SUPPLEMENTARY METHODS

Construction of TÜ165 CAR, iIL-12-secreting TÜ165 CAR and iEGFP-secreting TÜ165 CAR vectors

The TÜ165 CAR was designed based on the scFv of mAb TÜ165, comprising the variable domains of light and heavy chains linked by a $(G_4S)_3$ peptide. The scFv was synthesized (Thermo Fisher Scientific) and cloned into the previously described ROR1-CAR-epHIV7 vectors [31] using *NheI* and *RsrII* restriction sites to replace the ROR1-specific scFv. Briefly, the TÜ165 CAR construct comprised the TÜ165 scFv connected to a "hinge-only" (12 amino acids, AA) short-spacer domain or a "hinge-CH2-CH3" (229 AA) long-spacer domain derived from IgG4-Fc (Uniprot: P01861) linked to the cytoplasmic domain of human 4-1BB (Uniprot: Q07011) followed by the cytoplasmic domain of isoform 3 of human CD3z (Uniprot: P20963). Downstream of the CAR construct, the plasmid encoded for a T2A ribosomal skip element and a truncated epidermal growth factor receptor (EGFRt).

To generate the iIL-12-secreting TÜ165 CAR and iEGFP-expressing TÜ165 CAR constructs, we flanked the complete CAR backbone, including the co-expressed truncated epidermal growth factor receptor (EGFRt), with restriction sites *AgeI* and *SalI* and cloned it into the previously described pRRL.PPT.NFATenh.synTATA.IL12.PGK.newMCS.GD2CAR.PRE and pRRL.PPT.NFATenh.synTATA.EGFP.PGK.newMCS.GD2CAR.PRE vectors to replace the GD2-CAR expression cassette [31]. The constructs were therefore so-called "all-in-one" vectors containing both an inducible IL-12 or inducible EGFP expression cassette drived by an inducible promotor element with six NFAT response elements and a synthetic promoter (NFATsyn) and a constitutive CAR expression cassette in a single vector.

Cloning and sequence details will be provided on reasonable request.

Generation and titration of lentiviral vectors

TÜ165 CAR, TÜ165 CAR iIL-12 or TÜ165 CAR iEGFP-encoding lentiviral particles were produced in 293T cells. In brief, 4-6x10^6 293T cells were seeded and transfected using the calcium phosphate method. Using second-generation packaging vectors, psPAX2 (5 µg) and pMD2.G (5 µg) were added to the cells together with 10 µg lentiviral vector plasmid in the presence of 200 nM chloroquine, and supernatants were harvested after 48 h and 72 h. Alternatively, the third-generation packaging vectors pcDNA3.HIV-1.GP.4×CTE (lentiviral gag/pol) (12 µg), pRSV-Rev (5 µg) and pMD.G (1.5 µg) were used with 10 µg lentiviral vector plasmid in the presence of 23 µM chloroquine, and supernatants were harvested after 36 h

and 48 h as previously described [31]. Supernatants were concentrated via ultracentrifugation at 24,200 x g for 2 h or 10,000 x g for 16 h at a temperature of 16 °C.

Titers of viral supernatants were determined by transducing $2x10^5$ Jurkat cells in the presence of 5 µg/mL Polybrene Infection / Transfection Reagent (Merck Millipore, Burlington, MA, USA). After 48 h, the transduction efficiency was assessed by staining EGFRt with biotin-anti-EGFRt and PE Streptavidin (Thermo Fisher Scientific, Waltham, MA, USA) followed by flow cytometric analysis. Titers were calculated from samples with EGFRt expression between 15% and 45%.