Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med 2018;378:449-59. DOI: 10.1056/NEJMoa1709919

Supplementary Appendix

Table of Contents

Supplementary Methods

CAR T Cell Manufacturing: Patient T cells were harvested, transduced, formulated, and released as previously described[1,2]. Gamma-retroviral 19-28z CAR gene transfer[2] was robust with a mean CAR transduction efficiency of 30%. The analysis of CD4:CD8 T cell ratios and T cell subsets of transduced T cells are shown in Fig. S7 in the Supplementary Appendix.

Assessment of 19-28z CAR T Cell Expansion: Presence of 19-28z CAR T cell was detected and quantified using a multiplex real time PCR assay as previously described[2]. Briefly, DNA was extracted from whole blood and 200ng was used in duplicate to amplify the vector and albumin gene. The results were converted to vector copy number (VCN) per mL based on white blood cell counts.

Figure S1

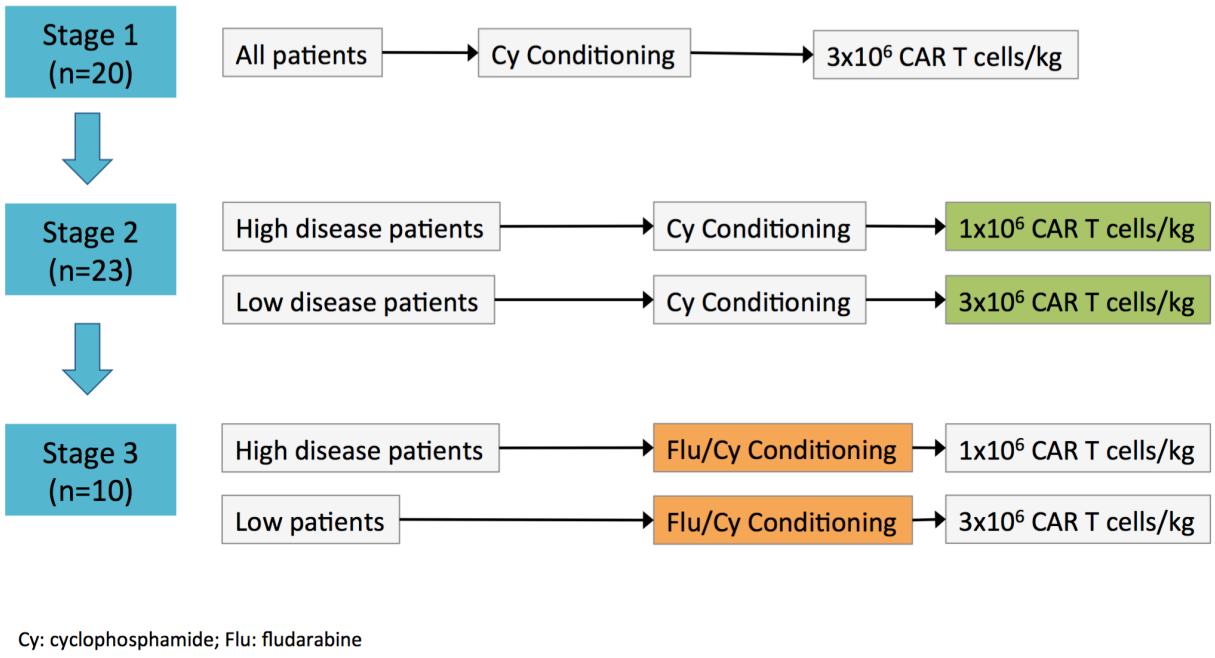
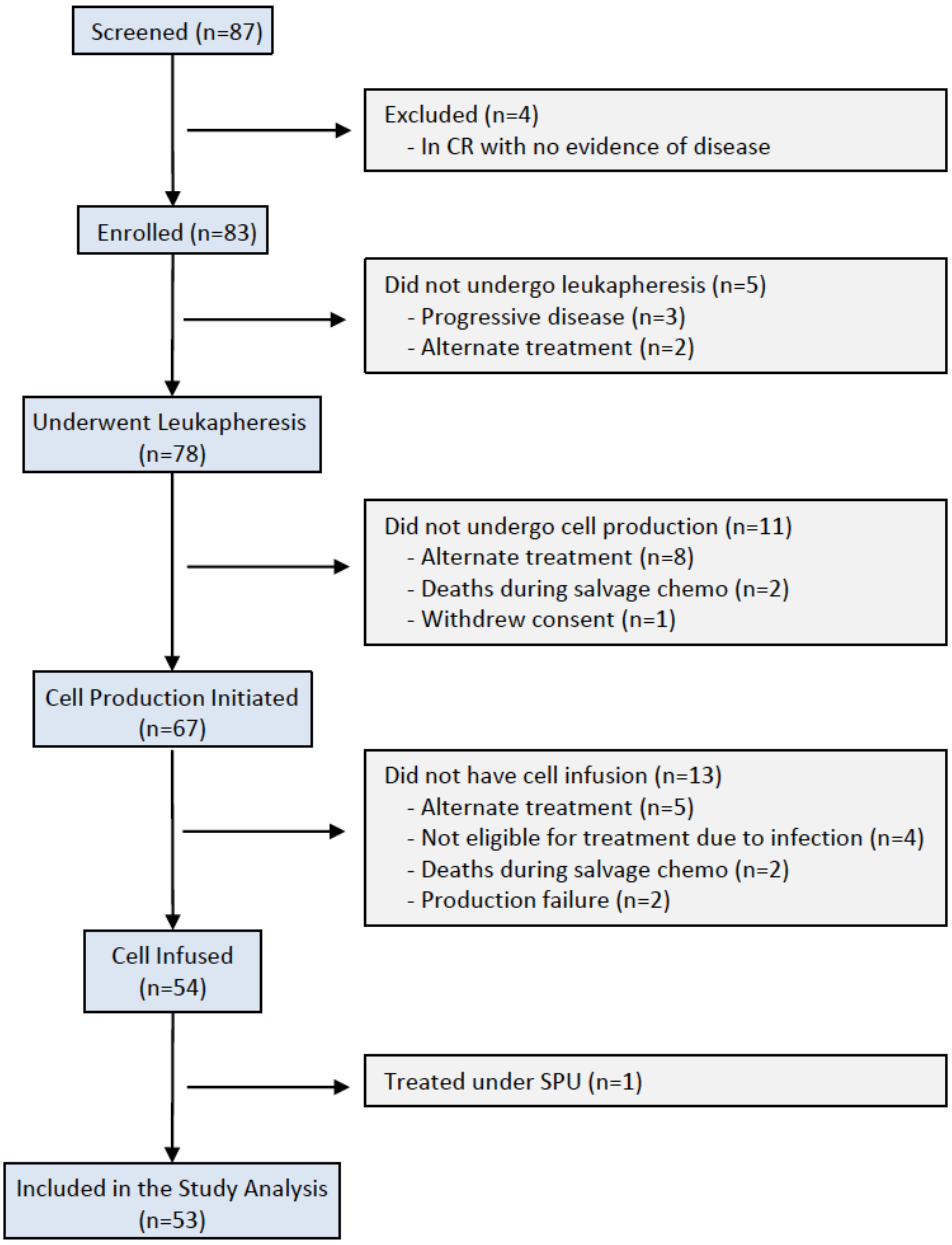


Figure S1. Study Design. The study included 3 stages. In Stage 1 of the study, all patients received cyclophosphamide (Cy) conditioning chemotherapy followed by $3x10^6$ 19-28z CAR T cells/kg. In Stage 2 of the study, patients were divided into two cohorts based on pretreatment disease burden. High disease patients included patients with ≥ 5% blasts in bone marrow (BM) or radiographically evident disease; low disease patients included patients with <5% BM blasts and with no evident extramedullary disease. High disease patients (n=12) received $1x10^6$ and low disease patients (n=11) received $3x10^6$ 19-28z CAR T cells/kg. In Stage 3, patients received Fludarabine + Cy chemotherapy while keeping the same two T-cell dosing scheme used in Stage 2.

Figure S2



SPU: Single patient use

Figure S2. Study Flow of All Participants. The diagram shows all study participant's course from the time of consent to treatment on study.

Figure S3

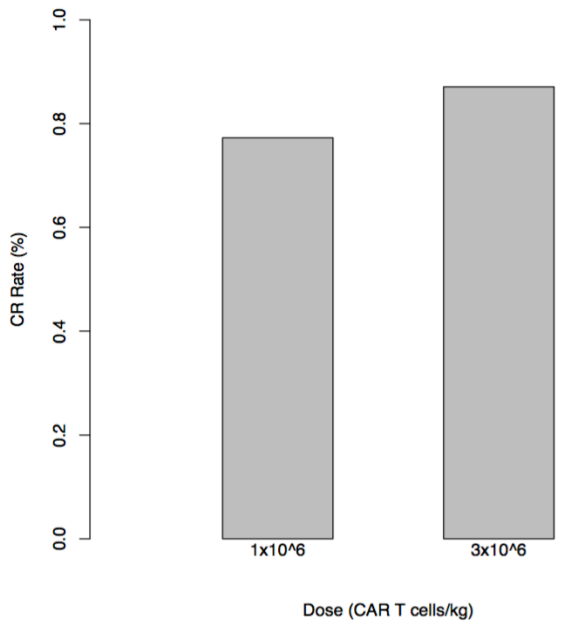


Figure S3. Impact of T Cell Dose on Response. Infused CAR T cell dose ($1x10^6$ vs. $3x10^6$ CAR T cells/kg) did not correlate with CR.

Figure S4

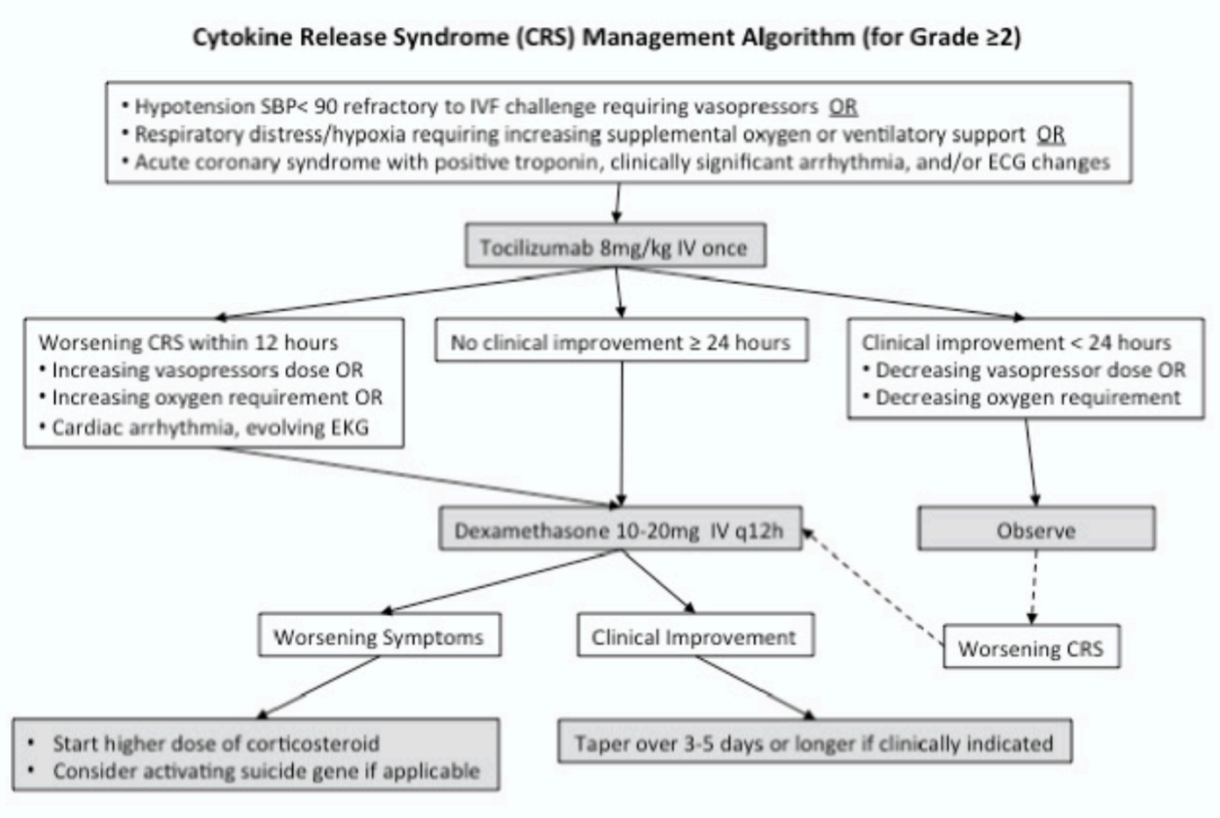


Figure S4. Cytokine Release Syndrome Management Algorithm at MSKCC. The diagram shows the updated management guideline of grade 2 or higher cytokine release syndrome in patients being treated with 19-28z CAR T cells at MSKCC.

Figure S5

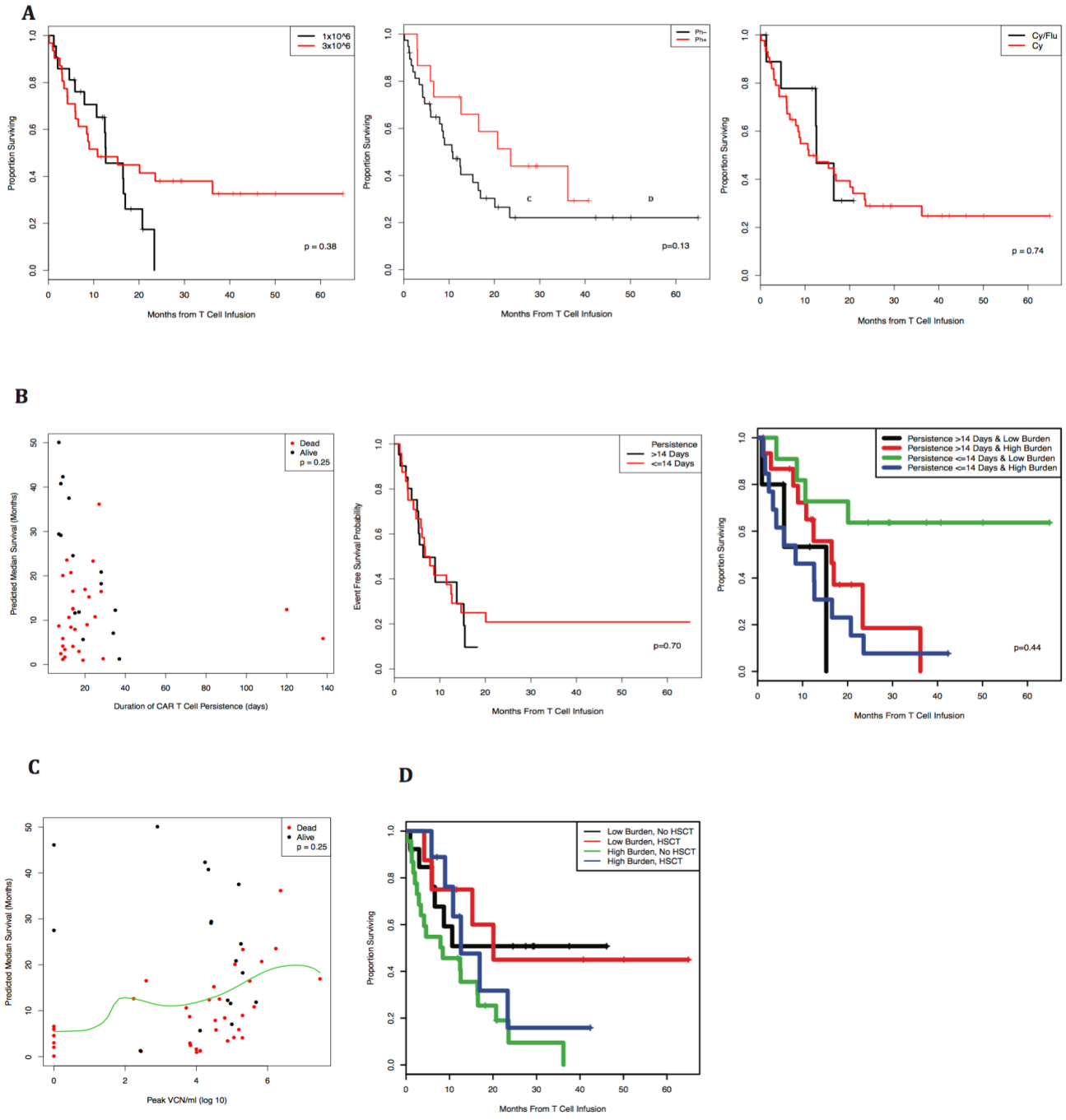


Figure S5. Impact of Clinical and Biological Variables on Long-Term Survival. A. CAR T cell dose ($1x10^6$ vs. $3x10^6$), Ph+ status and different conditioning chemotherapy (Flu vs. Flu/Cy) did not impact OS. **B.** Duration of CAR T cell persistence did not impact OS, when analyzed by all groups,

stratified by median duration of T cell persistence (14 days), and by pretreatment disease burden and persistence. **C.** Absolute magnitude of CAR T cell expansion did not impact OS. **D.** OS of four groups of patients who achieved initial response after CAR T cells, divided by disease burden and post-CAR T therapy allogeneic HSCT status.

Figure S6

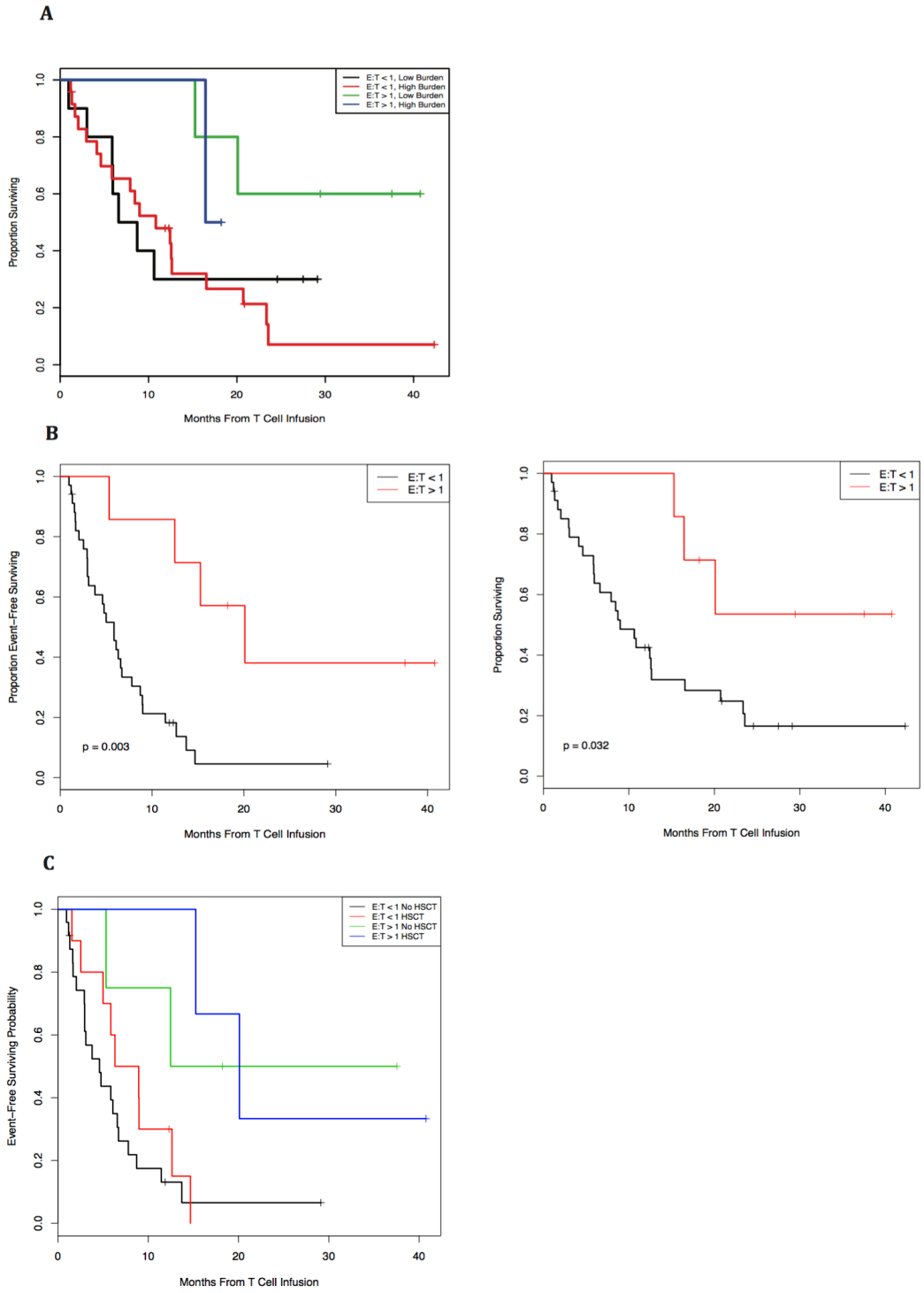


Figure S6. CAR T Cell Expansion in Proportion to Tumor Burden Significantly Correlated with Long-Term Survival After 19-28z CAR T Cells. A. A peak CAR T cell expansion to tumor burden

ratio better predicted OS compared to pretreatment disease burden. **B.** Median event-free survival, overall survival and 2-year overall survival of patients with a peak CAR T cell expansion to tumor burden ratio >1 were 20 months, not reached, and 54%, respectively vs. 6 months, 9 months and 17%, respectively, in patients with a ratio <1. **C.** Overall survival of patients according to the peak CAR T cell expansion to tumor burden ratio and post-CAR allogeneic status.

Figure S7

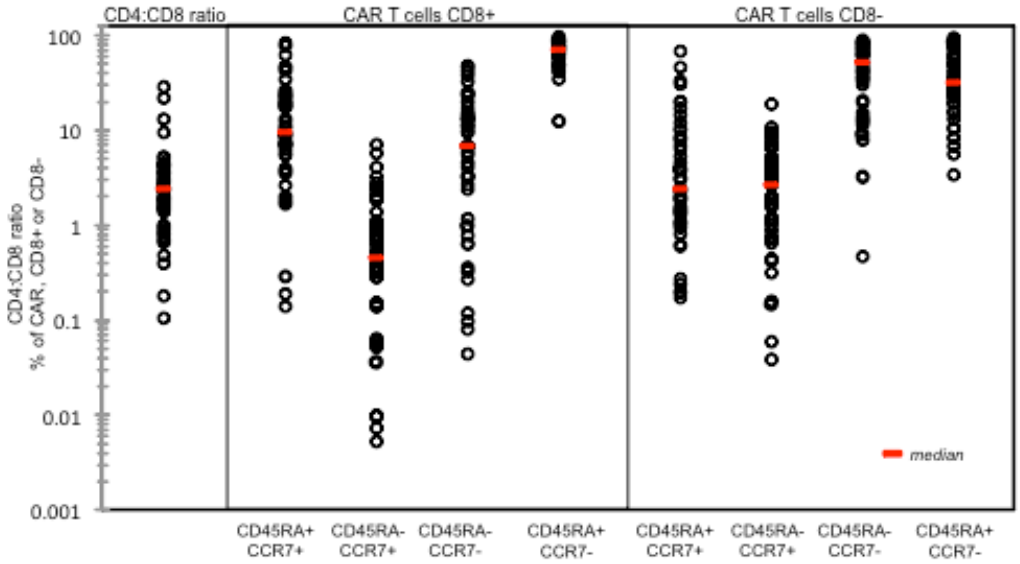


Figure S7. CD4:CD8 Ratio and T Cell Phenotypes of CAR T Cell Subsets. Analysis of CAR T cell subsets in 51 manufactured products included CD4:CD8 ratio, percent naïve (CD45RA+, CCR7+), central memory (CD45RA-, CCR7+), effector memory (CD45RA-, CCR7-) and effector (CD45RA+, CCR7-) subsets from both CD8+ and CD8- transduced T cell populations.

Table S1: Cytokine Release Syndrome Grades Definitions

Grade	Definitions
Grade 1	Mild symptoms, requiring observation or symptomatic management only (*e.g.* antipyretics, antiemetics, pain medications, etc.)
Grade 2	Moderate symptoms Hypotension requiring any vasopressors < 24 hours, or Hypoxia or dyspnea requiring supplemental oxygen <40% (up to 6L NC)
Grade 3	Severe symptoms Hypotension requiring any vasopressors ≥24 hours, or Hypoxia or dyspnea requiring supplemental oxygen ≥40%
Grade 4	Life-threatening symptoms Hypotension refractory to high-dose vasopressors* Hypoxia or dyspnea requiring mechanical ventilation
Grade 5	Death

*Refractory to vasopressors was defined as failure to reach target blood pressure despite the use of high-dose vasopressors ≥ 3 hours. Definitions of high dose vasopressors included: norepinephrine ≥20 μg/min, dopamine ≥10 μg/kg/min, phenylephrine ≥200 μg/min, or epinephrine ≥10 μg/min.

Reference:

1. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Science translational medicine 2013;5:177ra38.

2. Hollyman D, Stefanski J, Przybylowski M, et al. Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy. J Immunother 2009;32:169-80.