Supplemental Materials and Methods

***Vector construction***

Construction of lentiviral vectors containing CD19-specific CARs with short, intermediate, or long spacers and a 4-1BB costimulatory domain, and with a long spacer and a CD28 costimulatory domain has been described ([1](#_ENREF_1),[2](#_ENREF_2)). To generate a short/CD28 construct, the 4-1BB costimulatory domain in the short/4-1BB construct was replaced by the 41 amino acid cytoplasmic domain of human CD28 (Uniprot Database: P10747) containing a LL🡪GG substitution located at position 186-187 of the native CD28 protein ([3](#_ENREF_3)). A CD19-CAR with both costimulatory domains (long/CD28\_4-1BB) was created by adding the 42 amino acid cytoplasmic domain of human 4-1BB (Uniprot Database: Q07011) between CD28 and CD3ζ of the long/CD28 construct. Construction of the long 4/2 spacer CAR was accomplished by replacing the first six amino acids of the CH2 domain of IgG4 (APEFLG) with the corresponding five amino acids of IgG2 (APPVA). The long 4/2NQ spacer included an additional Asn297 to Gln mutation ([4](#_ENREF_4)). ROR1-specific R11-CARs were created by exchanging the CD19-specific FMC63 scFv with the scFv of the ROR1-specific mAb R11 ([5](#_ENREF_5)). All vectors encoded a truncated epidermal growth factor receptor (EGFRt) sequence ([1](#_ENREF_1)) downstream of the CAR linked by a T2A ribosomal skip element.

1. Wang X, Chang WC, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood 2011;118(5):1255-63.

2. Hudecek M, Lupo-Stanghellini MT, Kosasih PL, Sommermeyer D, Jensen MC, Rader C, et al. Receptor Affinity and Extracellular Domain Modifications Affect Tumor Recognition by ROR1-Specific Chimeric Antigen Receptor T Cells. Clin Cancer Res 2013;19(12):3153-64.

3. Nguyen P, Moisini I, Geiger TL. Identification of a murine CD28 dileucine motif that suppresses single-chain chimeric T-cell receptor expression and function. Blood 2003;102(13):4320-5.

4. Leatherbarrow RJ, Rademacher TW, Dwek RA, Woof JM, Clark A, Burton DR, et al. Effector functions of a monoclonal aglycosylated mouse IgG2a: binding and activation of complement component C1 and interaction with human monocyte Fc receptor. Molecular immunology 1985;22(4):407-15.

5. Yang J, Baskar S, Kwong KY, Kennedy MG, Wiestner A, Rader C. Therapeutic potential and challenges of targeting receptor tyrosine kinase ROR1 with monoclonal antibodies in B-cell malignancies. PloS one 2011;6(6):e21018.