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Favorable Activity and Safety Profile of Memory-Enriched CD19-Targeted Chimeric Antigen Receptor T Cell Therapy in Adults with high-risk Relapsed/Refractory ALL

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Abstract

Purpose: A phase 1/2 study evaluating the safety and activity of memory-enriched CD19-directed chimeric antigen receptor (CD19-CAR) T cells in adults with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL).

Experimental Design: In phase 1, we tested sequentially two cell populations for CAR transduction: 1) central memory (Tcm) or 2) naïve, stem and central memory (Tn/mem) T cells. The study employed an activity constrained for toxicity design to determine the recommended phase 2 dose (RP2D), which was tested in phase 2.

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Results: The Tcm cohort was closed early due to lack of activity. The 200×10^6 Tn/mem-derived CD19-CAR T cell dose was found to be safe, active, and was declared the RP2D. At RP2D, 58 participants underwent leukapheresis and 46 received CD19-CAR T cells. Median age for treated participants was 38 years (22–72). Twenty-nine (63%) participants had relapsed post-allogeneic hematopoietic cell transplantation (alloHCT), 18 (39%) had Philadelphia-like genotype and 16 (35%) had extramedullary disease (EMD) at lymphodepletion. Three (7%) participants had grade 3 cytokine release syndrome (CRS), and none had grade ≥4 CRS. Eight (17%) participants had grade ≥3 neurotoxicity, including one fatal cerebral edema. Forty (87%) patients achieved complete remission (CR)/CR with incomplete hematologic recovery, 2 (4%) progressed, and 4 (9%) were unevaluable for response. Among 42 response-evaluable participants, 16/17 with Philadelphia-like ALL and 13/15 with EMD at LD responded. Twenty-one (53%) responders underwent alloHCT consolidation, which was associated with improved relapse-free survival (aHR=0.16, 95%CI:0.05–0.48; P=0.001).

Conclusion: Tn/mem-derived CD19-CAR T cells were safe and active, including in Philadelphia-like ALL and EMD.

Keywords

Acute lymphoblastic leukemia; ALL; CD19CAR; chimeric antigen receptor; extramedullary disease; Ph-like

INTRODUCTION

Treatment of patients with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) using CD19-targeted chimeric antigen receptor (CD19-CAR) T cells has yielded remarkable remission rates in both pediatric and adult patients (1–6). Until recently, tisagenlecleucel (CTL-019, Kymriah) was the only approved CD19-CAR T cell therapy for r/r B-cell ALL in children and young adults up to age 25 years (2). On October 1st, 2021, brexucabtagene (KTE-X19, Tecartus) was granted the FDA approval as the first CD19-CAR T cell therapy for adults with r/r B-cell ALL without an upper age restriction. This approval was supported by the encouraging results of the phase 1/2 ZUMA-3 trial that yielded a complete remission (CR)/CR with incomplete count recovery (CRi) rate of 71% in 55 adult patients treated with KTE-X19 (6,7). Despite enthusiasm for the approval of CAR T cell therapy for adults with ALL, this novel therapy conveys significant risks of toxicity, with rates of grade ≥3 cytokine release syndrome (CRS) and neurotoxicity of 23–31% and 25–50% in adults, respectively (1,6–8). Because older adults (≥50 years) are expected to endure severe toxicities related to CAR T cell therapy poorly, physicians may be reluctant to offer this promising therapeutic to this age group, despite the FDA approval. Indeed, most of the published CD19-CAR T cell experience in ALL involves children and relatively younger adult patients (2,3,5,9), with few reports addressing CAR T cell activity and toxicity in middle aged and older adult patients (1,4,6).

Cases of r/r ALL often involve extramedullary disease (EMD) including sanctuary sites, such as the central nervous systems (CNS) (10,11). Unfortunately, ALL that has relapsed with EMD generally responds poorly to both conventional chemotherapy and novel drugs including the CD3/CD19 bispecific T-cell engager, blinatumomab (12,13). Therefore,

relapsed ALL with EMD remains an area of unmet need. Early observations suggest that CD19-CAR T cell therapy is a promising treatment for advanced B-cell ALL with EMD involvement (1,4,14–16). However, this experience remains limited to few reports as CAR T cell studies in ALL historically have excluded patients with isolated EMD without marrow involvement. Another area of unmet need in ALL is the Philadelphia (Ph)-like genotype, a subtype of B-cell ALL that confers resistance to standard chemotherapy and is associated with inferior survival outcomes (17,18). Outcomes of novel therapeutics in patients with r/r Ph-like ALL are generally lacking, including the efficacy of CD19-CAR T cell therapy in this context (19).

Therapeutic efficacy and treatment-related toxicity may be influenced by attributes of the CD19-targeted CAR construct (e.g., costimulatory domain) as well as the T cell manufacturing platform used to generate the CAR T cell product. Different CD19-CAR T cell platforms may use unselected peripheral blood mononuclear cells (PBMC) or may select distinct T cell populations for transduction and/or infusion, with the primary aim of maximizing the activity of the modified T cells by enhancing expansion and/or persistence and reducing T cells exhaustion. Preclinical studies suggest that using a more uniform T cell product with a less-differentiated T cell phenotype improves antitumor activity (20). Central memory T cells (Tcm) are of a particular interest for transduction since they have the potential of stemness with self-renewal capacity and multipotency, and therefore, the potential for a longer persistence relative to unselected PBMCs (21–23). We have assessed Tcm-derived CD19-CAR T cells in B-cell non-Hodgkin's lymphoma following autologous hematopoietic stem cell transplantation in a series of phase 1 clinical trials and demonstrated safety and feasibility of this platform (20). However, early data from these trials, which suggested that Tcm-derived CD19-CAR T cells had limited expansion and efficacy, in combination with additional preclinical studies from our laboratory, led us to modify our manufacturing platform to include naïve and stem cell memory T cells (Tn/mem) in the starting population for transduction (24).

Here we report the results of a phase 1/2 clinical trial (NCT02146924) in adult patients with r/r B-ALL investigating a memory-enriched Tcm and Tn/mem cell starting population engineered to express a CD19-specific, CD28-costimulatory CAR (CD19:28z-CAR).

PATIENTS AND METHODS

Clinical protocol design

This is a prospective single-center, open-label, phase 1/2 dose finding study approved by the City of Hope Institutional Review Board (IRB #13447) under BB-IND 15918 and registered on ClinicalTrials.gov as NCT02146924. The trial followed the treatment schema depicted in Figure 1A. Patients or the allogeneic donors of previous recipients of allogeneic hematopoietic cell transplantation (alloHCT) underwent leukapheresis for isolation of T cells for *ex vivo* selection and lentiviral transduction. Salvage chemotherapy was allowed per the treating physician discretion. Patients received lymphodepletion (LD) with 500 mg/m^2 cyclophosphamide and 30 mg/m^2 fludarabine on Days −5, −4 and −3 prior to CD19-CAR T cell infusion. Only one infusion of CAR T cells was planned for each individual participant in the study.

This trial tested CAR T cells derived from either isolated Tcm (i.e., central memory only) or Tn/mem (i.e., central memory, naïve and stem cell memory) cell populations; the manufacturing platform is depicted in Supplemental Figure 1, with differences between Arm A (Tcm) and Arm B (Tn/mem) highlighted. Tcm or Tn/mem cells were transduced with a lentiviral vector [CD19R(EQ)28ζ-T2a-EGFRt_epHIV7] encoding a CD19-CAR that included a CD28 costimulatory domain, as well as a truncated epidermal growth factor receptor (EGFRt) for T cell tracking. The primary objectives for the trial were 1) to examine the safety and activity of CD19-CAR T cellular immunotherapy and 2) to determine the recommended phase 2 dose (RP2D). The primary endpoints were 1) safety as determined by dose-limiting toxicity (DLT) and the full toxicity profile, and 2) activity, defined as achievement of complete response [CR or CRi] post CAR T cells infusion.

The study used an activity constrained for toxicity (ACT) design, considered an extension of the toxicity equivalence range (TEQR) design of Blanchard and Longmate (25), which defines the dose escalation and de-escalation rules for determining the maximum tolerated dose (MTD) based on a target range of acceptable toxicity. Briefly, the ACT design dose escalates for lack of activity (defined as rate of CR and CRi), while dose de-escalating for toxicity. The lowest acceptable level of activity was 66%, so the equivalence range was set to 66%−100% with a target response level of 100%. The toxicity constraint level was set at 51% for a dose level, so that in a cohort of 3 participants, we de-escalated if ≥2 of 3 participants experienced toxicity meeting the DLT definition. Dose escalation ended when 12 participants were studied at one dose. The RP2D was the dose closest to the target toxicity of 0.25 and below 0.51 based on isotonic regression. Relapse-free survival (RFS) was defined as the time from CAR T cell infusion to relapse or death from any cause, whichever was observed first. Overall survival (OS) was defined as the time from CAR T cell infusion to death due to any cause. Participants who received disease-directed treatment(s) such as ponatinib, another CAR T cell infusion or a second allogeneic HCT post-relapse during follow-up (treatments that were deemed to impact relapse and/or survival risk), were censored at the disease-directed treatment start date.

CAR T cell Dose Levels.—The trial included three possible dose levels (DL) of 10×10^6 (de-escalation dose −1), 50×10^6 (DL 1; starting dose for Arm A) and 200×10^6 (DL 2; starting dose for Arm B) CAR+ T cells in a single infusion.

Toxicity and disease assessment.—Toxicity was assessed using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE v4.03). Neurotoxicity was graded by the CTCAE as the study was initiated before the introduction of immune effector cell associated neurotoxicity syndrome (ICANS) grading. We used Lee et al. criteria to define CRS grade (26). Patients were followed for possible DLTs during the 28 days following T cell infusion. DLT was defined as: 1) any grade 3 or higher toxicity with an attribution of definitely or probably related to the infusion of the T cells (excepting expected infusion-related reactions, CRS lasting less than 72 hours, and grade 3 neurotoxicity lasts ≤7 days); 2) any grade 3 or higher autoimmune toxicity; and 3) grade 5 toxicity with an attribution of possibly, probably or definitely related to the infusion of the Tcm or Tn/mem cells. Response was assessed by the NCCN guidelines definition

for CR and CRi for ALL (27). For extramedullary sites, response was assessed per the response criteria for extramedullary and CNS disease in the revised International Working Group Criteria for malignant lymphoma (28). PET/CT scan was performed as part of response assessment in patients with EMD. Minimal residual disease (MRD) was assessed by multicolor flow cytometry in Ph-negative ALL and by quantitative PCR for *BCR/ABL1* for Ph+ ALL. MRD negativity was defined as <1 leukemic cells per 10,000 viable cells (<0.01%).

Patient eligibility: Eligible patients were at least 18 years old with a diagnosis of r/r CD19+ B-cell ALL or lymphoblastic lymphoma (LL) following previous therapy at the time of enrollment. Patients with EMD irrespective of bone marrow (BM) involvement and those with CNS-2 or asymptomatic CNS-3 were eligible. Patients who had previously undergone alloHCT were eligible as long as documented to have ≤ Grade 2 graft versus host disease (GVHD) and were tapered off of all immunosuppressants prior to LD. Patients with prior allogeneic donors were allowed to use T cells manufactured from donor PBMC. All patients enrolled and treated on this trial gave written informed consent before participation; trials were conducted in accordance with the Declaration of Helsinki.

Generation of Tcm and Tn/mem-derived CD19-CAR T cells

Patient or donor PBMC were depleted of CD14+ monocytes and CD25+ Tregs, followed by positive selection of CD62L+ T cells. For Arm A, Tcm-derived CAR T cells were purified, transduced, formulated, and released as previously described (20). Differences between Arms A and B are described in Supplemental Figure 1, with text in red applying only to Arm A. For Arm B, we modified the published manufacturing procedure by omitting CD45RA depletion to include both naïve and stem/memory T cells, in addition to central memory T cells in the starting population for lentiviral transduction.

Sample processing and storage for correlatives

Patient samples (peripheral blood, BM and CSF) were processed and stored as described previously (20).

Statistical Analysis

OS and RFS were estimated using the Kaplan-Meier method. Cox proportional hazard models with hypothesis-driven variable selection were used to evaluate the association between potential risk factors and RFS. P values were two-sided with a significance level of 0.05. Landmark survival analyses were performed with a landmark time of day 28 post CAR T cell infusion, when disease response was assessed. All data were analyzed, and all graphs were plotted using R version 4.1.1 (R Foundation, Vienna Austria; https://www.r-project.org).

Data Availability Statement

The data generated in this study are available upon request from the corresponding author.

RESULTS

Phase 1 study using Tcm (Arm A) and Tn/mem (Arm B) products

Four patients were treated with Tcm-derived CD19-CAR T cells (Arm A); 2 patients received 50×10^6 CAR+ cells (DL 1) and 2 patients received 200×10^6 CAR+ cells (DL 2). The median manufacturing time for Tcm products was 19.5 (range: 15–22) days. The median age was 27 (range, 23–31) years. There was one DLT in a participant who received cells at DL 2 who developed prolonged grade 3 CRS. No response was observed, and all patients developed progressive disease. Correlative studies on peripheral blood collected following CAR T cell infusion illustrated poor expansion [median peak of 0.20% CAR+ cells (range, 0.1%−4.6%) in CD3+ population] and persistence of Tcm-derived CAR T cells (Supplemental Figure 2). Therefore, this cohort was closed, and we enrolled additional patients on the Tn/mem platform (Arm B) that used a product modified to include naive and stem/memory T cells for transduction by omitting the CD45RA depletion step. Fifteen patients received Tn/mem-derived products at DL 2 (200×10^6). No DLTs attributed to CAR T cells were observed in this cohort. Two patients were unevaluable for response (T cells below allowable dose, n=1; CD19-negative EMD progression post LD, n=1). All 13 response-evaluable patients achieved CR/CRi. Therefore, DL 2 (200×10^6) was declared the RP2D, and we opened a phase 2 expansion cohort. Patient characteristics, correlatives and outcomes for the full Tn/mem cohort are described in the section below, which combines all patients treated on phase 1/2 with Tn/mem derived products at the RP2D.

Phase 1/2 Patient Characteristics for Tn/mem CAR T cells (Arm B)

We enrolled a total of 58 patients on the Tn/mem 200×10^6 dose phase 1 (15 patients described above) and 2 (expansion) cohorts (Figure 1B and Table 1) who underwent T cell collection, including 12 allogeneic donor collections. We successfully manufactured products for 56 (97%) patients. One patient received cells on an emergency use protocol and 5 patients progressed or developed infectious complications before LD. Fifty patients received LD, of whom 4 did not receive CAR T cells due to CD19-negative disease (n=1) or infectious complications (n=3), and 46 of whom received CAR T cells. For the 46 treated patients, the median manufacturing time and time between apheresis and infusion were 12 (range: 11–18) days and 47 (range: 29–182) days, respectively. The median CAR T cell viability was 89.5% (range: 67.5–95.8%). The immunophenotype of the final CAR T cell products were similar to freshly isolated Tn/mem cells, with the exception that CD8+ cells were enriched in CAR T cell products (Supplemental Figure 3).

Patient characteristics for those who received CAR T cells at RP2D (n=46) are in Table 1. Briefly, the median age was 38 years (range, 22–72), with 15 (33%) patients ≥ 50 years. Twenty-eight (61%) patients were male and 24 (52%) were of Hispanic ethnicity. The median number of prior lines of therapy was 3 (range, 1–9); 29 (63%) patients had prior alloHCT, and 29 (63%) and 15 (33%) patients failed blinatumomab and inotuzumab before enrollment, respectively. Sixteen (35%) patients had poor-risk cytogenetics (29), including 7 (15%) with Philadelphia-chromosome positive (Ph+) disease and 4 (9%) with *KMT2A* (*MLL*) rearrangement. Eighteen of 39 (46%) patients who were evaluated had Ph-like genotype signature detected by RNA sequencing, microarray, and/or FISH studies.

Thirty-nine (85%) patients received bridging therapy after T cell collection, including 21 (46%) patients who received high-intensity therapies, such as hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) or fludarabine, cytarabine and filgrastim (FLAG)-based regimens. EMD was present in 24 (52%) and 16 (35%) patients at enrollment and LD, with 9 and 4 patients having disease involving the CNS, respectively. At LD, EMD-CNS only was observed in 3 patients, non-CNS in 12 patients, and combined CNS and non-CNS in 1 patient (Supplemental Table 1). Fifteen (33%) patients had a history of CNS involvement during their ALL course before CAR T cell infusion. At the time of LD, 37 (80.4%) participants had BM blasts ≥ 5% and/or active EMD, 8 (17.4%) had MRD, and 1 (2.2%) was in MRD-negative CR.

Toxicity (Phase 1 and 2 patients treated with the Tn/mem CAR T cells)

Two (4%) patients experienced DLTs; one patient developed prolonged grade 3 CRS and one patient developed fatal grade 5 cerebral edema. Grade 3 CRS was observed in 3 (7%) patients, and no patients experienced ≥ grade 4 CRS. The median onset of CRS was 4 days (range, 0–11) after T cell infusion, and the median duration of CRS was 4 days (range, 1–14). CRS per the ASTCT consensus grading (30) was classified retrospectively as grade 1 in 19% (n=9), grade 2 in 63% (n=29), grade 3 in 7% (n=3) and grade 4 in 4% (n=2) of patients. Eight (17%) patients experienced grade ≥3 neurotoxicity (grade 3, n= 6; grade 4, n=1; grade 5, n= 1) at least possibly attributed to CAR T cells (Table 2). The median onset to maximum grade neurotoxicity was 7 days (range, 1–35) post infusion and the median duration of neurotoxicity was 1 day (range, 1–12). The patient who developed cerebral edema (grade 5 neurotoxicity) had bacterial meningitis just before LD which was adequately treated with antibiotics -including removal of a ventriculoperitoneal shunt. The post-mortem autopsy revealed evidence of parenchymal involvement by ALL. For the 15 older patients (≥ 50 years), 2 (13%) and 3 (20%) developed grade ≥3 CRS and neurotoxicity at least possibly attributed to CAR T infusion, respectively. Tocilizumab and dexamethasone were administered for CRS with or without neurotoxicity in 36 (78%) and 27 (59%) patients, respectively. The majority of tocilizumab doses were administered for prolonged grade 1 CRS and were given concurrently with a single or few doses of dexamethasone to prevent rebound neurotoxicity and CRS grade progression (Supplemental Figure 4). Twenty (43%) patients had grade ≥3 prolonged cytopenia at 6 weeks post CAR T cell infusion regardless of attribution, including 14 (30%) and 12 (26%) with prolonged grade ≥3 neutropenia and thrombocytopenia, respectively, of whom 69% and 57% eventually recovered to grade ≤2, while the rest either relapsed, underwent transplant or died before count recovery. The median time from CAR T cell infusion to grade ≤2 count recovery was 63 (range; 45–100) and 91 (range: 57–244) days for neutrophils and platelets, respectively. Of the 40 participants who survived ≥42 days post infusion, those with prolonged cytopenia that lasted beyond day 42 tended to be younger (median: 31 vs. 44 years), received more lines of prior therapies (median: 4 vs. 2), had a prior alloHCT (75% vs. 55%) and lower BM blasts at the time of LD (median: 1% vs. 18%), compared to participants without prolonged cytopenia. Table 2 depicts grade ≥3 adverse events at least possibly attributed to CAR T cell therapy.

Response

Forty of 46 patients (87%) achieved CR/CRi (Figure 1B), 2 (4%) patients progressed, and 4 (9%) patients were unevaluable for response (infection, n=2; cerebral edema, n=1; received T cells below allowable dose, n=1). When analysis is restricted to patients evaluable for response, the best CR/CRi rate was 95% (40/42). Of the 39 evaluable responders who had MRD assessment post infusion, 95% (37/39) had MRD-negative remission. Of the 17 response-evaluable patients with Ph-like ALL, 16 responded with corresponding CR/CRi rate of 94%. Of the 15 evaluable patients with EMD at the time of LD, 2 patients failed to respond (one had Ph-like ALL with CNS involvement with concurrent BM involvement; one had 11q23/*KMT2A* rearrangement, multiple skin lesions, marrow involvement and early CD19- disease progression) with corresponding CR/CRi rate of 87%. Three of 4 patients with CNS involvement at LD responded. All response-evaluable older patients (≥50 years) (n=12) achieved CR/CRi. Twenty-one (53%) responders proceeded directly to alloHCT consolidation post CAR T cell therapy while in remission (Figure 1B), including 10 patients who received their second alloHCT. Notably, 6/13 patients with EMD at LD who achieved CR/CRi after infusion underwent alloHCT consolidation. The median time to transplant was 79 days (range, 50–192) from the time of CAR T cell infusion.

Survival outcomes

With a median follow up of 9.9 months (range, 0.3–65.8 months), the median OS for the 46 patients who received CAR T cells was not reached. For the 40 responders, with a median follow up of 11.4 months (range, 3.3–65.8) post CAR T-cell infusion, the median OS was not reached, and the median RFS was 17.1 months (95%CI: 6.9 to NA). The 12-month OS and RFS were 63.2% (95%CI: 44.9–76.9; Figure 2A) and 52.6% (95%CI:35.5–67.1; Figure 2B), respectively. A pre-specified Cox proportional hazards regression model was fitted to evaluate the adjusted association between RFS and time varying status for alloHCT consolidation (yes vs. no), Ph-like status (yes vs. no/unknown), BM blasts at LD (<5% vs. ≥5%) and presence of EMD at LD (yes vs. no) among the responders (Table 3). Consolidation with alloHCT post CAR T cells was associated with superior RFS [adjusted hazard ratio (aHR)=0.16; 95% CI: 0.05–0.48; *P*= 0.001] while harboring Ph-like genotype was associated with a trend toward inferior RFS [aHR=2.26; 95% CI: 0.86–5.94; P= 0.097], compared to the absence of consolidative alloHCT and Ph-like genotype, respectively. Figure 2C and Figure 2D show OS and RFS curves according to alloHCT consolidation in responders, respectively. In the exploratory univariate analysis, age, sex, failing prior alloHCT, source of T cells, bridging therapy intensity, and prior exposure to blinatumomab and inotuzumab were not associated with RFS among responders. Supplemental Table 2 illustrates univariate analysis for all factors.

Overall, 13/40 (33%) responders relapsed with a median time to relapse of 3.9 months (range, 2.1–17.1). Ten (77%) relapses were CD19+, while 2 (15%) patients developed CD19-negative relapse, and 1 (8%) had unknown CD19 status. There were 9 relapses isolated to the BM, 2 relapses as isolated EMD including one in the CNS, and 2 patients had combined EMD (one in the CNS) and BM relapse. Among the 19 participants who responded and received CAR T cell infusion without alloHCT consolidation, 11 relapsed with a median time of 3.4 months (range, 2.1–10.3) post CAR T-cell infusion, 3 patients

died in remission, and 5 patients remained alive and in remission at last contact or date of censoring with a median follow up of 9.7 months (range, 3.3–51.8) including 1 patient who was censored at day 99 post infusion due to initiating ponatinib maintenance. For the 21 patients who underwent alloHCT consolidation in remission after CAR T cells, 1 patient died at 2.5 months post-transplant due to infection, 2 patients relapsed (6.9- and 12.4-months post-transplant) of whom 1 died after relapse and 1 received second CAR T-cell infusion thus was censored at the date of second infusion for OS, 2 patients died in remission, and 16 patients remained alive and in remission. The 100-day non-relapse mortality rate post-transplant was 5% (n= 1). Causes of death for participants treated at the RP2D are shown in Supplemental Table 3.

CAR T cell *in vivo* expansion and persistence

Patient peripheral blood samples were evaluated for presence of CD19-CAR T cells by flow cytometry using cetuximab, which recognizes EGFRt in the lentiviral construct. CAR T cells expanded *in vivo*, and the expansion peaked at day 7–14, and with a median peak of 24.0% CAR+ cells (range 0.8%−77.0%) in CD3+ population (n=41) (Figure 3A). The median expansion for Tn/mem cohort was 120-fold higher compared with Tcm CAR cohort. Of the 24 patients with available samples, CAR T cells were detected in the CSF in all 24 (100%) patients, with a median of 23.3% (range 6.2%−75.4%) CAR+ T cells per CD3+ cells on day 28 post infusion. The median %CAR T cells in the CSF among patients who experienced 0–1 grade neurotoxicity and ≥2 grade neurotoxicity were 22.8% (range, 6.2–54.9%) and 25.2% (range, 15.2–75.4%), respectively (Supplemental Figure 5B).

We utilized a quantitative PCR assay to detect the WPRE transgene within the lentiviral vector to determine transgene copy number and enable quantitative tracking of CD19-CAR T cells in the blood (Figure 3B). Expansion of CAR T cells by WPRE was predominantly observed in the first 28 days post infusion. No patterns of peak WPRE by neurotoxicity (grade 0–1 vs. ≥2), CRS (grade 0–1 vs. ≥2), or disease burden at LD (defined as ≥5% marrow blasts +/− EMD) were observed (Figure 3C-E). Cytokine levels were measured at prespecified timepoints in the first four weeks post CAR T cell infusion. Figure 3F illustrates the median fold change from baseline (pre-LD) levels for each individual cytokine.

DISCUSSION

Unique to our CAR T cell study is the memory-enriched manufacturing platform, which uses a naïve/memory T cell-enriched T cell product that is lentivirally transduced to express CD19:28z-CAR. The selected T cell population for CAR transduction includes central memory, stem cell memory and naïve T cell populations. This Tn/mem manufacturing platform is the same as our Tcm-derived platform,(20) with the exception that CD45RA depletion was omitted (Supplemental Figure 1). While preclinical data suggested potential persistence advantage for Tcm transduced cells,(21–23) we observed disappointing activity, expansion and persistence in the first two dose levels on this trial using the Tcm platform (Supplemental Figure 2), potentially due to the long manufacturing time and selection process that yielded fewer cells for CAR transduction, and ultimately a high fold expansion

to achieve therapeutic cell numbers. Because fold expansion can negatively affect CAR T cell potency, we believe that the difference in manufacturing time/fold expansion between the Tcm and Tn/mem arms likely contributed to differences we observed in clinical activity. (31–35) Therefore, based on data from early laboratory and clinical studies perform at our institution (24), we modified our manufacturing platform to select Tn/mem cells for CAR T cell production, with which we have established promising antileukemic activity.

We successfully selected Tn/mem cells for transduction and manufactured CAR T cells at the targeted dose for 97% of enrolled patients. We showed feasibility and comparable efficacy for CAR T cell products manufactured from donor cells compared to autologous products. The percentage of patients enrolled on our study who did not receive CAR T cell infusion was 21% (12/58 patients), which is unsatisfactorily high, yet consistent with prior experience of autologous CAR T cell therapy in r/r ALL (1,2,6). The innovation of rapid CAR T cell production as well as recent advancements in the arena of allogenic off-the-shelf cellular therapy may overcome this limitation and allow for the broader utilization of CAR T cell therapy for ALL, especially in patients with proliferative disease (36,37).

We showed that Tn/mem CD19-CAR T cell therapy administered at the RP2D was safe in adults with r/r ALL, with low rates of grade 3 CRS (Table 2) and no grade ≥4 CRS. The rate of grade ≥3 neurotoxicity was also lower compared to previously published experiences in adults with ALL (1,6–8), however, definition of CAR related toxicities varied from one study to another. Prolonged cytopenias have been observed after CAR T cell therapies, however, the exact pathogenesis remains poorly understood and definitions have varied by study (38–40). Here, we observed severe prolonged cytopenia in 43% of patients by day 42, but eventually toxicities were downgraded in the majority of patients over time. This favorable safety profile was observed despite the adult patient population, 33% of whom were ≥50 years old. This favorable safety profile may be the result of our unique CAR construct and/or T cell selection process or may also be the consequence of our management approach for toxicity that included the early administration of a single dose of dexamethasone along with tocilizumab for grade 2 CRS and prolonged grade 1 CRS (Supplemental Figure 4). Nonetheless, one participant in our cohort experienced fatal cerebral edema on day 6 post infusion, a recognized albeit rare toxicity of CD19-CAR T cell therapy (41).

Treatment with Tn/mem-derived CD19-CAR T cells infused at the RP2D led to excellent remission rate in heavily pretreated adults with r/r B-ALL. Moreover, over half of responders were successfully transitioned to a curative consolidation with alloHCT. Notably, high response rates were observed across various high-risk ALL features including patients failing prior alloHCT, blinatumomab and inotuzumab.

ALL relapsed at extramedullary sites is encountered frequently at the time of treatment failure and is a difficult to treat scenario. Disappointingly, the presence of EMD confers inferior response to blinatumomab, and is overly represented at the time of blinatumomab failure.(12,13) Our study included a large proportion of patients with EMD relapse at the time of enrollment and LD (Table 1). Most patients with EMD responded to CAR T cell therapy, and half of them were transitioned to a curative alloHCT in remission. The activity

of Tn/mem-derived CD19-CAR T cell therapy in patients with EMD relapse is consistent with previous experience with different CAR constructs in the setting of EMD.(4,42) We further demonstrated that CAR T cells traffic efficiently to the CSF, as we identified CAR T cells in the CSF in all evaluable patients on day 28 post infusion (Supplemental Figure 5). Our observation of CSF trafficking, also observed by others (3,5,8,14,43), emphasizes that CAR T cells represent a potential therapy for ALL patients who suffer CNS relapse. Furthermore, these findings and others underscore the potential role of CAR T cell therapy in ALL with EMD as the preferred salvage therapy, considering the limitations of other salvage therapeutics.

Ph-like ALL has emerged as difficult-to-treat subset of B-cell ALL and is associated with poor outcomes with traditional therapies (17,18,44). Our patient population was enriched for Ph-like ALL, likely due to the high proportion (52%) of Hispanic patients, a demographic that most often develops Ph-like ALL (18,45). We illustrated that the excellent response to CAR T cell therapy extended to Ph-like ALL genotype, with 16/17 (94%) evaluable patients with Ph-like ALL responding, a finding that is imperative and warrants a larger confirmatory study. If confirmed, these results suggest that patients with Ph-like ALL could benefit from the introduction of CAR T cell therapy as an early salvage therapy, as well as provide impetus to investigate CAR T cell therapy as an alternative consolidation approach in this high-risk disease.

Consistent with several other experiences in adults with r/r B-cell ALL (14,43,46,47), we showed that consolidation with alloHCT was necessary to attain durable remission post response to CD19-CAR T cell therapy. In our study, 15/19 (79%) patients who did not undergo alloHCT consolidation in remission eventually died, relapsed or were censored due to starting a new therapy within 1-year post infusion. In contrast, we observed outstanding RFS in responders who underwent alloHCT consolidation, despite the large proportion of patients with advanced disease and/or who had failed prior transplant. Nonetheless, the question of CAR T cells as a single-standing treatment versus a bridging therapy toward transplant in adults with r/r B-ALL remains arguable, and the decision is influenced by CAR construct and projected persistence, disease setting (i.e., early versus advanced), disease burden and whether or not the patient is a candidate for transplant.

In conclusion, our Tn/mem CD19-CAR T cell therapy at RP2D has shown a promising safety profile in adults with r/r ALL, with low rates of grade 3 CRS and no grade ≥4 CRS. Treatment led to notable responses, including in patients with Ph-like ALL and EMD, and a high rate of alloHCT consolidation in highly refractory patients that led to durable remission. Given these encouraging results, we are developing a clinical study exploring the safety and activity of Tn/mem CD19CAR T cells as a consolidation approach for older adults with ALL in CR1. Furthermore, we are extending the Tn/mem-enriched CAR T cell platform to other CAR targets in r/r ALL as well as to a variety of malignancies with unique targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF TRANSLATIONAL RELEVANCE

Memory-enriched CD19-directed chimeric antigen receptor (CD19-CAR) T cell therapy demonstrated favorable safety profile and yielded an excellent response rate in adults with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (ALL). Our study cohort included high-risk patients with r/r ALL who had limited available salvage options, such as patients who had failed prior novel therapies and allogeneic hematopoietic cell transplant (alloHCT), older adults, ALL with Philadelphia-like genotype and patients with extramedullary relapse. The incidence of severe cytokine release syndrome (CRS) and neurotoxicity were relatively low following therapy with CD19-CAR T cells, with no grade ≥4 CRS. Long-term outcomes for patients who responded to CD19-CAR T cells and underwent subsequent consolidation with alloHCT were outstanding. The intriguing safety and response data from this study support further development of this promising therapy in high-risk adults with ALL in early stages of the disease to improve long term outcomes.

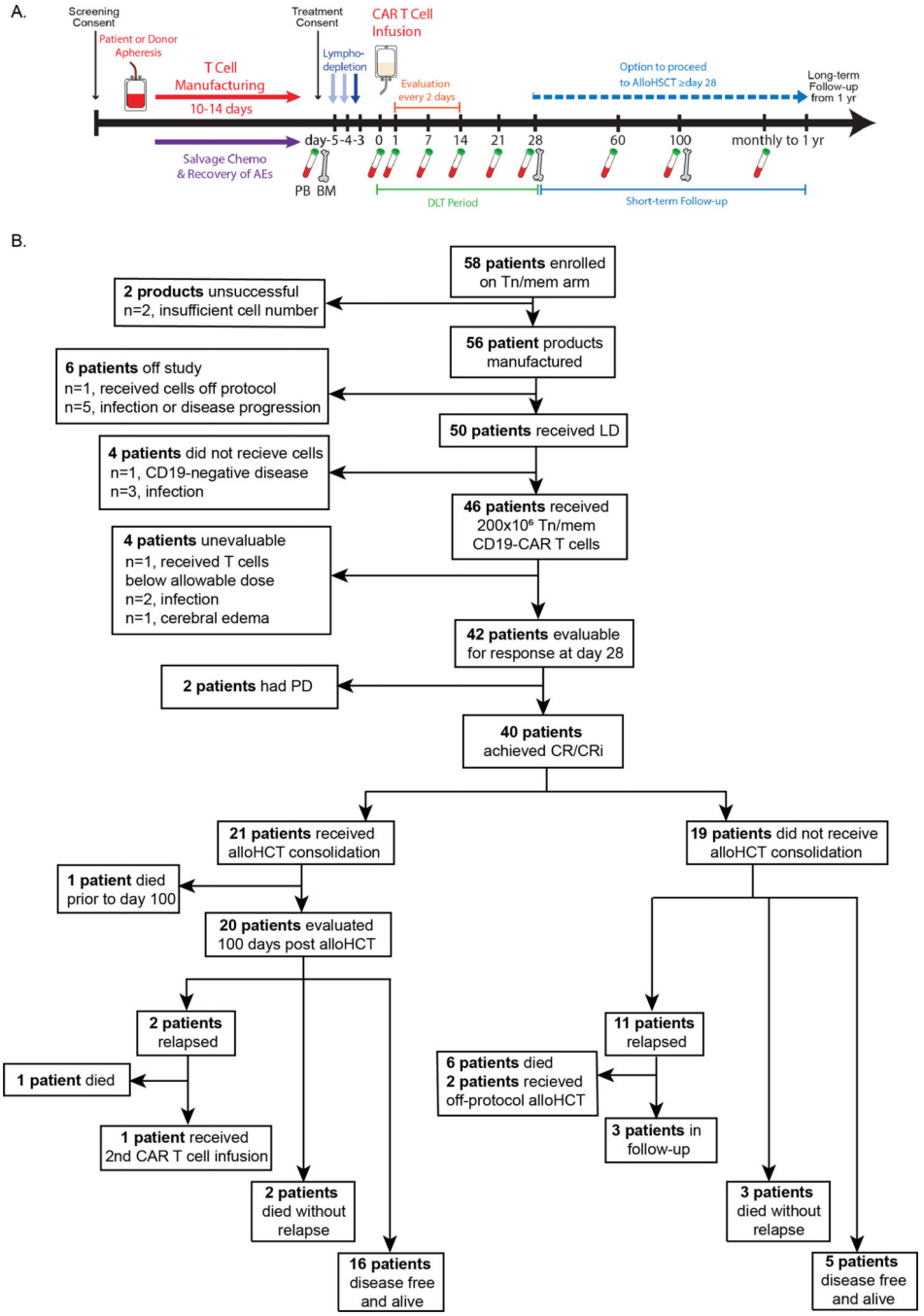


Figure 1. Treatment schema and consort diagram.

A. Patients on both arms of the trial received CAR T cells manufactured from patient or donor apheresis products following lymphodepletion. Patients were followed 3 times/ week during the first two weeks post-infusion and then weekly until the end of the dose limiting toxicity (DLT) period before entering short-term follow-up. Patients had the option to proceed to allogeneic hematopoietic cell transplantation (AlloHCT) 28 days post CAR T cell infusion. **B**. Consort diagram of patients enrolled on the Tn/mem arm. The outcome of

all patients (n=58) who were enrolled to receive 200×10^6 Tn/mem-derived CD19-CAR T cells is depicted.

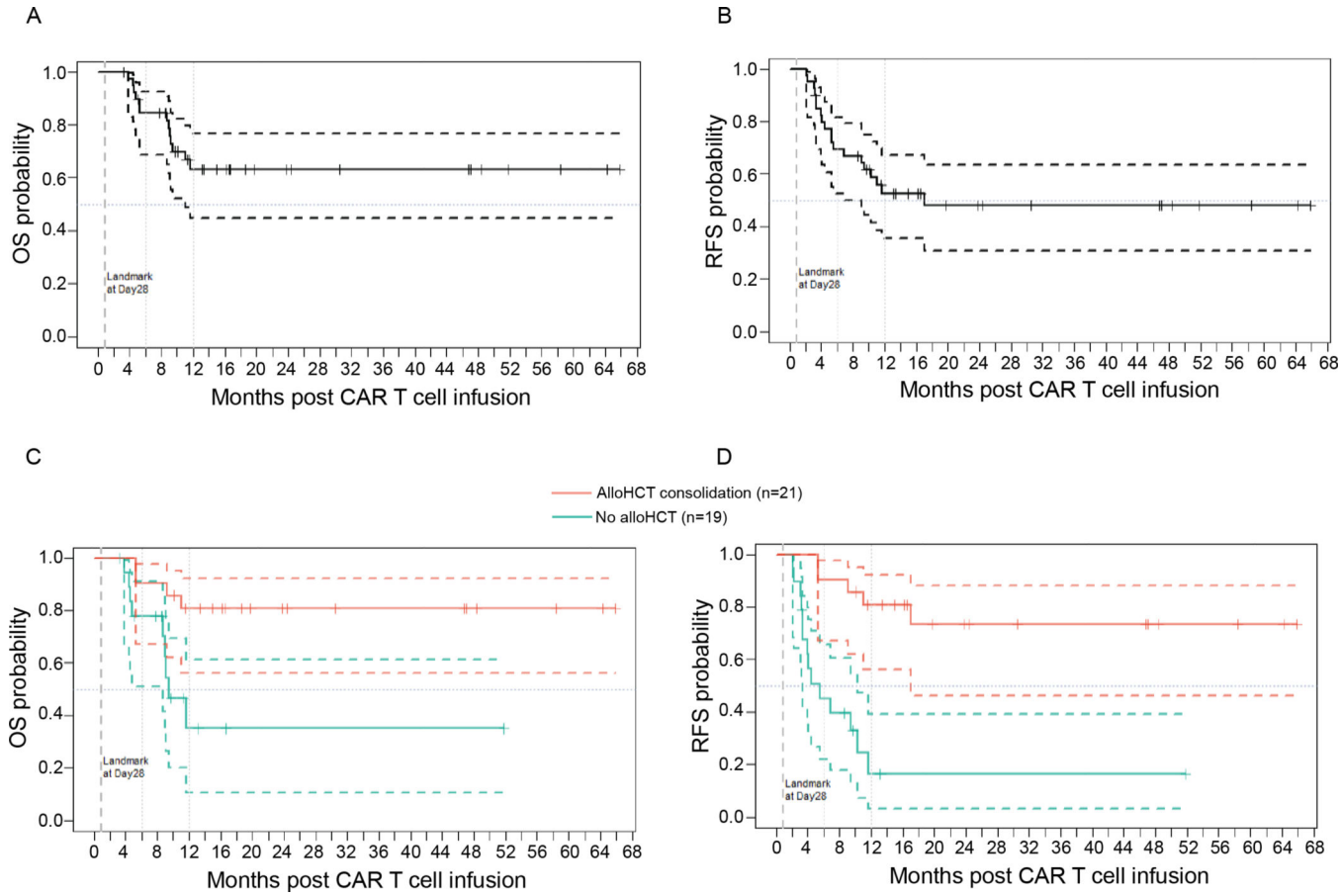


Figure 2. Survival outcomes post CD19-CAR T cell therapy.

A. Overall survival and **B.** Relapse-free survival for the 46 patients treated with Tn/mem-derived CD19-CAR T cells. **C.** Overall survival and **D.** Relapse-free survival for the patients who received alloHCT consolidation post-CAR T cell therapy.

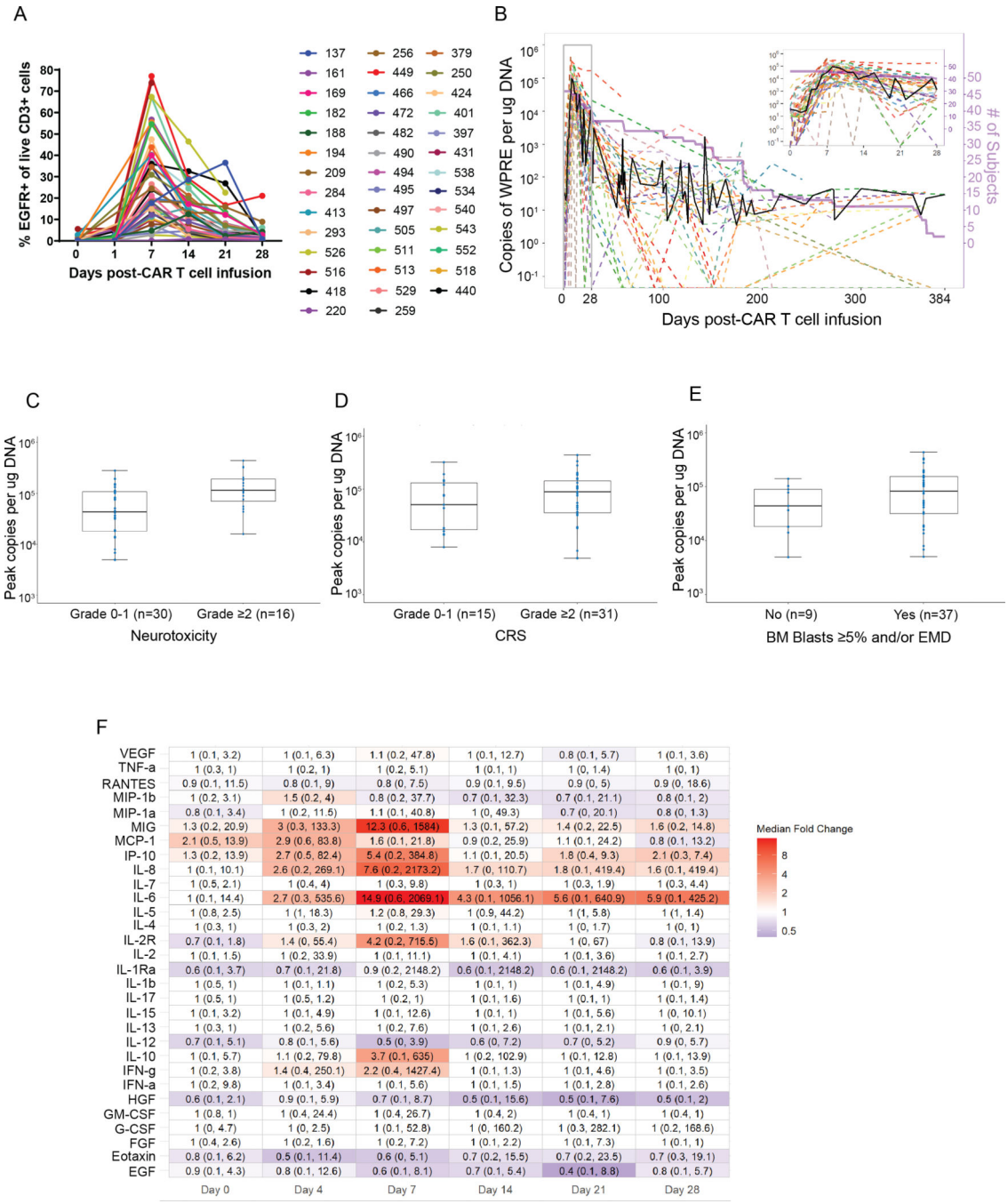


Figure 3. Expansion of Tn/mem-derived CD19-CAR T cells.

A. CAR+ cells were detected using cetuximab in patient blood at the indicated timepoints post infusion of CD19-CAR T cells. Percentage of EGFR+ cells of live CD3+ cells for each UPN are presented. **B.** CAR+ cells in patient blood were detected by qPCR analysis of the WPRE transgene encoded by the lentiviral vector. Black line indicates median copies of WPRE per μg DNA; purple line indicates the number of subjects at each corresponding timepoint. Correlation between peak copies of CAR+ cells by WPRE and grade of **C.** neurotoxicity, **D.** CRS or **E.** Blasts ≥5% and/or extramedullary disease (EMD) at the time

of lymphodepletion. **F.** Serum cytokines were measured at the indicated timepoints post infusion of CAR T cells. Cytokine levels at Days 0, 4, 7, 14, 21, and 28 were divided by the pre-LD level, and the medians at each time point were plotted as a heatmap to illustrate the median fold change from pre-LD level for each cytokine. We computed the average lower limit of quantification (LLOQ) and average upper limit of quantification (ULOQ) across all plates for each cytokine, by which we imputed the out-of-range (OOR) below, OOR above, and extrapolated values that fall outside of the thresholds.

Table 1.

Patient Demographics and Baseline Disease Assessment

Variables	Median (range) or n (%)		
	Received 200×10^6 Tn/mem CAR T cells		
	Yes, n=46	No, n=12	All Enrolled, n=58
Age at consent (yrs)	37 (22, 72)	40 (26, 72)	38 (22, 72)
Age at infusion (yrs)	38 (22, 72)	n/a	n/a
Age at infusion ≥50 yr	15 (33)	n/a	n/a
Sex			
Female	18 (39)	6 (50)	24 (41)
Male	28 (61)	6 (50)	34 (59)
Race			
Asian	3 (6.5)	0 (0)	3 (5.2)
Black	1 (2.2)	0 (0)	1 (1.7)
Caucasian	39 (85)	11 (92)	50 (86)
Multi: Pacific Islander, Asian	1 (2.2)	0 (0)	1 (1.7)
Unknown	2 (4.3)	1 (8.3)	3 (5.2)
Ethnicity			
Hispanic or Latino	24 (52)	8 (67)	32 (55)
Non-Hispanic or Latino	22 (48)	4 (33)	26 (45)
Cytogenetic risk at diagnosis			
Good risk	8 (17)	0 (0)	8 (14)
Poor risk	16 (35)	7 (58)	23 (40)
Standard risk	16 (35)	5 (42)	21 (36)
Undetermined	6 (13)	0 (0)	6 (10)
CG: PH-LIKE			
No	21 (46)	2 (17)	23 (40)
Not tested	7 (15)	6 (50)	13 (22)
Yes *IGH-CRLF2* *P2RY8-CRLF2* *CRLF2* overexpression *ZC3HAV1-ABL2* *BICD2-JAK2*	18 (39) 10 4 2 1 1	4 (33) 3 1 0 0 0	22 (38) 13 5 2 1 1
Number of prior lines of therapies	3 (1, 9)	4 (2, 7)	4 (1, 9)
Prior blinatumomab	29 (63)	9 (75)	38 (66)
Prior inotuzumab	15 (33)	2 (17)	17 (29)
Prior Allogeneic HCT	29 (63)	8 (67)	37 (64)
History of CNS disease	15 (33)	5 (42)	20 (34)
T-cell collection			
Donor	6 (13)	6 (50)	12 (21)
Patient	40 (87)	6 (50)	46 (79)

Variables	Median (range) or n (%)		
	Received 200×10^6 Tn/mem CAR T cells		
	Yes, n=46	No, n=12	All Enrolled, n=58
BM Blasts ≥5% and/or EMD at enrollment	39 (85)	12 (100)	51 (88)
BM Blasts ≥5% and/or EMD at LD	37 (80)	n/a	n/a
BM Blasts% at LD	12 (0, 95)	n/a	n/a
EMD at enrollment	24 (52)	4 (33)	28 (48)
EMD at LD	16 (35)	n/a	n/a
Isolated EMD (BM Blasts% <5%)	11		
CNS only Non-CNS EMD Combination of CNS and non-CNS EMD	3 12 1		
Bridging cytoreduction therapy intensity			
High intensity	21 (46)	n/a	n/a
Low intensity	18 (39)	n/a	n/a
No bridging cytoreduction	7 (15)	n/a	n/a

Table 2.

Adverse events at least possibly attributed to CAR T cell therapy

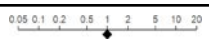
Adverse Events of Special Interest[a]						
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**	**Total (%)**
Neurotoxicity	20	8	6	1	1	36 (78.3)
Headache	12	9	4			25 (54.3)
Confusion	12	2	1			15 (32.6)
Dizziness	10					10 (21.7)
Lethargy	6	3				9 (19.6)
Somnolence	1	1	2	1		5 (10.9)
Tremor	5					5 (10.9)
Dysphasia	3		1			4 (8.7)
Encephalopathy	1		2	1		4 (8.7)
Memory impairment	4					4 (8.7)
Cognitive disturbance	3					3 (6.5)
Peripheral sensory neuropathy	3					3 (6.5)
Dysarthria	1					1 (2.2)
Edema cerebral					1	1 (2.2)
Presyncope		1				1 (2.2)
Seizure		1				1 (2.2)
Cytokine release syndrome	12	28	3			43 (93.5)
Other Adverse Events [a]	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**	**Total (%)**
Febrile neutropenia			31			31 (67.4)
Lymphocyte count decreased			6	9		15 (32.6)
Fibrinogen decreased			5	5		10 (21.7)
Hypoxia			8			8 (17.4)
Neutrophil count decreased			1	6		7 (15.2)
Blood bilirubin increased			5	1		6 (13)
Platelet count decreased				6		6 (13)
Anemia			5			5 (10.9)
White blood cell decreased			2	3		5 (10.9)
Hypotension			5			5 (10.9)
Hypertension			4			4 (8.7)
Pain			3			3 (6.5)
Fever			3			3 (6.5)
Hypophosphatemia			2	1		3 (6.5)
Nausea			2			2 (4.3)
Activated partial thromboplastin time prolonged			2			2 (4.3)
Alanine aminotransferase increased			1	1		2 (4.3)

Adverse Events of Special Interest[a]						
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**	**Total (%)**
Aspartate aminotransferase increased			1	1		2 (4.3)
Hypertriglyceridemia			2			2 (4.3)
Diarrhea			1			1 (2.2)
Vomiting			1			1 (2.2)
Fatigue			1			1 (2.2)
Hepatic failure				1		1 (2.2)
Weight gain			1			1 (2.2)
Anorexia			1			1 (2.2)
Hypokalemia			1			1 (2.2)
Hyponatremia			1			1 (2.2)
Tumor lysis syndrome			1			1 (2.2)
Generalized muscle weakness			1			1 (2.2)
Acute kidney injury				1		1 (2.2)
Hematuria			1			1 (2.2)

[a]Toxicity was assessed using CTCAE v4.03. CRS grade was defined using Lee et al. criteria.

Table 3.

Landmark Analysis at Day 28 Multivariable Cox Proportional Hazards Model for Relapse-Free Survival

Variable	Group	N	Number of events	Adjusted HR (95%CI)	Forest Plot: Adjusted HR (95% CI)	p-value
Time-varying status: Received Post CAR T Therapy Allo HCT Consolidation in Remission	No	19	14	Ref.		
	Yes	21	5	0.16 (0.05, 0.48)		0.001
Ph-like	No/ Unknown[#]	24	8	Ref.		
	Yes	16	11	2.26 (0.86, 5.94)		0.097
BM Blasts at Lymphodepletion (%)	[0,5)	19	8	Ref.		
	[5,95]	21	11	2.3 (0.74, 7.11)		0.148

Variable	Group	N	Number of events	Adjusted HR (95%CI)	Forest Plot: Adjusted HR (95% CI)	p-value
Extramedullary disease at Lymphodepletion	No	27	13	Ref.		
	Yes	13	6	1.1 (0.34, 3.55)		0.871