Supplementary Materials:
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Supplementary Figure 2
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Supplementary Fig. 1: Manufacturing of CD19 CAR-T cells. A) The strategy for manufacturing of CD19 CAR-T cells using the CliniMACs is shown. The leukapheresis product is split into two aliquots. CD4⁺ cells are selected from one of the aliquots using the CD4 CliniMACs reagent. CD8⁺ T_CM cells are enriched from the second aliquot by a two-step method involving depletion of CD4⁺, CD14⁺ and CD45RA⁺ cells followed by selection of CD62L⁺ cells from the CD4⁺/CD14⁺/CD45RA⁺-depleted fraction. The selected CD4⁺ cells and CD4⁺/CD14⁺/CD45RA⁺/CD62L⁺ cells are separately stimulated with anti-CD3/CD28 paramagnetic beads, transduced with a lentiviral vector encoding the CD19 CAR transgene and EGFRt and expanded in vitro for 15 - 20 days before formulation in a CD4⁺/EGFRt⁺: CD8⁺/EGFRt⁺ ratio of 1:1 for infusion. In some patients with severe lymphopenia or circulating blasts, CD8⁺ cells were selected rather than CD8⁺ T_CM cells (not shown). B) Flow cytometry plots from a representative two-step CD8⁺ T_CM enrichment are shown, illustrating the isolation of the CD4⁺/CD14⁺/CD45RA⁻/CD62L⁺ fraction that contains T_CM-enriched CD3⁺ cells and CD3⁻ cells. C) The CD8⁺ T_CM enriched cell product contains CD3⁻ myeloid cells (SS hi/CD13⁺/CD15⁺/CD16 hi; top) and basophils (SS lo/CD13⁺/CD123⁺; bottom). D) Representative flow cytometry plots
show enrichment in EGFRt⁺ CAR expression in transduced CD4⁺ (top) and CD8⁺ (bottom) T cells between LCL stimulation and product release. E) Data from all B-ALL patients (n = 27) showing enrichment of EGFRt⁺ cells in transduced CD4⁺ (left) and CD8⁺ (right) T cells between LCL stimulation (pre-LCL) and product release.

Supplementary Fig. 2: Immunophenotype of infused EGFRt⁺ CAR-T cells. A) Immunophenotype of CD4⁺/EGFRt⁺ CAR-T cells. B) Immunophenotype of CD8⁺/EGFRt⁺ CAR-T cells manufactured from bulk CD8⁺ T cells or CD8⁺ T_CM cells.

Supplementary Fig. 3: Resolution of bulky extramedullary disease after therapy with a low dose of CAR-T cells manufactured from a defined T cell subset composition. PET-CT scans before and after CAR-T cell therapy are shown from 2 patients with extramedullary B-ALL who achieved CR after lymphodepletion chemotherapy and infusion of CAR-T cells at DL 1 (2 x 10⁵ CAR-T cells/kg).

Supplementary Fig. 4: Immune responses directed against the CAR transgene products. A) Pre- and post-infusion PBMC were stimulated twice at weekly intervals with CD19 CAR-transduced autologous T cells. The capacity of the pre-infusion and post-infusion cultured to lyse CD19 CAR-transduced autologous T cells (black) and non-transduced autologous T cells (white) was evaluated by ⁵¹chromium release assay. One representative patient of 5 is shown. B-C) The post-infusion T cell line from a patient that exhibited specific lysis of autologous CAR-T cells was stimulated with pools of overlapping peptides from the CAR construct, and peptide pools that induced IFN-γ
secretion higher than that induced by T cells alone in an ELISpot assay were identified. Peptides 51, 55, 59 and 60 are located within the murine scFv. D) Peptides in the murine scFv that are predicted with a percentile rank < 5% (IEDB Analysis Resource, http://tools.immuneepitope.org/mhci) to bind HLA molecules expressed by the patient’s CAR-T cells are indicated. Lower percentile scores indicate stronger binding.

**Supplementary Table 1:**

Patients treated with CAR-T cells at DL 1 or 2 who received dexamethasone with or without tocilizumab for CRS and neurotoxicity.
SUPPLEMENTARY FIGURE 1

A

CliniMACS CD4+ selection

LEUKAPHESIS

CliniMACS CD4+/CD14+/CD45RA+ depletion

CD3/CD28 bead stimulation, transduction and expansion (15-20 days)

Formulation CD4+/EGFRt+ : CD8+/EGFRt+ = 1:1

CliniMACS CD62L+ selection

CD3/CD28 bead stimulation, transduction and expansion (15-20 days)

B

Leukapheresis

CD14

CD4

CD4+/CD14+/CD45RA-depleted

CD62L

CD4

CD45RA

CD62L

CD4/CD14/CD45RA-depleted and CD62L selected

CD3+

CD62L

C

SS

FS

CD3-

High SS

Low SS

CD13

CD14

CD15

CD16

CD19

CD123

D

Pre-LCL

Release

EGFRt

EGFRt

EGFRt

EGFRt

EGFRt

EGFRt

CD4

CD4

CD8

CD8

E

Percent EGFR+ in CD4+ T cells

Percent EGFR+ in CD8+ T cells

Pre-LCL

Release

Pre-LCL

Release
Patient 1:
Bilateral renal and bone infiltration

Patient 2:
Retroperitoneal, para-aortic, and right iliac lymphadenopathy and right scapular infiltration.
SUPPLEMENTARY FIGURE 4

A

Before CAR-T cell infusion

After infusion #1

day 28

After infusion #2

day 16

Effector:target ratio

- Autologous CAR-T cells
- Untransduced autologous T cells

B

Number of spots per 25,000 cells

Peptide pools

C

Peptide pools

D

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**SUPPLEMENTARY TABLE 1**

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*NCI CTCAEv4.03

¶¶ Died on day 122 in CR with persistent neurotoxicity