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A Phase 1 Clinical Trial of NKTR-255 with CD19-22 CAR-T Cell Therapy for Refractory B-cell Acute Lymphoblastic Leukemia

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Abstract:
While chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized the treatment of B-cell malignancies, many patients relapse and therefore strategies to improve antitumor immunity are needed. We previously designed a novel autologous bispecific CAR targeting CD19 and CD22 (CAR19-22), which was well tolerated and associated with high response rates but relapse was common. Interleukin-15 (IL15) induces proliferation of diverse immune cells and can augment lymphocyte trafficking. Here, we report the results of a phase 1 clinical trial of the first combination of a novel recombinant polymer-conjugated IL15 receptor agonist (NKTR-255), with CAR19-22, in adults with relapsed / refractory B-cell acute lymphoblastic leukemia. Eleven patients were enrolled, nine of whom successfully received CAR19-22 followed by NKTR-255. There were no dose limiting toxicities, with transient fever and myelosuppression as the most common possibly related toxicities. We observed favorable efficacy with eight out of nine patients (89%) achieving measurable residual disease negative remission. At 12 months, progression-free survival for NKTR-255 was double that of historical controls (67% vs 38%). We performed correlative analyses to investigate the effects of IL15 receptor agonism. Cytokine profiling showed significant increases in IL15 and the chemokines CXCL9 and CXCL10. The increase in chemokines was associated with decreases in absolute lymphocyte counts and CD8+ CAR T-cells in blood and ten-fold increases in CSF CAR-T cells, suggesting lymphocyte trafficking to tissue. Combining NKTR-255 with CAR19-22 was safe, feasible and associated with high rates of durable responses (NCT03233854).-

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Title

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Running Title

NKTR-255 and CAR19-22 in R/R B-ALL

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Data Sharing Statement

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Key Points

1. Combining CAR-T cells targeting CD19 & CD22 with a recombinant, polymer-conjugated IL15 receptor agonist (NKTR-255) was safe and feasible.
2. NKTR-255 was associated with increases in cytokines (IL15 and IFNγ) and related chemokines (CXCL9, CXCL10).

Abstract

While chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized the treatment of B-cell malignancies, many patients relapse and therefore strategies to improve antitumor immunity are needed. We previously designed a novel autologous bispecific CAR targeting CD19 and CD22 (CAR19-22), which was well tolerated and associated with high response rates but relapse was common. Interleukin-15 (IL15) induces proliferation of diverse immune cells and can augment lymphocyte trafficking. Here, we report the results of a phase 1 clinical trial of the first combination of a novel recombinant polymer-conjugated IL15 receptor agonist (NKTR-255), with CAR19-22, in adults with relapsed / refractory B-cell acute lymphoblastic leukemia. Eleven patients were enrolled, nine of whom successfully received CAR19-22 followed by NKTR-255. There were no dose limiting toxicities, with transient fever and myelosuppression as the most common possibly related toxicities. We observed favorable efficacy with eight out of nine patients (89%) achieving measurable residual disease negative remission. At 12 months, progression-free survival for NKTR-255 was double that of historical controls (67% vs 38%). We performed correlative analyses to investigate the effects of IL15 receptor agonism. Cytokine profiling showed significant increases in IL15 and the chemokines CXCL9 and CXCL10. The increase in chemokines was associated with decreases in absolute lymphocyte counts and CD8+ CAR T-cells in blood and ten-fold increases in CSF CAR-T cells, suggesting lymphocyte trafficking to tissue. Combining NKTR-255 with CAR19-22 was safe, feasible and associated with high rates of durable responses (NCT03233854).

Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy targeting CD19 (CAR19) has transformed the treatment of relapsed/refractory B-cell malignancies including acute lymphoblastic leukemia (B-ALL). However, relapse after CAR19 occurs in the majority of patients and is associated with dismal outcomes.[1,2] Antigen loss is a frequent cause of immune evasion, thus CAR-T constructs targeting alternate antigens may overcome this mechanism of relapse.[3,4] Our group previously generated a bispecific CAR-T targeting CD19 and CD22 with a 4-1BB costimulatory domain (CAR19-22).[5] In a phase 1 trial, CAR19-22 was safe with no dose-limiting toxicities (DLTs) and led to complete remissions (CR) in 100% of B-ALL patients (n = 17) with measurable residual disease (MRD) negativity in 88%.[6] However, relapse occurred in ten patients leading to a median progression free survival (PFS) of 5.8 months (95% CI 2.6 – NR). Five of ten patients retained CD19 and CD22 expression at the time of relapse indicating mechanisms other than antigen loss may be responsible for disease progression as other studies have suggested.[7] Improving the antitumor response of CAR19-22 in an antigen agnostic manner may therefore promote durable remissions.

IL15, a proinflammatory member of the common gamma chain family of cytokines, has been proposed as a novel immunotherapy that may synergize with CAR-T therapy through multiple mechanisms.[8] IL15 drives proliferation of NK, NK-T and CD8+ T cells and promotes differentiation of CD8+ T effector cells into memory T cells.[8–11] IL15 also promotes tissue migration of NK and CD8+ T.[12] Enhanced homeostatic proliferation of T cells following lymphodepleting chemotherapy is mediated, in part, by IL15,[9] and in

patients who received axicabtagene ciloleucel, peak IL15 levels correlated with CAR expansion and response.[13,14] Several preclinical models demonstrate that enhanced IL15 signaling increases the potency of adoptively transferred T cells.[10,11,15,16] Together, evidence supports a role for physiologic elevations in circulating IL15 in augmenting the potency of adoptive cell therapies and that IL15 administration could enhance the efficacy of CAR-T therapy through multiple mechanisms.

Previous attempts to leverage the salutary antitumor effects of IL15 have been limited by its short half-life.[12,17] To address this, NKTR-255 was developed as a novel recombinant IL15 receptor agonist attached to a polyethylene glycol (PEG) moiety. NKTR-255 has been shown to prolong IL15 half-life and activate NK and CD8+ T cells durably.[18] In pre-clinical studies, NKTR-255 improves CAR19 proliferation and persistence in murine models of lymphoma and *in vitro* assays of human cell lines.[19] We conducted a phase 1 trial combining NKTR-255 with CAR19-22 to evaluate the safety and feasibility of this approach in relapsed / refractory B-ALL.

Methods

Trial Design and Oversight

This phase 1 single-center, single-arm dose-escalation study was approved by Stanford University's institutional review board (IRB# 41382) and registered with clinicaltrials.gov (NCT03233854). Patients provided informed consent in accordance with the Declaration of Helsinki. A data safety and monitoring committee oversaw the trial conduct.

Experimental Interventions

The CAR19-22 construct includes a murine anti-CD19 FMC63 single-chain variable fragment (scFv) linked to a fully human anti-CD22 m971 scFv (αCD19 vH-αCD22 vL-linker-αCD22 vH-αCD19 vL) with the following additional domains: human CD8 hinge and transmembrane, 4-1BB costimulatory, and CD3ζ activation. Patients underwent apheresis and CAR19-22 manufacturing in a Miltenyi CliniMACS Prodigy closed-system device as previously described.[6] Patients received standard lymphodepletion (LD) with fludarabine 30mg/m2 on days -5, -4, -3 and cyclophosphamide 500mg/m2 on days -5, -4 (Supplemental Figure 1). The CAR19-22 dose of 3 x 10^6 cells/kg was the recommended phase 2 dose (RP2D) identified in the phase 1 trial.[6] Escalating doses of intravenous NKTR-255 were tested in a 3x3 design: dose level 1 = 1.5 mcg/kg; dose level 2 = 3.0 mcg/kg; dose level 3 = 6.0 mcg/kg. NKTR-255 was administered on day +14 (D14); patients were eligble to receive additional monthly NKTR-255 infusions for a maximum of 6 cycles; however, feasibility was determined based on ability to receive D14 NKTR-255. Criteria for NKTR-255 discontinuation were pre-specified in the protocol and are listed in the supplemental methods. We initially planned for a total of ten subjects to allow for at least three patients to receive NKTR-255 at each dose level, however, there was insufficient lentiviral vector to allow for additional trial enrollment and therefore only two patients received NKTR-255 dose level 3.

Eligibility

Adult patients (≥18 years old) with B-ALL with stable or progressive disease (refractory) after a single line of therapy (chemotherapy or TKI) or who relapsed after achieving CR

were eligible for enrollment. MRD-only relapses required confirmation with a second test within four weeks. CD19 expression via immunohistochemistry or flow cytometry was required; CD22 expression was not. A full list of inclusion and exclusion criteria is included in the supplement.

Endpoints

The primary outcomes of this study were feasibility and safety. Feasibility was measured by the number of patients who successfully received CAR19-22 and D14 NKTR-255. Safety of CAR19-22 was evaluated for all patients (n = 11) and safety of NKTR-255 was evaluated for those who received the combination (n = 9). Incidence and severity of adverse events (AEs) at each dose level were recorded with the goal of identifying the RP2D, defined as the highest dose level of NKTR-255 tested and tolerated without dose limiting toxicity (DLT) after CAR19-22.

AEs were graded using the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE).[20] American Society for Transplantation and Cellular Therapy consensus grading was used to assess cytokine release syndrome (CRS), immune effector cell associated-neurotoxicity (ICANS) and immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS).[21,22]

Secondary endpoints were assessed for patients who received NKTR-255 (n = 9) and included pharmacokinetics of NKTR-255 as measured by IL15 levels in blood and efficacy as measured by progression free survival (PFS). Exploratory endpoints

included cytokine profiling, CAR19-22 expansion in blood, bone marrow, and cerebrospinal fluid (CSF), and long-term CAR19-22 persistence. MRD was assessed by multiparameter flow cytometry, polymerase chain reaction or next generation sequencing of the immunoglobulin receptor (Clonoseq) (Adaptive Biotechnologies, Seattle, WA).

Controls

For secondary and exploratory endpoints, patients in this study were compared to a non-contemporaneous control cohort of patients who received CAR19-22 as part of the initial phase 1 clinical trial without the addition of NKTR-255. Control patients were enrolled between December 2017 and October 2020 and were matched primarily based on disease burden at time of screening, as leukemic burden has been shown to most closely associate with toxicity and efficacy in ALL CAR-T studies.[1,23,24] Low disease burden was defined as <5% marrow involvement of ALL and absence of bulky extramedullary disease.

Correlative Analyses

We aimed to collect peripheral blood samples prior to LD, during the first week after infusion, on D14 before and after NKTR-255 infusion, D15, D16, D21 and D28 for patients receiving NKTR-255. We aimed to collect samples for controls prior to LD, on D0 and then weekly thereafter until at least D28. Details for laboratory assessment of correlates are provided in the supplemental methods. Briefly, cytokines were quantified in batch via multiplexed immunofluorescence (Luminex). A CD19 anti-idiotype

monoclonal antibody (generous gift from MD Anderson) was used to quantify CAR+ T cells by flow cytometry including CD4+ and CD8+ subsets in an assay hereafter referred to as CARFACS in peripheral blood, bone marrow, and CSF.[6,25] DNA was extracted and measured via quantitative PCR (qPCR) using previously published primers.[6]

Statistical Analyses

Continuous variables were compared using Mann-Whitney U tests with paired methods as needed. Progression free survival was estimated using the Kaplan-Meier method. All statistical analyses were performed in R version 4.2.2 (10/31/2022).

Results

Patient Characteristics & Feasibility

Eleven patients were enrolled between February 2022 and July 2023. CAR19-22 manufacturing was successful in all patients and all eleven were infused. Two of eleven patients (18%) who received CAR19-22 were ineligible to receive NKTR-255 at D14 due to active grade 3 infection in one case and ongoing grade 3 IEC-HS in the other. The baseline characteristics of these 2 patients were similar to the 9 patients who received NKTR-255 (Supplemental Table 1).

Among the nine patients who received the combination of CAR19-22 and NKTR-255, a majority had Ph-negative disease (89%) and received multiple lines of prior therapy (median 3, range 1-5) including prior allogeneic hematopoetic stem cell transplantation

(HCT) (56%), blinatumomab (56%), inotuzumab (44%), and CAR T-cells (11%) (Table 1). Eight of the nine patients had low tumor burden and one patient had high burden due to the presence of bulky extramedullary disease.

The cohort of control patients previously treated with CAR19-22 was older (median age 49 vs 36 years), less likely to have Ph-negative disease (63%) and received more lines of therapy (median 4, range 2-10) including higher rates of prior transplant (75%) (Table 1). Rates of prior CAR19, blinatumomab and inotuzumab were similar between the two cohorts. All eight patients had low tumor burden at screening. Three of the historical control patients received CAR19-22 at a lower dose than the RP2D (1.5×10^6 cells/kg vs 3.0×10^6 cells/kg), however, no dose response was observed in the prior CAR dose-finding study.[6]

Safety

Four out of the nine (44%) patients who ultimately received NKTR-255 experienced CRS after CAR19-22 (Table 1). All cases of CRS were grade 1 and resolved prior to the first dose of NKTR-255. Four of the eight (50%) control patients developed CRS after CAR19-22; three cases were grade 2 and the remaining case was grade 1. There were no cases of ICANS either before or after NKTR-255; one patient in the control cohort experienced ICANS (Table 1). There was one case of IEC-HS related to CAR19-22 in the NKTR-255 cohort that occurred in one of the two patients who did not receive NKTR-255; there were no cases of IEC-HS in the control cohort.

No dose-limiting toxicities related to NKTR-255 were observed. Fevers after NKTR-255 were common, occurring after 55% of patients' first infusion and 55% of subsequent infusions (Table 2). The onset of fever was typically between 4 and 12 hours after infusion and resolved within 24 hours of onset. All fevers after NKTR-255 were grade 1 or 2 in severity and managed with acetaminophen. No episode of fever required treatment with steroids or cytokine blockade (e.g. tocilizumab, anakinra).

Cytopenias were also common after NKTR-255 (Table 2). Anemia (44%) and thrombocytopenia (55%) were common after the first infusion and recurred at lower rates upon subsequent NKTR-255 doses (anemia 16%, thrombocytopenia 8%). Cytopenias typically were typically self-limited, although one patient had persistent grade 3 neutropenia and grade 4 thrombocytopenia after CAR19-22 plus D14 NKTR-255 that were ongoing at time of pre-planned allogeneic transplantation (Supplemental Figure 2B-C). While neutropenia occurred at all dose levels, both patients treated at the highest dose level of NKTR-255 (6.0 ug/kg) experienced grade 3 or 4 neutropenia compared to 2 cases of grade 3 - 4 neutropenia at lower doses (Supplemental Table 2). Supportive care for neutropenia followed institutional protocols including G-CSF use for absolute neutrophil counts (ANC) less than 1,000 cells / uL and mold and bacterial prophylaxis. There were no bacterial or fungal infections after treatement with NKTR-255. Cytopenias were also common amongst the control cohort after CAR19-22 including anemia (n = 6, 75%), neutropenia (n = 7, 88%) and thrombocytopenia (n = 5, 63%). One control patient developed prolonged grade 3-4 panctyopenia after CAR19-22 without count recovery (Supplemental Figure 2D-F).

Patients received a median of 2 cycles of NKTR-255 (range, 1-5 cycles) (Figure 1A). Causes for NKTR-255 discontinuation included ALL relapse (n = 3), allogeneic HCT (n = 2), infection (n = 1), inflammation (n = 1), and clinician decision (n = 2). The discontinuation related to infection occurred in a patient who developed a viral respiratory tract infection that was deemed unlikely related to NKTR-255 but took several weeks to resolve. The discontinuation due to inflammation occurred in a patient with abdominal pain and rise in CRP after starting hormone replacement therapy prior to the sixth dose of NKTR-255. Both the pain and rise in CRP were self-limited and deemed unrelated to NKTR-255.

Efficacy

Eight out of nine patients (89%) achieved a CR with or without hematologic recovery, all without detectable MRD (Table 3). Two patients received allogeneic HCT in CR (Figure 1A). Three patients (33%) relapsed (one with loss of CD19), all within six months of CAR19-22. For patients who received NKTR-255, 12 month PFS was 67% and with 14.4 months of followup, the median PFS has not been reached (Figure 1B). Similar results were obtained if treating allogeneic transplantation as a censoring event (Figure 1C) and in the intention-to-treat population, which includes the two patients who were ineligible for NKTR-255 infusion (Supplemental Figure 3). After median follow up of 24.5 months, the median PFS of the control cohort was 3.9 months and 12 month PFS was 38% (Supplemental Figure 4).

IL15 Pharmacokinetics & Cytokine Dynamics

During the first week after CAR19-22, we observed no significant difference in IL15 levels between the interventional and control patients (Figure 2A). However, after D14 NKTR-255, we observed significant increases in IL15 compared to pre-infusion levels on the same day (median mean fluorescent intensity [MFI] 15,898 vs 69, p = 0.039) (Figure 2B). IL15 levels gradually returned to pre-infusion baseline by D21. From D14 prior to NKTR-255 infusion to D15, we observed significant increases interferon gamma (IFNγ) (median MFI 35.0 vs 71.3, p = 0.031) and related pro-inflammatory cytokines, namely IL10 (median MFI 78.0 vs 272.3, p = 0.031) and IL6 (median MFI 119.0 vs 184.0, p = 0.031) (Figure 2C). We also observed significant increases in chemokines induced by IFNγ including CXCL9 (median MFI 3,020 vs 8,508, p = 0.031) and CXCL10 (median MFI 5,394 vs 14,277, p = 0.031). Among common γ chain cytokines, we observed significant increases in IL4 levels on D14 post infusion (median MFI 28.0 vs 116.8, p = 0.023), but not IL2 or IL7. In the subset of patients for whom serial blood samples were available after D28 (n = 4), we continued to observe increases in IL15 after additional NKTR-255 infusions (Supplemental Figure 5).

CAR Expansion, Persistence & Trafficking

All patients who received CAR19-22 and NKTR-255 had detectable CAR+ T cells in the blood during the first month after infusion. After D14 NKTR-255 infusion, there was no change in CAR19-22 as measured by qPCR (Figure 3A) or CARFACS (Figure 3B). However, on D15 compared to D14 pre-infusion, we observed a decrease in CD4+ (median 0.65 vs 1.45 cells / uL, p = 0.109) and CD8+ CAR19-22 cells (median 0.43 vs

2.38 cells / uL, p = 0.148) (Figure 3C, 3D). Given the relatively larger decrease in CD8+ CAR19-22 cells in blood, the CD4:CD8 ratio rose after D14 NKTR-255 administration (0.776 vs 1.38, p = 0.055) (Figure 3E).

We next assessed total lymphocyte dynamics at the same timepoints after D14 NKTR-255. On D15, absolute lymphocyte counts (ALC) decreased significantly in NKTR-255 treated patients compared to D14 pre-infusion (median 95 vs 460 cells / uL, p = 0.016) (Figure 3F). By D28, we observed a significant rebound of lymphocytes compared to D15 (median ALC 95 vs 1,160 cells / uL, p = 0.021). The vast majority of these rebounding lymphocytes did not express the CAR construct as assessed by flow cytometry (Supplemental Figure 6). At their peak on D21, 42% of the rebounding lymphocytes were CD3+ indicating expansion of both T cells and non-T cell lymphocyte subsets (Figure 3F). CAR19-22 remained detectable in peripheral blood up to D180 in both control and NKTR-255 treated patients at similar levels (Supplemental Figure 7).

Given the role of IL15 in lymphocyte trafficking, the increases in CXCL9 and CXCL10 and decreases in ALC and CD8+ CAR19-22 in blood, we hypothesized that NKTR-255 may be driving lymphocytes into tissues. We therefore profiled CAR levels in blood and CSF in two patients in the NKTR-255 cohort with confirmed CNS disease. ALCs and CD8+ CARs decreased in blood in both patients following NKTR-255 administration (Figure 4A, 4B). Both patients had a ten-fold increase in CAR+ cells in CSF with the increase in patient 1 occurring on D17 compared to D28 in patient 2 (Figure 4C), suggestive of trafficking to CNS. In comparison, among three control patients with CSF

samples available at D28, we did not see elevations in CAR+ cells in CSF: two patients had fewer than one white blood cell per microliter; the third patient had ten-fold fewer CAR+ cells than the two NKTR-255 treated patients (median 0.00911 vs 0.292) (Figure 4C).

We also assessed CAR levels via flow cytometry in bone marrow for patients with aspirates available. There was a numerically higher percentage of CD3+ cells in NKTR-255 treated patients (median 42.0% vs 23.7%, p = 0.4) (Supplemental Table 4). However, the proportion of these CD3+ cells expressing the CAR construct was low in both groups and there was no evidence of enhanced migration of CAR19-22 to bone marrow in NKTR-255 treated patients (Supplemental Table 3).

Discussion

Our trial represents the first attempt to combine a recombinant cytokine product with CAR-T cell therapy and demonstrated this approach to be both feasible and safe. All patients had successful manufacturing and infusion of CAR19-22, and 88% received at least one dose of NKTR-255. The most common AEs in patients receiving NKTR-255 were fevers, chills, and myelosuppression which were manageable with supportive care and were comparable to toxicities seen after CAR19-22 alone. We did not observe DLT at any of the doses of NKTR-255 tested; however, we did note more prominent cytopenias at the highest dose of 6.0 mcg/kg.

345 Outcomes in this population were favorable with high rates of MRD-negative responses
346 (88%). Our prior study of CAR19-22 showed similarly high initial response rates but
347 58% of patients relapsed within 6 months of CAR infusion. With median follow up of
348 14.4 months, only three patients receiving combination therapy (33%) relapsed, which
349 may suggest administration of NKTR-255 helps prevent early disease recurrence
350 though the non-randomized nature of our study precludes definitive efficacy
351 assessment. While longer-term follow up is needed to assess for late relapses, all
352 relapses in the initial phase 1 trial of CAR19-22 occurred within 6 months of CAR
353 infusion as was seen in the current study's control cohort.
354
355 Our correlative analyses suggest NKTR-255 may influence lymphocyte trafficking to
356 tissues. After NKTR-255 administration, we observed dramatic increases in cytokines
357 typically secreted from activated T-cells, including CXCL9 and CXCL10, that promote
358 migration of lymphocytes into tissues where they exert their effects on tumor cells. This
359 hypothesis is supported by the decrease in CD8+ CAR-T cells and endogenous
360 lymphocytes in peripheral blood and the increase in CAR19-22 in CSF of patients with
361 confirmed CNS disease shortly after NKTR-255 administration. Prior studies of
362 recombinant IL15 also support the notion that IL15 activates T-cells and promotes
363 migration of lymphocytes and NK cells to tissue.[12,17] After this decrease in lymphocytes
364 and CD8+ CAR19-22, we observed a rebound in a heterogenous group of lymphocytes
365 including both CAR and non-CAR cells as has been described in a non-human primate
366 model of NKTR-255.[19] Further studies should address the nature of these lymphocytes

and how they interact with CAR-T cells to shape the immune response against leukemia.

Our study has several important limitations. The non-contemporaneous control arm received the same CAR19-22 construct and had similar disease burden, but without randomization differences in outcomes may reflect residual confounding. The relatively small study cohort prohibited more robust matching. Comparisons between the two cohorts should therefore be interpreted with caution. Further, we were unable to replace subjects who did not receive NKTR-255 due to limited availability of lentiviral vector for CAR19-22 manufacturing. Longer-term follow up and larger sample sizes are needed to assess both late relapses and overall survival. Although we hypothesize that NKTR-255 may improve lymphocyte trafficking to tissue and activate both CAR and non-CAR mediators of antitumor immunity, we did not have enough contemporaneous tissue and blood samples to robustly test this.

In conclusion, our study demonstrates that administration of a novel CAR-T cell product followed by infusion of a pegylated IL15 receptor agonist is feasible. We speculate that NKTR-255 may improve lymphocyte trafficking to tissue and activate both CAR and non-CAR mediators of antitumor immunity. Larger, randomized trials are ultimately needed for accurate assessment of efficacy and toxicity. Future correlative studies to understand the effect of NKTR-255 on CAR trafficking, function, and phenotype are warranted.

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Conflicts of Interest

D.B.M. reports consulting for Kite Pharma-Gilead, Juno Therapeutics-Celgene, Novartis, Janssen, and Pharmacyclics. Research support from Kite Pharma-Gilead, Allogene, Cargo therapeutics, Pharmacyclics, Miltenyi Biotec, and Adaptive Biotechnologies. C.L.M. is a founder, holds equity and consults for CARGO Therapeutics, Link Cell Therapies and GBM NewCo; holds equity and consults for Ensoma, Red Tree Capital; consults for Immatics; receives research funding from Tune therapeutics and Lyell Immunopharma; receives royalties from NIH and Stanford for CD22-CAR and holds multiple patents related to CAR T cell therapies. S.S.: reports research funding for Magenta Therapeutics, BMS, Allogene, Janssen, Novartis. Consultancy to Magenta Therapeutics, BMS, Janssen, Sanofi, Oncopeptides, Takeda, Regeneron, Abbvie, Pfizer. The remaining authors report no relevant disclosures.

Authorship Statement

DBM, CLM, and LSM conceived and designed the study. HS, CJ, PS, NJ, MH, EE, SM, BS, NA, AK, AMK, SA, SB, SD, HH, LJ, VK, ML, RL, EM, LM, RN, AR, SS, JS, MS,

411 WKW, SF, MF, DBM, CLM and LSM collected the data and wrote the manuscript. All
412 authors contributed to writing and editing the manuscript.
413

Tables

Table 1. Baseline characteristics.

	NKTR-255 + CAR19-22 (n = 9)	CAR19-22 Historical Controls (n = 8)
Age (median, years)	36	49
Hispanic	5 (56%)	5 (62%)
Ph-negative	8 (89%)	2 (25%)
Prior lines of therapy (range)	3 (1-5)	4 (2-10)
CD19 expression	9 (100%)	8 (100%)
CD22 expression*	7 (78%)	7 (88%)
Prior blinatumomab	5 (56%)	5 (63%)
Prior inotuzumab	4 (44%)	4 (50%)
Prior CAR19**	1 (11%)	1 (13%)
Prior HCT	5 (56%)	6 (75%)
Low disease burden	8 (89%)	8 (100%)
CNS Disease	3 (33%)	4 (50%)
CAR19-22 Dose 1 x 10^6 cells/kg 3 x 10^6 cells/kg	 0 9 (100%)	 3 (37%) 5 (63%)
CRS related to CAR19-22 Grade 1 Grade 2	 4 (44%) 0 (0%)	 1 (13%) 3 (38%)
ICANS related to CAR19-22 Grade 1	 0 (0%)	 1 (13%)
NKTR-255 Dose 1.5mcg/kg 3.0mcg/kg 6.0mcg/kg	 3 (33%) 4 (44%) 2 (22%)	 - - -
Number of NKTR-255 Infusions (median, range)	2 (1-5)	-

*Two patients in the NKTR-255 cohort and one in the control group did not have a formal CD22 evaluation. ** One patient in the NKTR-255 cohort previously received the same CAR19-22 and achieved CR and underwent allogeneic HCT prior to eventual relapse and was retreated with CAR19-22 + NKTR-255 post-HCT. One patient in the control cohort received an investigational CAR19 product twice before CAR19-22.

422 Table 2. Safety of NKTR-255.

	Cycle 1 (n = 9 infusions) N (%)				Subsequent Cycles (n = 12 infusions) N (%)			
	Gr 1	Gr 2	Gr 3	Gr 4	Gr 1	Gr 2	Gr 3	Gr 4
Anemia	-	4 (44)	-	-	1 (8)	-	1 (8)	-
Neutropenia	-	-	2 (22)	2 (22)	2 (17)	-	1 (8)	-
Thrombocytopenia	4 (44)	-	-	1 (11)	1 (8)	-	-	-
Fevers	4 (44)	1 (11)	-	-	5 (42)	-	-	-
Chills	2 (22)	-	-	-	2 (17)	-	-	-
Nausea	2 (22)	-	-	-	-	-	-	-
Vomiting	1 (11)	-	-	-	-	-	-	-
Myalgias	-	-	-	-	2 (17)	-	-	-
Infusion Reaction	1 (11)	1 (11)	-	-	-	-	-	-
Fatigue	-	1 (11)	-	-	-	-	-	-
Headache	-	1 (11)	-	-	-	-	-	-
Dizziness	1 (11)	-	-	-	-	-	-	-
Sinus tachycardia	1 (11)	-	-	-	-	-	-	-
Hypotension	-	-	-	-	-	-	1 (8)	-
Dyspnea	-	-	-	-	-	-	1 (8)	-
Hypoxia	-	-	-	-	-	-	1 (8)	-
Diarrhea	-	-	-	-	-	1 (8)	-	-

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425 Table 3. Efficacy of CAR19-22 with NKTR-255

	CAR19-22 + NKTR-255 (n = 9)	**CAR19-22 control (n = 8)**
CR/CRi	8 (89%)	7 (88%)
MRD-negative	8 (89%)	6 (75%)
Progression/Relapse	3 (33%)	5 (62%)
Consolidative HCT	2 (23%)	1 (13%)
6-month RFS (%, 95% CI)	67% (42 – 100%)	38% (15 – 92%)
12-month RFS (%, 95% CI)	67% (42 – 100%)	38% (15 – 92%)

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Figure Legends.

Figure 1. Clinical Outcomes. (A) Swimmer plot for patients who received CAR19-22 and NKTR-255. 3 patients received NKTR-255 dose level 1 (1.5 ug/kg, green) 4 patients received dose level 2 (3.0 ug/kg, orange) and 2 patients received dose level 3 (6.0 ug/kg, blue). Circles represent doses of NKTR-255, squares represent allogeneic stem cell transplant, and X's represent relapse. (B) Progression free survival for patients who received NKTR-255 (n = 9) with events as death or relapse and without censoring for allogeneic HCT. (C) PFS considering allogeneic HCT as censoring event.

Figure 2. (A) IL15 during the first month after CAR19-22 infusion. Shown are medians and interquartile ranges for MFIs of IL15 for control (orange) and NKTR-255 (teal) patients. NKTR-255 administration is denoted by the red dashed line. The number of observations at each timepoint is shown below the graph. PRE indicates timepoints prior to lymphodepleting chemotherapy. Control patients did not have samples at D15 or D16 available for analysis. Cytokine levels before and after NKTR-255 administration for patients with both samples available for analysis. (B) compares D14 pre-infusion to post-infusion (n = 8) (C) compares D14 pre-infusion to D15 (n = 6). P-values were calculated with the paired Mann-Whitney U test. Cytokines were grouped according to common gamma chain cytokines (top), IFNγ-related (middle) and proinflammatory cytokines (bottom).

Figure 3. CAR19-22 expansion and persistence. Shown are CAR19-22 levels (medians with interquartile ranges) in peripheral blood in the first month after CAR infusion as measured by (A) qPCR and (B) CARFACS. NKTR-255 administration is denoted by the red dashed line. The number of observations at each timepoint is shown below the

graph. (C) CD4 and (D) CD8 CAR subsets assessed by CARFACS. (E) CD4:CD8 CAR+ ratio during the first month after CAR19-22. (F) Absolute lymphocyte count (circles) and total CD3+ T-cells by CARFACS (triangles).

Figure 4. CAR trafficking. CAR dynamics for patients with CNS leukemia. Shown are (A) peripheral blood absolute lymphocyte count, (B) CD8+ CAR-T cells, and (C) CSF absolute white blood cell count (circles) and CAR19-22+ cells (triangles) for NKTR-255 patients with CNS disease (n = 2). (D) WBC count (circles) and CAR+ cells in CSF on D28 evaluated by flow cytometry for the same NKTR-255 patients (teal, n = 2) and the historical control patient with evaluable CSF (orange, n = 1).

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Figure 1

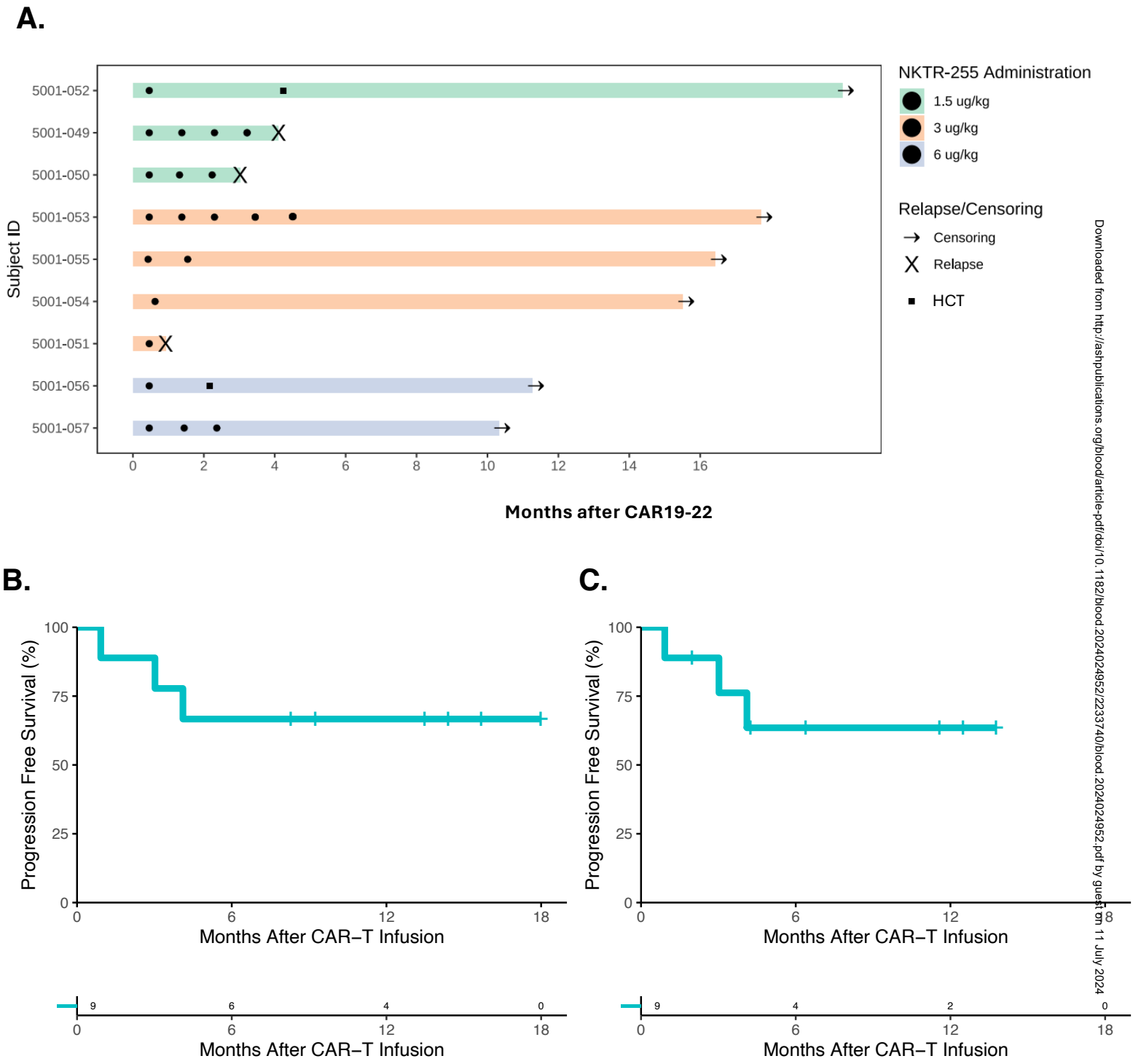


Figure 2

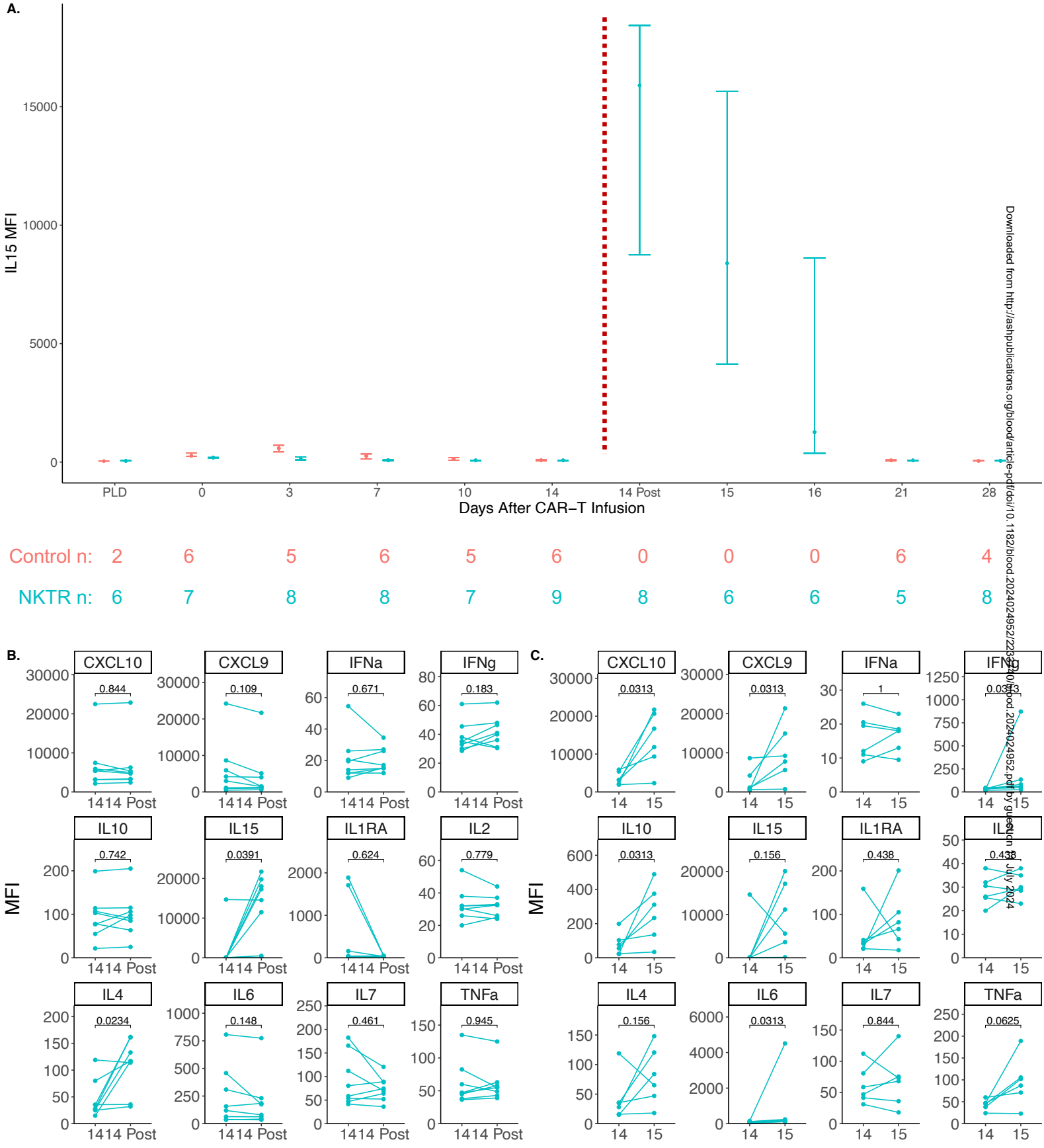


Figure 3

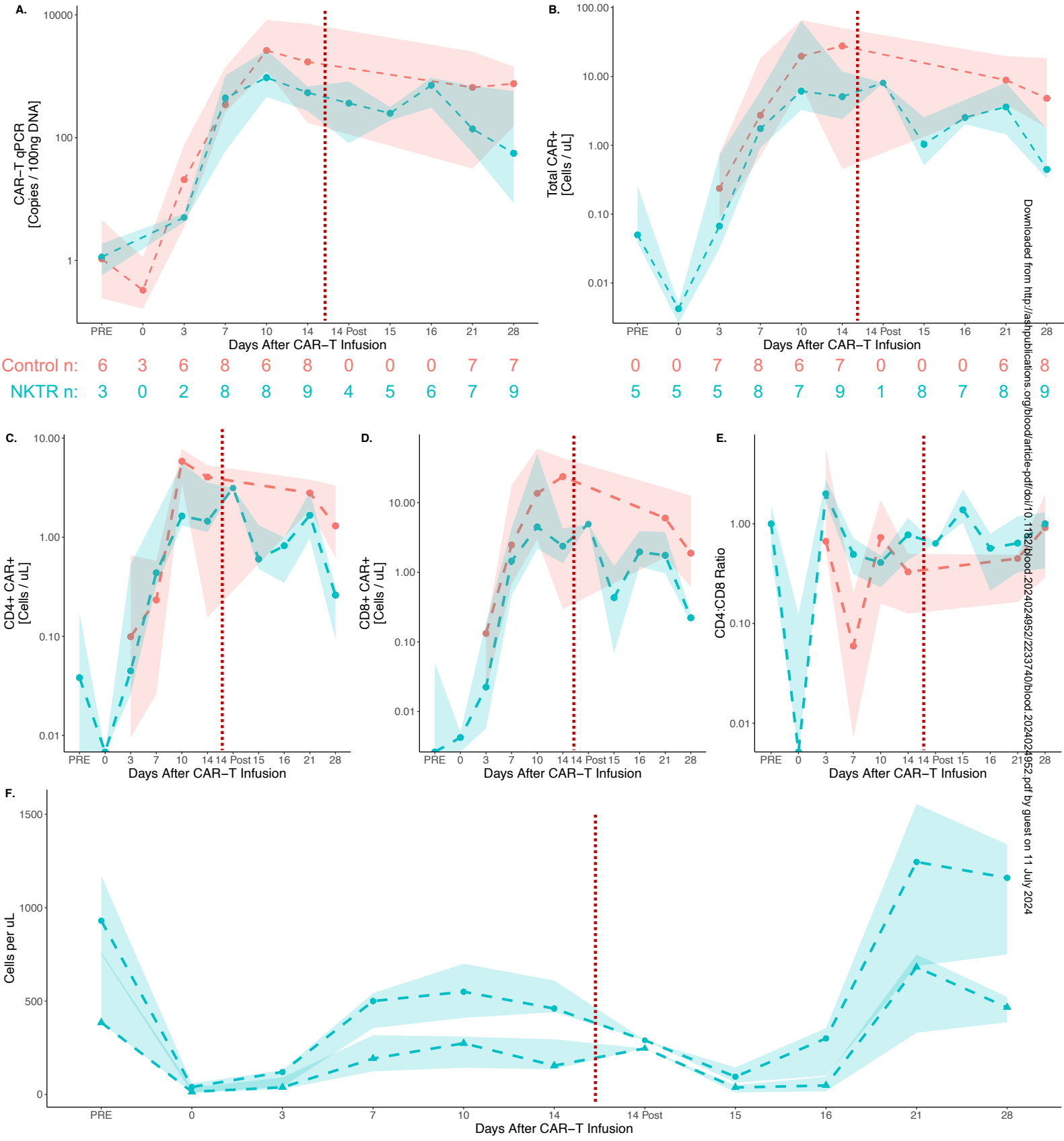
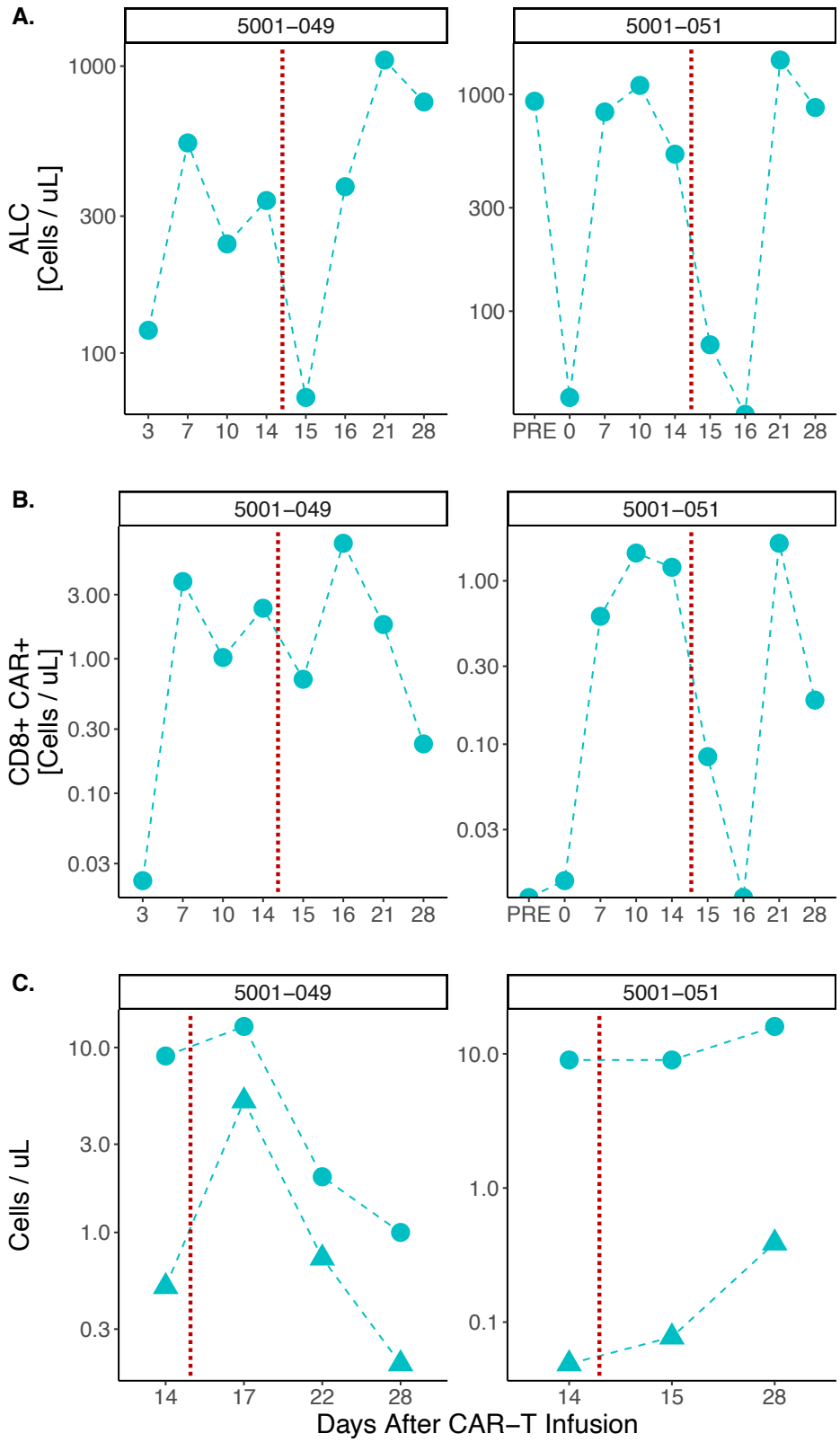
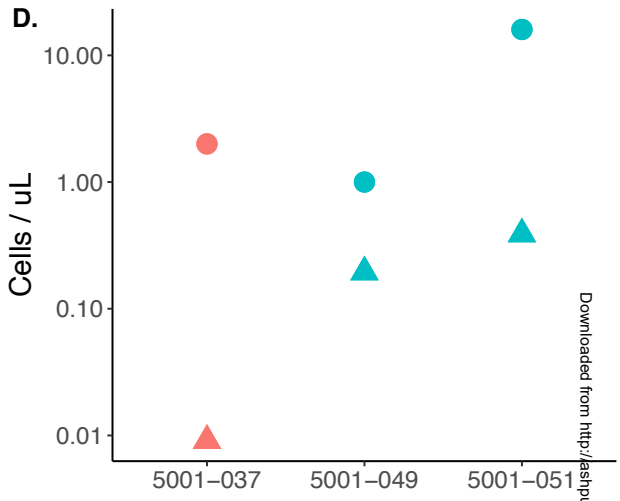


Figure 4



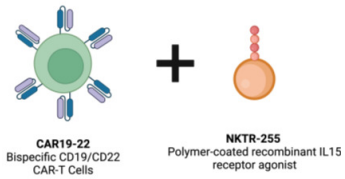


A Phase 1 Clinical Trial of NKTR-255 with CD19-22 CAR-T Cell Therapy for Refractory B-cell Acute Lymphoblastic Leukemia

Context:

CAR19-22 was associated with high response rate but frequent relapses in R/R B-ALL.

We hypothesized a rational combination of CAR19-22 with NKTR-255 (recombinant IL15) would be safe and feasible.



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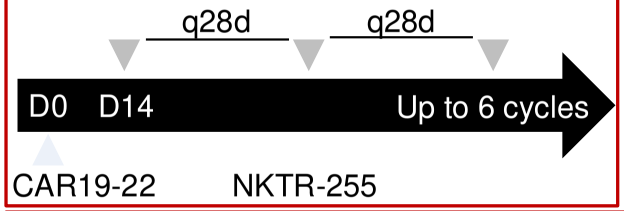
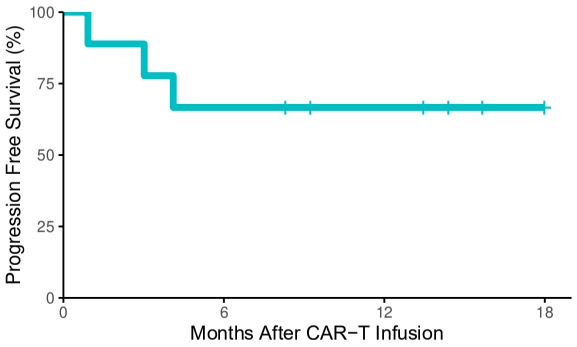


Clinical Outcomes:

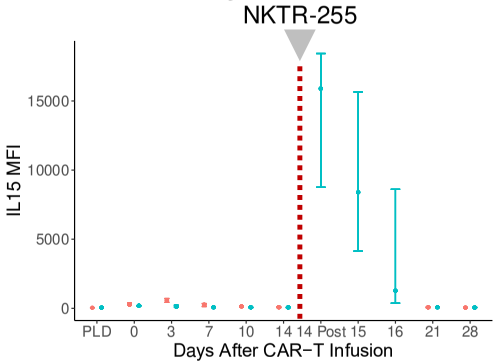


Combining NKTR-255 with CAR19-22 was feasible.

No dose limiting toxicities were seen.

Responses were durable in a heavily pre-treated population.

Correlative Analyses:



NKTR-255 was associated with increases in cytokines (IL15, IFNg) and chemokines (CXCL9, CXCL10).

Dynamic changes in lymphocyte and CAR levels in blood occurred after NKTR-255.

1. **Combining CAR19-22 and NKTR-255 was safe and feasible.**
2. **NKTR-255 was associated with increases in cytokines (IL15 and IFNγ) and related chemokines (CXCL9, CXCL10).**

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