Background Autologous CAR T cell therapies have revolutionized the treatment of hematologic malignancies but have inherent disadvantages that hinder widespread access, including complex logistics and manufacturing limitations. These challenges may be overcome with off-the-shelf allogeneic CAR T cells derived from healthy donor T cells. Although allogeneic CAR T cells provide immediate availability to patients and scalable manufacturing, they may be susceptible to allorejection and have reduced persistence, limiting clinical responses. To address this challenge, we developed an anti-rejection CD70 CAR capable of selectively depleting activated (CD70+) host lymphocytes. We previously showed that this approach rendered allogeneic CD19 CAR T cells resistant to allorejection while also enhancing antitumor activity by endowing dual targeting in CD70+CD19+ lymphoma models. Here, we describe an optimized construct for site-specific integration (SSI)-based co-expression of the anti-rejection CD70 CAR and a CD19 CAR from a single locus. The resulting CAR T cell product showed high homogeneity, enrichment of CD70 CAR/CD19 CAR double positive cells, efficacy comparable to CAR T cells expressing only a CD19 CAR, and resistance to allorejection.

Methods TALEN® gene-editing technology combined with adeno-associated virus (AAV) transduction was employed to knock-in CAR constructs into the T Cell Receptor Alpha Constant (TRAC) locus. Constructs encoding a CD70/CD19 tandem CAR (single CAR containing both CD70 and CD19 single-chain variable fragments) or a dual CAR (CD70 CAR and CD19 CAR separated by a self-cleaving peptide) were tested. Cytotoxicity was assessed in vitro and in vivo using a Raji lymphoma model. Anti-rejection activity of the CD70/CD19 CAR T cells was assessed in mixed lymphocyte reaction (MLR) assays.

Results SSI of the CD70/CD19 dual CAR transgene in activated T cells was highly efficient and resulted in a high percentage and yield of CD70 CAR/CD19 CAR double positive cells (~80–99%), which showed improved functionality compared to cells expressing tandem CAR constructs. Enrichment and expansion of dual CAR+ cells was likely enhanced due to CD70-dependent activation during the manufacturing process. Despite this, these cells preserved T cell memory subsets, efficiently eliminated Raji cells in vitro and in vivo, and resisted allorejection, suggesting that both CARs retain their independent functions.

Conclusions Manufacturing of CD70/CD19 CAR T cells by SSI of a bicistronic construct successfully yields high percentages and numbers of CAR+ cells without significantly compromising CAR T cell effector or anti-rejection functions. This novel off-the-shelf allogeneic CD70/CD19 CAR T cell product is a promising candidate for clinical evaluation.

Ethics Approval All procedures performed on animals were reviewed and approved by an Institutional Animal Care and Use Committee and were conducted in accordance with established guidelines.

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