Journal for ImmunoTherapy of Cancer

Efficacy of second CAR-T (CART2) infusion limited by poor CART expansion and antigen modulation

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To cite: Holland EM, Molina JC, Dede K, *et al*. Efficacy of second CAR-T (CART2) infusion limited by poor CART expansion and antigen modulation. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004483. doi:10.1136/jitc-2021-004483

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-004483).

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Accepted 19 April 2022

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ABSTRACT

Chimeric antigen receptor T-cells (CART) are active in relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL), but relapse remains a substantial challenge. Reinfusion with the same CART product (CART2) in patients with suboptimal response or antigen positive relapse following first infusion (CART1) represents a potential treatment strategy, though early experiences suggest limited efficacy of CART2 with CD19 targeting. We report on our experience with CART2 across a host of novel CAR T-cell trials. This was a retrospective review of children and young adults with B-ALL who received reinfusion with an anti-CD19, anti-CD22, or anti-CD19/22 CART construct on one of 3 CAR T-cells trials at the National Cancer Institute (NCT01593696, NCT02315612, NCT0344839) between July 2012 and January 2021. All patients received lymphodepletion (LD) pre-CART (standard LD: 75 mg/m^2 fludarabine, 900 mg/m^2 cyclophosphamide; or intensified LD: 120 mg/m^2 fludarabine, 1200 mg/m^2 cyclophosphamide). Primary objectives were to describe response to and toxicity of CART2. Indication for CART2, impact of LD intensity, and CAR T-cell expansion and leukemia antigen expression between CART infusions was additionally evaluated. Eighteen patients proceeded to CART2 due to persistent (n=7) or relapsed antigen positive disease (n=11) following CART1. Seven of 18 (38.9%) demonstrated objective response (responders) to CART2: 5 achieved a minimal residual disease (MRD) negative CR, 1 had persistent MRD level disease, and 1 showed a partial remission, the latter with eradication of antigen positive disease and emergence of antigen negative B-ALL. Responders included four patients who had not achieved a CR with CART1. Limited cytokine release syndrome was seen following CART2. Peripheral blood CART1 expansion was higher than CART2 expansion (p=0.03). Emergence of antigen negative/dim B-ALL in 6 (33.3%) patients following CART2 contributed to lack of CR. Five of seven (71.4%) responders received intensified LD pre-CART2, which corresponded with higher CART2 expansion than in those receiving standard LD (p=0.029). Diminished CAR T-cell expansion and antigen downregulation/loss impeded robust responses to CART2. A subset of patients, however, may derive benefit from CART2 despite suboptimal response to CART1. Intensified LD may be one strategy to augment CART2 responses, though further study of factors associated with CART2 response, including serial monitoring of antigen expression, is warranted.

Key message

⇒ Suboptimal response and relapse following chimeric antigen receptor T-cell (CART) infusion remain significant challenges in children and young adults receiving CART therapy for relapsed/refractory B-cell acute lymphoblastic leukemia. Reinfusion of the same CART construct (CART2) represents a potential treatment strategy. Evaluation of CART2 outcomes across a host of novel CART constructs and targets revealed minimal CART2 toxicity, with a subset of patients deriving benefit from CART2 despite suboptimal CART1 response. Notable biological limitations, including diminished CART expansion and target antigen downregulation/loss, however, impeded both robust CART2 responses and duration of remission. Given the potential need for and benefit of CART2, strategies to augment CART2 responses, including increased cell dose, routine use of intensified lymphodepletion and postremission consolidative approaches warrant further study.

INTRODUCTION

Chimeric antigen receptor T-cells (CART) have induced remarkable remission rates of 70%–90% in children and young adults with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL).[1–6] Many patients, however, will not achieve a durable remission with CART therapy alone. Relapse following CART therapy remains a significant challenge: approximately 50% will relapse in the first year,[2 7 8] with many retaining expression of the CART target antigen. In patients with residual disease or antigen-positive relapse following CART1, reinfusion of the same CAR T-cell product (CART2) represents a potential treatment strategy; which is to

be distinguished from infusion of a unique CAR T-cell construct (eg, targeting an alternate antigen). Early experiences with CD19 CART reinfusion in B-ALL suggest limited efficacy of CART2.[9 10] Diminished CART expansion, persistence, and development of anti-CAR immune response inhibiting CAR T-cell activity are potential mechanisms of CART2 failure. Further characterization of factors associated with CART2 response is needed.

We evaluated complete remission (CR) rates following CART2 in patients who received reinfusion across several of our phase I CAR T-cell trials using a host of CART constructs and targets. To elucidate factors associated with CART2 response, we additionally evaluated indications for CART2, CART expansion and antigen expression following CART2, and the impact of pre-CART2 lymphodepletion (LD) intensity.

METHODS

Study population

This was a retrospective review (NCT03827343) of children and young adults with r/r B-ALL receiving reinfusion of the same CART product (CART2) on one of three institutional review board approved phase I CAR T-cell trials at the National Cancer Institute (NCI) between 7/2012 and 1/2021. Patients received anti-CD19-28ζ (NCT01593696), anti-CD22-.BBζ (NCT02315612), or anti-CD19/CD22.BBζ (NCT0344839) CAR T-cells. CART2 was offered only to patients who had (1) relapse following a CR with CART1 or (2) suboptimal CART1 response with evidence for CART1 expansion in whom strategies to augment CART2 response were feasible (eg, intensified LD, increased cell dose or reduced disease burden pre-CART2). Patients with disease progression without CART1 expansion were not offered CART2. Four (22.2%) patients received CART2 by individualized compassionate use following CART1 infusion on-study (online supplemental file).

Patients were treated at one of three CAR T-cell dose levels: 3×10^5 cells/kg, 1×10^6 cells/kg, or 3×10^6 cells/kg. Conditioning pre-CART1 and -CART2 was composed of standard LD (fludarabine 75 mg/m^2 and cyclophosphamide 900 mg/m^2) or intensified LD (fludarabine 120 mg/m^2 and cyclophosphamide 1200 mg/m^2), the latter of which was routinely considered for reinfusion based on our initial experiences of suboptimal responses with CART2. Standard FLAG regimen with fludarabine (25 mg/m^2), cytarabine (2000 mg/m^2), and filgrastim (5 µg/kg) was used in 2 CD19 CART patients but was subsequently deemed as suboptimal LD.[5]

Objectives

The primary objective was to evaluate CART2 CR rate and toxicity incidence. Secondary objectives included identifying indication for CART2, characterizing CART1 versus CART2 expansion, describing pre-CART1 and pre-CART2 antigen expression, and evaluating the impact of LD intensity on CART2 outcomes.

Disease assessments

Bone marrow disease was assessed by standard bone marrow morphology and classified as M1 (<5%), M2 (5%–25%), or M3 (>25%). Flow cytometry (FC) was used to evaluate disease at baseline, day 28 (±4 days) post-CART infusion and at best response. The NCI Flow Cytometry Laboratory performed minimal residual disease (MRD) assessment with validated limit of detection of ALL blasts at 0.002% of total cells.[11 12] Disease in the cerebrospinal fluid (CSF) was analyzed by routine cytopathology and FC. Non-central nervous system (CNS) extramedullary disease (EMD) was assessed with 18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/CT.

Objective marrow response was defined as achieving a bone marrow CR (with or without detectable MRD) or partial remission (PR) following CART infusion (online supplemental file). The lower limit of detection for CAR-positive (CAR$^+$) T-cells in the bone marrow by FC was 0.1% for CD19% and 1% for CD22 CAR.

Toxicity evaluations

Cytokine release syndrome (CRS) and neurological toxicity were prospectively evaluated on all studies. For the purposes of consistency, all CRS grading has been reconciled with the American Society for Transplantation and Cellular Therapy CRS consensus criteria.[13] As ICANS grading was not established prior to 2018 and cannot be retrospectively applied to patients and trials conducted prior to this time, neurologic toxicity was assessed by CTCAE and considered severe if the AE was ≥grade 3.

Statistical analyses

Standard descriptive statistics were used to characterize demographics of the cohort. Given the small sample size, non-parametric tests were used for all analyses. Wilcoxon signed-rank tests were used for paired analyses and Mann-Whitney U tests used for all unpaired analyses and performed with Prism GraphPad using threshold of significance $p<0.05$.

RESULTS

Patient characteristics

Eighteen of 136 (13.2%) patients with B-ALL receiving CART1 proceeded to CART2 with the same CART product. Median age at CART1 and CART2 was 18.5 years (range, 7–30 years) and 19 years (range, 8–31 years), respectively. Prior to CART1, patients were heavily pretreated and received a median of 6 lines of therapy (range, 2–13), with 77.8% (n=14) having undergone prior hematopoietic stem cell transplantation (HSCT). A substantial portion had previous immunotherapy exposures: 8 (44.4%) received blinatumomab or inotuzumab ozogamicin and 8 (44.4%) received an alternate anti-CD19- and/or CD22 CART therapy prior to CART1 at our institution (table 1). No patient had interim HSCT between CART1 and CART2.

Table 1 Patient demographics and therapy prior to CART1

	All B-ALL patients n=18
Age at CART1, median (range), years	18.5 (7–30)
Age at CART2, median (range), years	19 (8–31)
Sex, n (%)	
Male	16 (88.9)
Female	2 (11.1)
Race, n (%)	
White	12 (66.7)
Asian	2 (11.1)
Multiple and unknown	4 (22.2)
Ethnicity, n (%)	
Hispanic	3 (16.7)
Non-Hispanic	15 (83.3)
Prior no of lines of therapy excluding CART1, median (range)	6 (2–13)
Prior HSCT, n (%) n=14	
1	11 (61.1)
>1	3 (16.7)
Prior Immunotherapy, n (%) n=8	
Prior blinatumomab	6 (33.3)
Prior inotuzumab ozogamicin	2 (11.1)
Prior alternate CAR T-cell therapy, n (%) n=9 (prior to CART1)	
Prior anti-CD19 CAR	6 (33.3)
Prior anti-CD22 CAR	2 (11.1)
Prior anti-CD19/22 CAR	1 (5.6)

B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; HSCT, hematopoietic stem cell transplantation.

Table 2 Disease status and treatment characteristics at CART1 and CART2

Characteristic	CART1 n=18 (%)	CART2 n=18 (%)
Disease status pre-CART Infusion		
Bone marrow		
MRD-negative CR	0	2 (11.1)*
M1	5 (27.8)	4 (22.2)
M2/M3	13 (72.2)	12 (66.7)
CNS		
CNS1	11 (61.1)	14 (72.2)
CNS1 with flow +disease	7 (38.8)	3 (16.7)
CNS3	0	1 (5.6)
Non-CNS extramedullary disease	5 (27.8)	4 (22.2)
CART dose		
CART1=CART2 dose	15 (83.3)	
CART1 >CART2 dose	2 (11.1)	
CART1 <CART2 dose	1 (5.6)	
Lymphodepletion pre-CART		
Standard LD at CART1 and CART2	7 (38.9)	
Standard LD at CART1, intensified LD at CART2	8 (44.4)	
Intensified LD at CART1, standard LD at CART2	1 (5.6)	
Combination standard LD and FLAG†	2 (11.1)	
Indication for CART2		
Stable disease following CART1	7 (38.9)	
Relapse post-CART1	11 (61.1)	
Time elapsed between CART1 and CART2, median (range), days	116.5 (35–373)	

MRD-negative CR, <0.01% (1×10–4) ALL blasts/MNC by flow cytometry; bone marrow classifications by morphology: M1 bone marrow, <5% blasts; M2 marrow, 5%–25% blasts; M3 marrow, >25% blasts. CNS1, 0 blasts detectable on cytospin; CNS2, WCC < 5/µL, cytospin positive for blasts; CNS3,WCCs ≥ 5 µL, cytospin positive for blasts.
*Concurrent CNS disease (one patient with stable disease, one patient with relapsed disease). Standard LD: fludarabine 25 mg/m^2 × 3 days (–4, –3, –2), cyclophosphamide 900 mg/m^2 × 1 day (–2). Intensified LD: fludarabine 30 mg/m^2 × 4 days (–5, –4, –3, –2), cyclophosphamide 600 mg/m2 × 2 days (–3, –2). FLAG: fludarabine 25 mg/m^2 × 5 days (1–5), cytarabine 2000 mg/m^2 × 5 days (1–5), filgrastim 5µg/kg (day –1 through ANC>1000 ×2 days after nadir).
†Two CD19 CART patients received standard LD and FLAG: one with FLAG pre-CART1 and standard LD pre-CART2 and the other with standard LD pre-CART1 and FLAG pre-CART2.
ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CNS, central nervous system; CR, complete remission; FLAG, fludarabine, cytarabine, filgrastim; LD, lymphodepletion; MRD, minimal residual disease.

Disease characteristics

All patients had active medullary disease at CART1. Over half (n=13, 72.2%) had high disease burden (M2/M3) marrow involvement while 5 (27.8%) had <5% blasts (M1). Seven (38.9%) had CNS disease detectable by FC, though all maintained CNS1 status. Non-CNS EMD was identified in 5 (27.8%) patients (table 2). Sites of EMD included the lymph nodes, liver, stomach, kidneys, pancreas, and skin (leukemia cutis), results for which have been recently reported.[14]

Pre-CART2, 16 of 18 (88.9%) had active medullary disease, 4 (22.2%) with an M1 marrow and 12 (66.7%) with high disease burden. Two (11.1%) patients had isolated CNS disease despite attaining a medullary MRD negative CR with CART1, and 4 (22.2%) had any CNS involvement (CNS1 with FC detectable disease, n=3; CNS3 white cell count ≥5 µL, cytospin positive for blasts, n=1). Four (22.2%) had persistent non-CNS EMD pre-CART2 (table 2).

Response to CART1 and indications for CART2

CART2 was indicated for suboptimal response (PR or stable disease (SD)) to CART1 in 7 (38.9%) patients or for antigen-positive relapse in 11 (61.1%) following CART1 (figure 1A, online supplemental table 1). Two patients proceeded to CART2 for persistent or relapsed

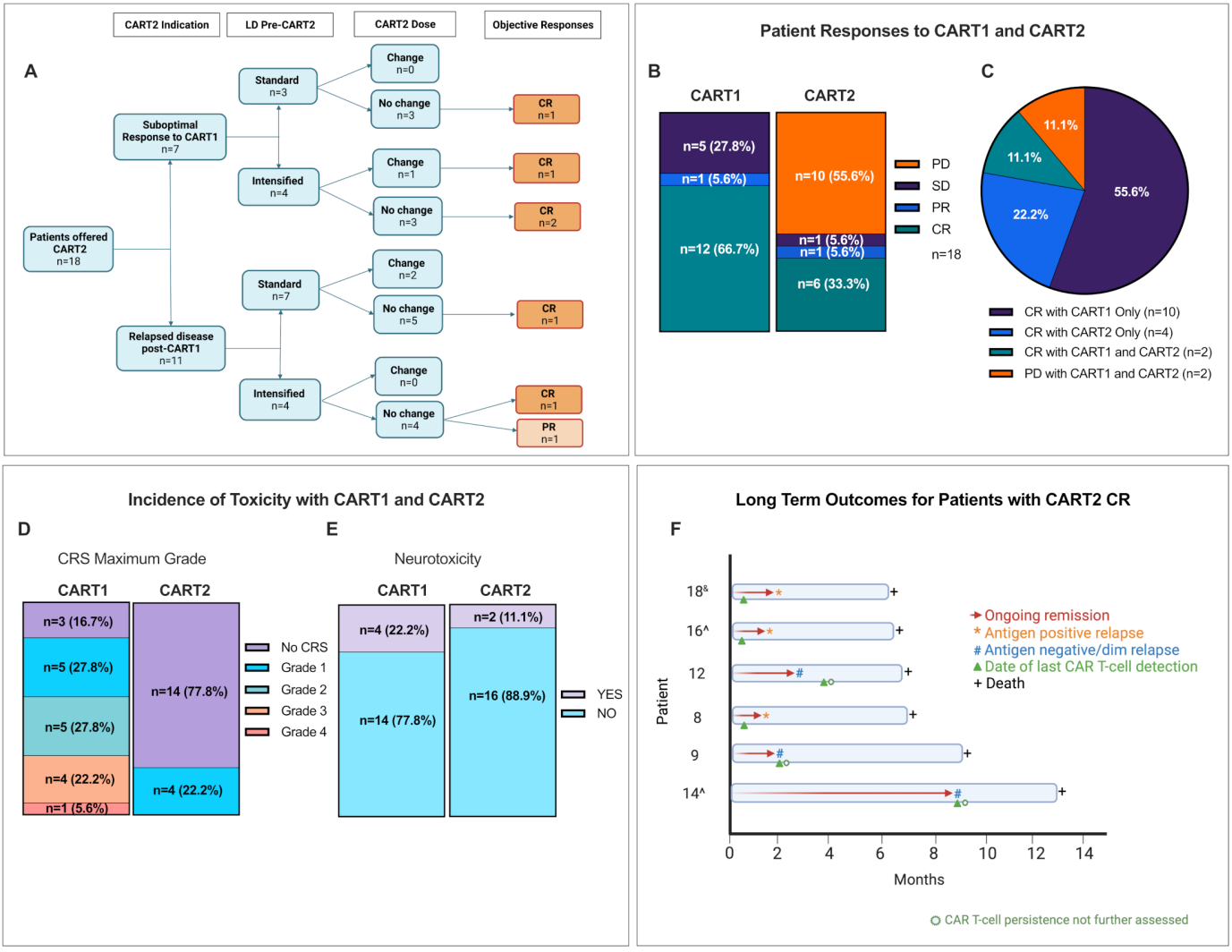

Figure 1 Patient responses to chimeric antigen receptor T-cells (CART1) and CART2. (A) Patients offered treatment with a reinfusion strategy (CART2) and modifications made to CART2 regimen. (B) Response to CART1 and CART2 at best response and (C) stratified by patients demonstrating a CR with CART1, CART2, or both CART1 and CART2. (D) Incidence of CRS and (E) neurotoxicity at CART1 and CART2. (F) Long-term course for 6 of 18 (33.3%) patients demonstrating a bone marrow CR with CART2 with timepoint of last CAR T-cell detection and disease phenotype at relapse indicated. ^Denotes patients who experienced central nervous system (CNS) relapse, while &indicates those with combined medullary and non-CNS extramedullary (EMD) relapse. Notably, patient 14 experienced relapse post-CART2 with isolated CNS disease and a myeloid sarcoma.[20] CR, complete remission; CRS, cytokine release syndrome; PD, progressive disease; PR, partial remission; SD, stable disease.

CNS disease despite attaining a medullary MRD negative CR with CART1. Median time between CART1 and CART2 was 116.5 days (range, 35–373 days). Seven (38.9%) patients received interim therapy to prevent or treat progressive disease between CART infusions (online supplemental table 2). No patient had interim HSCT. All patients offered CART2 had evidence of CART1 expansion, with 6 (33.3%) having residual low-level detectable CART1 cells prior to initiation of CART2 LD.

LD and CAR T-cell dose

Fifteen (83.3%) patients were treated at the same dose level for CART1 and CART2. Cell dose was lowered in 2 CD19 CART patients due to prior dose-limiting toxicity at a higher dose level. One CD19/22 CART patient received a higher CART2 dose relative to CART1 due to suboptimal CART1 response (figure 1A, table 2). All patients received LD pre-CART1 and -CART2. Eight (44.4%) patients received intensified LD pre-CART2 (figure 1A, table 2).

Response and toxicity with CART2

All 18 patients proceeded to CART2 using a cryopreserved aliquot of their original CART1 product. Seven of 18 (38.9%) patients demonstrated objective marrow response to CART2, with 6 (33.3%) attaining a morphologic CR; 5 of whom received CD22 CART (4 with CR). Five of seven (71.4%) marrow responders achieved an MRD negative CR, and one had persistent MRD level disease by FC. One patient with a bone marrow PR showed

clearance of antigen positive disease but emerged from CART2 with antigen loss. Among four patients with CNS involvement, two (50.0%) had full eradication of CNS disease with CART2, including the patient with CNS3 disease who had simultaneous eradication of non-CNS EMD.

Among seven CART2 responders, four had not achieved a CR with CART1 (PR, n=1; SD, n=3). Of these four, three (75%) received intensified LD, including one patient who also had improved disease control pre-CART2 and one who received a higher CART2 dose. One CART1 non-responder who required early steroids due to rapid disease progression abrogating CART1 expansion achieved a CR with CART2 following standard LD with lower disease burden (figure 1B,C).

Fifteen (83.3%) patients had CRS with CART1, with 5 (27.8%) experiencing severe (≥grade 3) CRS (figure 1D). In contrast, CRS occurrence and severity was limited following CART2. The majority (n=14, 77.8%) did not develop CRS with CART2, likely due to poor expansion. Maximum CRS grade 1 was observed in four patients (22.2%), with all toxicities transient and reversible.

Few experienced symptoms of neurological toxicity with CART1 or CART2, all of which were mild (grade 1 or grade 2). Four of 18 (22.2%) had neurotoxicity following CART1, while 2 (11.1%) had neurotoxicity with CART2 (figure 1E). Of four patients with CART1-associated neurotoxicity, only one subsequently experienced neurotoxicity with CART2.

Long-term survival across six patients achieving a CR with CART2 was limited. No patient was eligible for consolidative HSCT and all patients experienced relapse, either with persistent CAR T-cells and antigen escape (n=3); or with loss of CAR T-cells and preserved antigen expression (n=3). Median duration of remission was 77 days (range, 54–292 days) (figure 1F). Relapse included two patients with CNS disease; one with combined EMD and medullary relapse and three with medullary relapse.

CART2 expansion

Peripheral blood absolute CAR T-cell expansion was substantially higher with CART1 (median, 24.05 cells/mL; range, 0.35–13 653.0 c/mL) than with CART2 (median, 1.69 c/mL; range, 0–2886.0 c/mL) (p=0.03). Specifically, 5 (27.8%) of 18 failed to demonstrate any CART2 expansion which correlated with non-response (figure 2A). CD22 CART expansion was generally higher with both CART1 and CART2, as previously reported (online supplemental file).[15] CAR T-cell trafficking to the CSF was seen in 5 (35.7%) of 14 patients where lumbar punctures were performed and largely correlated with those who also had more robust PB expansion (online supplemental file).

Factors associated with response to CART2

We additionally interrogated potential associations between LD intensity, disease status, and CART2 response. Intensified LD pre-CART2 corresponded with higher CART2 expansion (median, 5.40 c/mL; range, 0.63–2886 c/mL) than in those receiving standard LD (median, 0 c/mL; range, 0–196.8 c/mL) (p=0.029) (figure 2A). Five of seven (71.4%) CART2 responders received intensified LD pre-CART2, with one concurrently receiving a higher CART2 dose. Pre-CART2 disease burden tended to be lower in marrow responders (median, 7.8% B-ALL/ MNCs by flow cytometry; range, 0%–62.9%) than in non-responders (median, 29.3% B-ALL/MNCs; range, 0%–95.0%) (p=0.16). Notably, five of seven (71.4%) responders had received interim therapy to treat or prevent progressive disease following CART1 (online supplemental table 2).

Antigen density and modulation

Paired analysis of antigen binding capacity (ABC) pre-CART1 and pre-CART2 revealed a trend toward decreased antigen expression pre-CART2 in a subset of patients (n=10) with evaluable serial data (figure 2B,C). In two (11.1%) with increased antigen expression pre-CART2, higher ABC did not correlate with CART2 response. Emergence of antigen negative/dim B-ALL in six (33.3%) patients following CART2 also contributed to suboptimal responses (figure 2D–F). Antigen modulation was evident post-CART1/pre-CART2 with partial expression (figure 2D) and diminution (figure 2E), highlighting the importance of serial monitoring of antigen expression.

DISCUSSION

CAR T-cells induce remissions in children and young adults with r/r B-ALL,[1–6] but relapse after and suboptimal response to first infusion (CART1) remain significant challenges. Optimization strategies for CART2 are needed, particularly for those with antigen positive relapse or poor CART1 response. Prospective study of reinfusion has been limited, and early data from retrospective reviews suggests that CART2 responses with CD19 targeting are suboptimal.[9 10] Our data describe outcomes of reinfusion across a host of CAR T-cell targets and constructs, providing biological insights into limitations of CART2 response and a strategic framework for considering CART2 optimization strategies.

Responses to CART2 were limited in our heavily pretreated B-ALL cohort. Objective response rate with CART2 was 38.9% in contrast to 72.2% with CART1. Poor CART2 response correlated with a significant lack of CART2 expansion, though intensified LD to enhance expansion represents a potential strategy to overcome rejection mechanisms. Decreased antigen expression pre-CART2 and antigen escape following CART2 was also observed. Our results suggest that diminished CAR T-cell expansion alongside antigen downregulation and loss impeded robust responses to CART2. Further exploration of the mechanisms underlying CART2 response is needed.

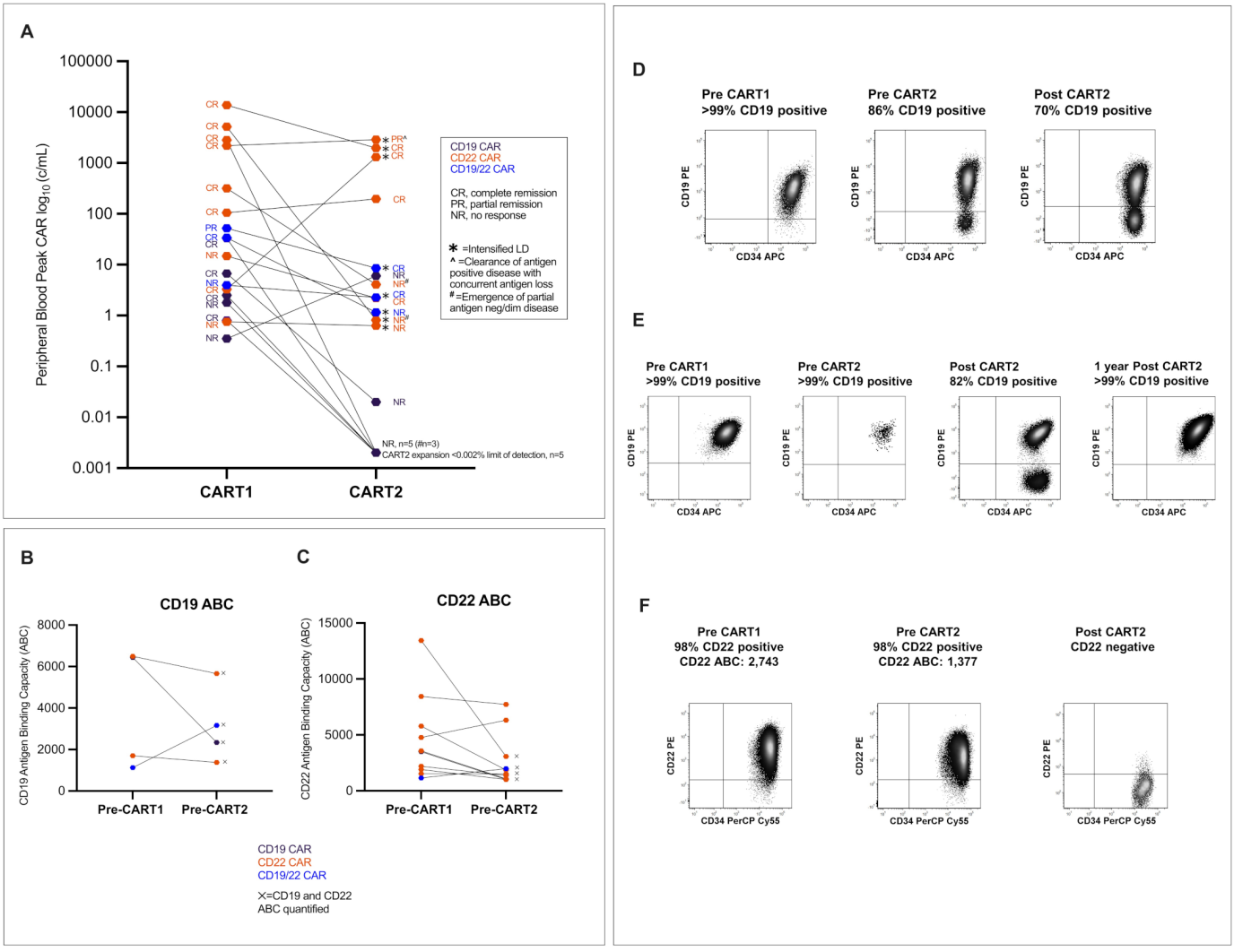

Figure 2 Antigen density and modulation. (A) Peripheral blood absolute CAR T-cell peak expansion at CART1 and CART2 with best response, intensity of lymphodepletion, and status of antigen expression indicated. (B) Paired analysis of CD19 antigen expression for four patients (CD19, n=1; CD22, n=2; CD19/22, n=1) with evaluable data prior to CART1 and CART2. Median pre-CART1 CD19 antigen binding capacity (ABC) was 4069 (range, 1124–6494) compared with median pre-CART2 CD19 ABC of 2753 (range, 1376–5657) (p=NS). (C) Paired analysis of CD22 antigen expression performed for 10 patients (CD19, n=1; CD22, n=8; CD19/22, n=1) with available serial data demonstrated a trend toward diminished CD22 ABC pre-CART2 (median, 1678; range, 1012–7727) compared with pre-CART1 ABC (median, 3537; range, 1150–13435) (p=0.084). Patients who had both CD19 and CD22 expression quantified are indicated. (D) Flow cytometry showing partial loss of CD19 expression from B-lymphoblasts after CART1 and CART2 (162 days apart) in CD19/22 CART patient 17. (E) CD19 CART patient three shows partial loss of CD19 expression after CART1 and CART2 (204 days apart), with disease regaining full expression of CD19 1 year post-CART2. (F) Flow cytometry showing diminished CD22 expression on CD22 CART patient 10's B-lymphoblasts after CART1, with disease becoming fully CD22 negative after CART2 (125 days after CART1). ABC, antigen binding capacity; CART, chimeric antigen receptor T-cells.

We also investigated indication for reinfusion as a potential prognostic factor affecting CART2 outcomes. In our small cohort, four of seven (57.1%) patients with suboptimal CART1 response had objective response to CART2. Of 11 who underwent CART2 for antigen positive relapse, only 3 (27.3%) had objective response (online supplemental table 1). Our numbers were small but suggest that even those without an initial response to CART therapy have potential to derive benefit from reinfusion. Furthermore, we demonstrate that CART2 has the potential to traffic to and eradicate CNS disease. Importantly, CART2 was strategically offered only to those who had evidence of CART1 expansion and for whom some aspect of CART2 infusion could be modified. This included: (1) change in CART dose; (2) change in LD or (3) change in pre-CART2 disease burden to collectively augment CART expansion and potential for response. We postulate that these factors collectively may explain the differences between our results and those of Myers *et al*, who found reinfusion ineffective in patients with CART1 non-response.[16] Consideration of individual patient parameters and

CART1 response will be critical to identifying who may benefit from CART2. Still, likelihood of response with CART2 remains low, and high relapse risk following CART2 limited long-term cure (figure 1F).[2 7 8] Therefore, in eligible patients achieving a CR with CART2, consolidation measures such as HSCT should be considered to extend durable remission.

Interestingly, nearly half (n=8, 44.4%) of patients in this analysis received prior CART therapy elsewhere before treatment on our studies. Of six with a CART2 CR, three (50%) had received an alternate CART construct before CART1 infusion. While we only report on CART1 and CART2 outcomes with our constructs here, we expect to see an increasing frequency of patients receiving multiple CART products as post-CART relapse occurs. Our findings suggest that receipt of an alternate CART construct does not preclude potential benefit from reinfusion, though the impact of multiple prior CART therapies on subsequent responses warrants investigation.

Limitations of our study included the heterogeneity of CART constructs analyzed and retrospective nature of our analysis. However, prospective reinfusion studies can be challenging to enroll on, as seen with the recently completed tisagenlecleucel reinfusion trial (NCT04225676). Furthermore, given challenges in testing and assessing the impact of immunogenicity on CART outcomes,[17 18] our study does not include these analyses. CART constructs incorporating fully humanized single chain variable fragments (scFvs) have been hypothesized to be less immunogenic than those with murine-derived components.[16 19] This may potentially explain the more robust CART2 expansion seen with our fully humanized CD22.BBζ construct (figure 2A) compared with the more immunogenic murine-based CD19 CART constructs (online supplemental file). Further study is needed to clarify how reinfusion strategies may differ between murine versus humanized CART constructs. Additionally, while our studies required active disease at CART2, given improved CART responses in low-disease burden, efforts to use reinfusion for relapse prevention are underway.[10] Such strategies have since been incorporated into our protocols.

In conclusion, our study provides important insights into CART2 outcomes, including with antigen targets beyond CD19. Future strategies to augment response to CART reinfusion may include strategic planning for modifications to CART2 such as routine use of intensified LD for patients with prior CART exposure. Consideration of the most appropriate antigen target for additional CART infusions will also be critical, particularly since antigen downregulation/loss impeded CART2 response. Given the limitations of CART reinfusion in an era with emerging novel CART constructs, our future efforts will further explore optimal timing of reinfusion strategies and when treatment with an alternate CART construct should be prioritized, especially in those receiving sequential CART infusions.

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Acknowledgements We gratefully acknowledge the study participants and their families, referring medical care teams, the faculty and staff of the NIH Clinical Center who provided their expertise in the management of the study participants, and the data managers and research nurses and patient care coordinators involved with this work. This work was supported in part by the Intramural Research Program, Center of Cancer Research, National Cancer Institute and NIH Clinical Center, National Institutes of Health (ZIA BC 011823, N.N.S). Figure 1A,F were created with BioRender.com.

Contributors EMH and NS wrote the first version of the manuscript. EMH, JCM, KD, BY, and NS, performed primary data analysis. DM, TZ, CMY, H-WW and MS-S, performed flow cytometry and analyzed results. SP, SH, DS, led efforts for CAR T-cell manufacturing for constructs described on this study. CM, TJF, LL, DWL, HS, BY and NS, provided patient care. No non-author wrote the first draft or any part of the paper. All authors contributed critically to the manuscript, reviewed the final manuscript, and have agreed to be coauthors.

Funding This work was supported in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research and the Warren Grant Magnuson Clinical Center. DWL consults for Harpoon Therapeutics, serves on external advisory boards for BMS/Juno Therapeutics and Amgen, and his institution (UVA) receives clinical trial support from Gilead/Kite Pharma. CM consults for Lyell, Syncopation, Nektar, Bristol Myers Squibb, Glaxo Smith Kline, Immatics, Neoimmune Tech, Apricity, Mammoth and Ensoma, holds equity in Lyell, Syncopation, Apricity, Mammoth and Ensoma and receives research funding from Lyell. All funding was provided by the NIH Intramural Research Program (ZIA BC 011823, NS).

Disclaimer The content of this publication does not necessarily reflect the views of policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by National Institutes of Health Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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