-T cells isolated from blood and spleen appeared to be antigen-experienced cells but lacked markers indicative of chronic activation and exhaustion. TAC-T cells isolated from spleens before and after the rechallenge were able to proliferate and kill tumor cells *ex vivo*.

Conclusions Here we report evidence that TAC-T cells controlled tumor cell growth through 11 rounds of repeated tumor rechallenge *in vitro*, protected mice against tumor rechallenge, and demonstrated long-term *ex vivo* proliferative and cytotoxic capabilities. These data indicate long-lasting T cell persistence and functionality against solid tumors.

Ethics Approval Animal studies performed for the work presented in this abstract were conducted under the Animal Utilization Protocol (AUP) # 20–10-37 and approved by the Animal Research Ethics Committee at McMaster University (Hamilton, ON, Canada).

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