378 CHIMERIC ENGULFMENT RECEPTOR (CER) T CELLS WITH A TLR2 DOMAIN SYNERGIZE WITH AN EGFR INHIBITOR TO TARGET NSCLC CELLS IN VITRO AND DEMONSTRATE APC-LIKE FUNCTION

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Background A barrier to successful adoptive cell therapy for solid tumors is target antigen heterogeneity and antigen escape. Activated T cells, including CAR T cells, have limited innate antigen capture/presentation capabilities.[1] We engineered a novel TIM-4 CER that bears a toll/interleukin-1 (TIR) signaling domain from TLR2 designed to induce both direct cell-mediated cytotoxicity and initiate secondary immune responses, leading to improved solid tumor clearance and durability of response. CER-T cells offer potential pairing with multiple small molecule drugs to enhance the TIM-4 target ligand. We tested for antigen-presenting cell (APC)-like function, characterized transcriptional signatures, and quantified cytotoxic responses against EGFR-mutant NSCLC cells in combination with the epidermal growth factor inhibitor (EGFRi) osimertinib.

Methods CER-1236 contains a TIM-4 extracellular signaling domain fused with a TLR2-TIR domain, CD28 and CD3zeta. Using PS-coated beads labeled with a pH-sensitive dye (pHrodo), we quantified CER-1236 phagocytic uptake by FACs and fluorescent microscopy. Cytotoxic and proliferative responses against osimertinib-treated H1975 NSCLC cells was tested in vitro. We quantified APC activity by evaluating autologous HPV E7 TCR T cell activation and proliferation following CER-1236 co-culture with HLA-matched HPV-16 E7+ SCC152 tumor cells. To characterize transcriptional states, we performed bulk RNA-sequencing at rest and following activation. TLR2 inhibition was used to examine signaling pathway induction.

Results In combination with subtherapeutic osimertinib doses, CER-1236 eliminated 97% of targets by 72h at low effector: target ratios. EGFR inhibition drove target-dependent proliferation, production of TH1 cytokines (IFN-gamma, TNFalpha) and granzyme-B. Upon co-culture with PS-coated beads, 60% of CER-1236 T cells demonstrated increased bead uptake compared to untransduced or CER T cells expressing a TIM-4 PS-binding mutant ($p<0.0001$), APC assays demonstrated higher activation of E7-TCR T cells by CER-1236 than untransduced T cells, indicating occurrence of antigen uptake and presentation. Both cytokine production and antigen presentation were inhibited by a TLR2 inhibitor. Finally, transcriptional profiling identified a distinct signature related to APC-like function upon CER-1236 stimulation with enrichment of NF-kB, MAP kinase, TNFalpha and chemokine signaling.

Conclusions Novel TIM-4-containing CER T cells that include a TLR2 signaling domain synergize with osimertinib to eliminate EGFR-mutant NSCLC cells in vitro. Notably, CER T cells capture and present tumor cell antigen, and demonstrate APC-like transcriptional signatures. Antigen presentation, alongside inducible, target-specific cytotoxic function in single T cells, represent a potential advantage to initiate host immune responses against novel antigens for solid tumors.

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