SNAP CAR T CELLS FOR PROGRAMMABLE ANTIGEN TARGETING

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Background Universal chimeric antigen receptors (CARs) are synthetic receptors that instead of directly binding to an antigen, recognize one or more adaptor molecules that bind to target antigens. Universal CARs are of high clinical interest due to their abilities to be tuned by adaptor dose, potentially avoiding toxicities, and to be targeted toward multiple antigens through combinatorial use of different adaptors, potentially avoiding relapse due to antigen loss.

Methods We developed a universal CAR, ‘SNAP-CAR,’ that carries out a self-labeling reaction to covalently attach to adaptor antibodies conjugated to a benzylguanine (BG) tag. SNAP-CAR-T2A-LNGFR was cloned and packaged into a gamma-retroviral expression system, for efficient transduction of primary human T cells. CAR T cells were co-incubated with different antibody adaptors at varying concentrations and target cells and then assayed by flow cytometry for T cell activation and target cell lysis and ELISA for cytokine production. To assess the in vivo activity of SNAP-CAR, NSG mice were challenged with HER2+ or CD20+ human leukemia or ovarian tumor xenografts. Mice were then treated with SNAP-CAR T cells and adaptor injections, and tumor size was measured by IVIS imaging.

Results In vitro experiments showed potent and specific SNAP-CAR function with co-administered adaptors targeting HER2, EGFR, and CD20 on cancer cell lines including activation of CD69 and CD107a markers, specific target cell lysis, and IFN-gamma production. Testing SNAP-CAR T cells in vivo in a human leukemia tumor xenograft NSG mouse model targeting HER2, we observed that SNAP-CAR T cells were able to significantly reduce tumor burden, leading to a lack of detectable tumors in the majority of mice. In another leukemia model targeting the CD20 antigen, SNAP-CAR T cells showed significant inhibition of tumor growth. While tumors in these mice relapsed, investigation demonstrated that relapsed cancer cells were CD20 negative, suggesting the importance of future multi-antigen targeting. Finally, evaluating two anti-HER2 adaptors with distinct binding epitopes in a human ovarian cancer xenograft model, we observed a significant tumor reduction with both adaptors compared to adaptor only and SNAP-CAR T cell only controls.

Conclusions Overall, these data demonstrate the potent and versatile antigen targeting abilities of SNAP-CAR T cells both in vitro and in vivo in human tumor xenograft models, suggesting future potential for treating liquid and solid tumor malignancies. Development of SNAP-CAR T cells for human use is underway.

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Ethics Approval All work in this study was approved by the University of Pittsburgh Institutional Biosafety Committee (IBC201900130). Animal work in this study was approved by the University of Pittsburgh Institutional Animal Care and Use Committee (23053145), and procedures were performed under their guidelines.

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