Background: Autologous T cells expressing chimeric antigen receptors (CARs) against CD30 have demonstrated high clinical efficacy in the treatment of relapsed or refractory CD30-positive classical Hodgkin lymphoma (cHL) [1]. In the allogeneic setting, we have treated nine CD30-positive lymphoma patients in an ongoing Phase 1 study (NCT04288726) with Epstein-Barr virus specific T cells (EBVST) expressing a CD30-specific CAR derived from the murine HRS-3 antibody. These allogeneic CD30.CAR EBVSTs show promising signs of efficacy with a favorable safety profile and no evidence of graft versus host disease (GVHD). However, low levels of infused CD30.CAR EBVSTs were detected in the peripheral blood of most patients seven days post infusion, suggesting that durable persistence of these cells remains a challenge. In this study, we seek to improve the persistence of the CD30.CAR EBVSTs with the humanization of the murine HRS-3 scFv to minimize immunogenicity, and the replacement of the current IgG1 spacer with a novel 4-1BB derived spacer to eliminate any off-target interactions.

Methods: CD30.CARs with humanized scFvs and novel spacers were designed and evaluated on their in vitro cytolytic potency and cytokine secretion profile. Top candidates were further tested for in vivo efficacy and safety in CD30-high tumor models.

Results: CD30.CARs with humanized scFvs preserved specificity and efficacy, and exhibited improved stability as compared to the parent HRS-3 CAR. We found that HRS-3 binds to the cysteine-rich domain 5 of the CD30 molecule, which enabled informed design of novel spacer candidates. In combination with the murine HRS-3 scFv, the novel spacer derived from the 4-1BB receptor decreased basal cytokine secretion compared to the current IgG1 spacer, while retaining cytolytic activity in vitro. Humanized CD30.CARs combined with the 4-1BB-derived spacer did not exhibit any nonspecific interactions with CD16+ immune cells, while displaying superior efficacy in vitro, better persistence in vivo in various humanized mouse models, and more importantly an improved safety profile in a leukemia model with high tumor burden.

Conclusions: Our re-engineered CD30.CAR construct, consisting of a humanized CD30.CAR and a 4-1BB-derived spacer, is likely to improve allogeneic CD30.CAR EBVST performance.

REFERENCE

Ethics Approval: This study was approved by the Agency for Science, Technology and Research (A*STAR) Institutional Animal Care and Use Committee (IACUC) under project approval number 211593.