T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial

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Summary

Background Chimeric antigen receptor (CAR) modified T cells targeting CD19 have shown activity in case series of patients with acute and chronic lymphocytic leukaemia and B-cell lymphomas, but feasibility, toxicity, and response rates of consecutively enrolled patients treated with a consistent regimen and assessed on an intention-to-treat basis have not been reported. We aimed to define feasibility, toxicity, maximum tolerated dose, response rate, and biological correlates of response in children and young adults with refractory B-cell malignancies treated with CD19-CAR T cells.

Methods This phase 1, dose-escalation trial consecutively enrolled children and young adults (aged 1–30 years) with relapsed or refractory acute lymphoblastic leukaemia or non-Hodgkin lymphoma. Autologous T cells were engineered via an 11-day manufacturing process to express a CD19-CAR incorporating an anti-CD19 single-chain variable fragment plus TCR zeta and CD28 signalling domains. All patients received fludarabine and cyclophosphamide before a single infusion of CD19-CAR T cells. Using a standard 3+3 design to establish the maximum tolerated dose, patients received either 1×10^6 CAR-transduced T cells per kg (dose 1), 3×10^6 CAR-transduced T cells per kg (dose 2), or the entire CAR T-cell product if sufficient numbers of cells to meet the assigned dose were not generated. After the dose-escalation phase, an expansion cohort was treated at the maximum tolerated dose. The trial is registered with ClinicalTrials.gov, number NCT01593696.

Findings Between July 2, 2012, and June 20, 2014, 21 patients (including eight who had previously undergone allogeneic haematopoietic stem-cell transplantation) were enrolled and infused with CD19-CAR T cells. 19 received the prescribed dose of CD19-CAR T cells, whereas the assigned dose concentration could not be generated for two patients (90% feasible). All patients enrolled were assessed for response. The maximum tolerated dose was defined as 1×10^6 CD19-CAR T cells per kg. All toxicities were fully reversible, with the most severe being grade 4 cytokine release syndrome that occurred in three (14%) of 21 patients (95% CI 3·0–36·3). The most common non-haematological grade 3 adverse events were fever (nine [43%] of 21 patients), hypokalaemia (nine [43%] of 21 patients), fever and neutropenia (eight [38%] of 21 patients), and cytokine release syndrome (three [14%] of 21 patients).

Interpretation CD19-CAR T cell therapy is feasible, safe, and mediates potent anti-leukaemic activity in children and young adults with chemotherapy-resistant B-precursor acute lymphoblastic leukaemia. All toxicities were reversible and prolonged B-cell aplasia did not occur.

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Introduction

B-precursor acute lymphoblastic leukaemia (B-ALL) is the most common malignancy in childhood. Newly diagnosed children have about 90% survival, but cure needs prolonged therapy with substantial short-term and long-term toxicities.[1,2] Adults with B-ALL have lower survival rates, partly because of a high frequency of subtypes with less chemosensitivity.[3,4] Irrespective of age, patients with primary or recurrent refractory B-ALL who do not have complete remission negative for minimum residual disease (MRD) with cytotoxic chemotherapy have dismal survival rates of less than 10%, and outcomes for these patients have not improved substantially in the last two decades.[5–7]

Chimeric antigen receptors (CARs) incorporate an antigen recognition sequence, such as a single-chain variable fragment (scFv) of a monoclonal antibody, with intracellular signalling domains that activate the T cell.[8] Although several case series have reported antitumour effects of autologous CD19-directed CAR T cells in patients with B-cell lymphoma,[9,10] chronic lymphocytic leukaemia,[11,12] and B-ALL,[12–15] results of an intention-to-treat protocol of sequentially enrolled patients treated with a consistent regimen has not been reported. In this phase 1 trial we define feasibility, toxicity, maximum tolerated dose, response rate, and biological correlates of response in 21 consecutively enrolled children and young adults

with refractory B-cell malignancies treated with CD19-CAR T cells.

Methods

Study design and participants

We did an open-label, phase 1 dose-escalation study of CD19-CAR T cells in children and young adults with ALL or non-Hodgkin lymphoma. Patients were screened and treated in the Pediatric Oncology Branch of the National Cancer Institute (NCI) at the Clinical Center of the US National Institutes of Health. Data are presented until July 18, 2014, and responding patients continue to be followed up for survival, relapse, and as mandated by the US Food and Drug Administration (FDA),[16] which requires 15 years of follow-up.

Eligible patients were aged 1–30 years with CD19+ B-ALL or non-Hodgkin lymphoma, relapsed or refractory to standard therapy plus at least one salvage regimen. Eligibility required measurable disease, adequate performance status, and organ function. Previous recipients of allogeneic haematopoietic stem-cell transplantation (HSCT) were eligible if more than 100 days post-transplant, without evidence of graft versus host disease (GVHD), and did not require immunosuppression. Patients with no detectable leukaemia in the CSF (CNS1), and those with CNS2 leukaemia (<5 white blood cells per μL and cytology positive for blasts) without clinically evident neurological changes, were eligible. Patients with CNS2 and neurological changes or CNS3 leukaemia (appendix) or isolated extramedullary leukaemia were ineligible. The protocol and its amendments were approved by the NCI institutional review board. Patients or their parents provided written, informed consent, and minor assent when appropriate, before participation.

See Online for appendix

A 3+3 dose-escalation schema was used. Patients whose CAR T-cell product did not meet the dose to which they were assigned did not inform dose escalation but were assessed for toxicity and for all other parts of the study. Because patients who had previously undergone allogeneic HSCT could have more severe toxicity (eg, GVHD or related to transplant-associated comorbidities) than patients without a history of HSCT, dose escalation for patients with HSCT was informed by toxicities recorded in the cohort that have not previously received HSCT, but not vice versa. According to protocol guidelines, 1×10^6 CAR T cells per kg was identified as the maximum tolerated dose, first in the non-HSCT group, at which time we had seen no GVHD in the post-HSCT group and all dose-limiting toxicities were associated with cytokine release syndrome. No evidence existed that toxicities differed in incidence or severity in patients with HSCT versus those without and we had noted a similar response rate in both HSCT and non-HSCT cohorts treated at the 1×10^6 cells per kg dose concentration. Therefore, the protocol was amended to expand the total number of patients treated at 1×10^6 cells per kg to 15 assessable patients to gain more experience with the therapy, with no difference in cell dose based on previous HSCT.

Procedures

Patients underwent leukapheresis to obtain peripheral blood mononuclear cells. CAR T-cell manufacturing commenced on the day of apheresis and was completed in 11 days according to published methods.[17] Clinical grade MSGV-FMC63-28Z retroviral vector supernatant was produced as previously described.[18] Cells were released for infusion if they met predefined release criteria for viability, sterility, and percent CAR transduction and were negative for replication-competent retrovirus by PCR as directed by the FDA.[16]

Patients were given fludarabine 25 mg/m² per day on days –4, –3, and –2 and cyclophosphamide 900 mg/m² per day on day –2. Cells were infused over 30 min on day 0, with a 7-day delay permitted to allow for resolution of intercurrent clinical conditions. Dose 1 was 1×10^6 CAR-transduced T cells per kg (within 20%) and dose 2 was 3×10^6 CAR+ T cells per kg (within 20%). Patients received prophylactic intrathecal chemotherapy during eligibility assessment, but prophylactic intrathecal chemotherapy was not administered after cell infusion.

Toxicity was monitored according to the protocol with NCI Common Terminology Criteria for Adverse Events v4.02 for grading severity of adverse events except for cytokine release syndrome, which was graded according to a revised grading system (appendix). Non-haematological dose-limiting toxicities was any toxicity of grade 3 or higher occurring within 28 days of the CD19-CAR T-cell infusion judged possibly related to the treatment regimen, with exceptions detailed in the appendix. Haematological dose-limiting toxicities was grade 4 toxicity (except lymphopenia) lasting more than 30 days if not attributable to underlying disease.

Response assessment was done on day 28 (within 4 days) after CD19-CAR T-cell infusion. MRD negative was defined as less than 0·01% marrow blasts by flow cytometry. Complete response was less than 5% marrow blasts, absence of circulating blasts, and no extramedullary sites of disease with absolute neutrophil count 1000 per μL or more and platelets 100 000 per μL or more. Complete response with incomplete count recovery was a complete response with cytopenia. Stable disease was disease that did not meet criteria for complete response, complete response with incomplete count recovery, or progressive disease. Progressive disease was defined as worse M status (appendix) or no change in M status but greater than 50% increase in absolute peripheral blast count. Response in lymphoma was defined according to Cheson and colleagues.[19] After completion of CD19-CAR therapy, patients achieving a MRD-negative complete response proceeded to HSCT if recommended by their treating physicians. All patients were followed up until relapse or death. Patients who

responded but had remaining or recurrent detectable disease were allowed to receive a second cycle of the lympho-depleting regimen and CAR T cells.

Flow cytometry was used to quantitate disease burden and CD19-CAR T cells in blood, marrow, and CSF using the anti-idiotype mAb 136.20.1 as described.[20] Circulating CAR T-cell numbers were calculated on the basis of estimated blood volume and measured absolute lymphocyte counts. CD19 site density on blasts was enumerated by flow cytometry according to manufacturer's instructions (QuantiBRITE Beads, BD Biosciences, San Jose, CA, USA). CD19-CAR T cell expansion was also measured by quantitative PCR (qPCR) (item AJHSOAH; Life Technologies, Grand Island, NY, USA), and adapted from published methods.[21] Briefly, measured CAR copies per 100 ng DNA were normalised to the input quantity of amplifiable DNA by measurement of the single-copy gene, *CDKN1a*. Plasma and CSF were cryopreserved before measurement of interferon γ, interleukin 2, tumour necrosis factor α (TNFα), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 6, and interleukin 10 in a multiplex format according to manufacturer's instructions (MesoScaleDiscovery, Gaithersburg, MD, USA).

Outcomes

Our primary objectives were to define the maximum tolerated dose of CD19-CAR T cells, to describe the toxicity of the regimen, and determine the feasibility of generating CD19-CAR T cells in this population. Secondary objectives sought to define response rate, measure expansion and persistence of CD19-CAR T cells in the peripheral blood, bone marrow, and CSF, and identify biological correlates of clinical outcomes and toxicity.

Statistical analyses

All 21 patients enrolled on the protocol as of June 20, 2014, were included in the analyses. For two patients whose cell expansion was insufficient to meet the prescribed cell dose, products were infused and patients were included in assessments of feasibility and response, but toxicities in these patients did not inform dose escalation, and additional patients were added to that cohort to assess toxicity. Overall survival and leukaemia-free survival probabilities were determined by the Kaplan-Meier method, using all enrolled patients to determine overall survival and those with MRD-negative response for leukaemia-free survival. Detailed descriptions of other statistical analyses are provided in the appendix.

Role of the funding source

No commercial interests were involved in the study. The investigational new drug application was held by CLM and all funding was provided by the NIH Intramural Research Program with the exception of the St Baldrick's Foundation Scholar award provided to DWL. The authors designed the study, collected, and interpreted the data, and wrote the report. DWL and CLM had complete access to all the data and had final responsibility for the decision to submit for publication.

Results

Between July 2, 2012, and June 20, 2014, 21 patients were enrolled and all received CAR T cells. Table 1 shows patient demographics and clinical characteristics. All were heavily pretreated with cytotoxic chemotherapy (appendix). Six patients with B-ALL had primary refractory disease and had never attained an MRD-negative remission despite many intensive chemotherapy regimens. Eight had previously undergone allogeneic HSCT. One patient had previously received CD19-CAR T-cell therapy at another institution with no response. Two patients had measurable CNS leukaemia. Median leukaemia burden was 26% marrow blasts (IQR 2·9–69·5), and median circulating CD3 cell count was 725 cells per μL (IQR 418–1032). Mean CD19 molecules measured per blast in individual patients was 8379 (SD 4921; appendix). Differences in number of CD19 molecules per blast in responding versus non-responding patients were not significant (p=0·078), although the power of this analysis is low to detect differences because of the small number of samples studied.

Mean CAR transduction efficiency was 66·0% (95% CI 55·1–76·8). Protocol-prescribed CD19-CAR T-cell doses were successfully produced for 19 of 21 patients for a 90% feasibility rate (95% CI 69·6–98·8). Patient 2 enrolled with marked lymphopenia (CD3 cell count 66 cells per μL) after a clofarabine-based regimen, received 28 000 CAR-transduced T cells per kg (3% of prescribed dose) and had stable disease. Patient 5 received 480 000 CAR+ T cells per kg (16% of prescribed dose), and had an MRD-negative complete response then underwent HSCT and remains disease-free 16 months after CD19-CAR therapy. Because the dose concentration was not met in these two patients, they were not assessed for maximum tolerated dose but were assessed for all other parts of the study. All patients received fresh CD19-CAR T-cell infusions on day 0, except patient 8, whose infusion was cryopreserved and delayed until day 5 for resolution of *Clostridium difficile* infection.

Patients 1–10 were enrolled during the dose-escalation phase. The first three assessable patients (two post-HSCT, one non-HSCT) received the starting dose 1 (1×10^6 cells per kg), and none had dose-limiting toxicity. Because the non-HSCT group did not inform dose escalation in the post-HSCT group, we escalated the non-HSCT cohort to dose 2 (3×10^6 cells per kg) and treated a third post-HSCT patient (patient 8) at dose 1 without dose-limiting toxicity before escalating that cohort. At dose 2, two patients of four (one non-HSCT and one post-HSCT) had dose-limiting toxicity (one grade 4 CRS, one grade 3 CRS), which defined the maximum tolerated dose for the entire cohort as 1×10^6 CAR+ T cells per kg.

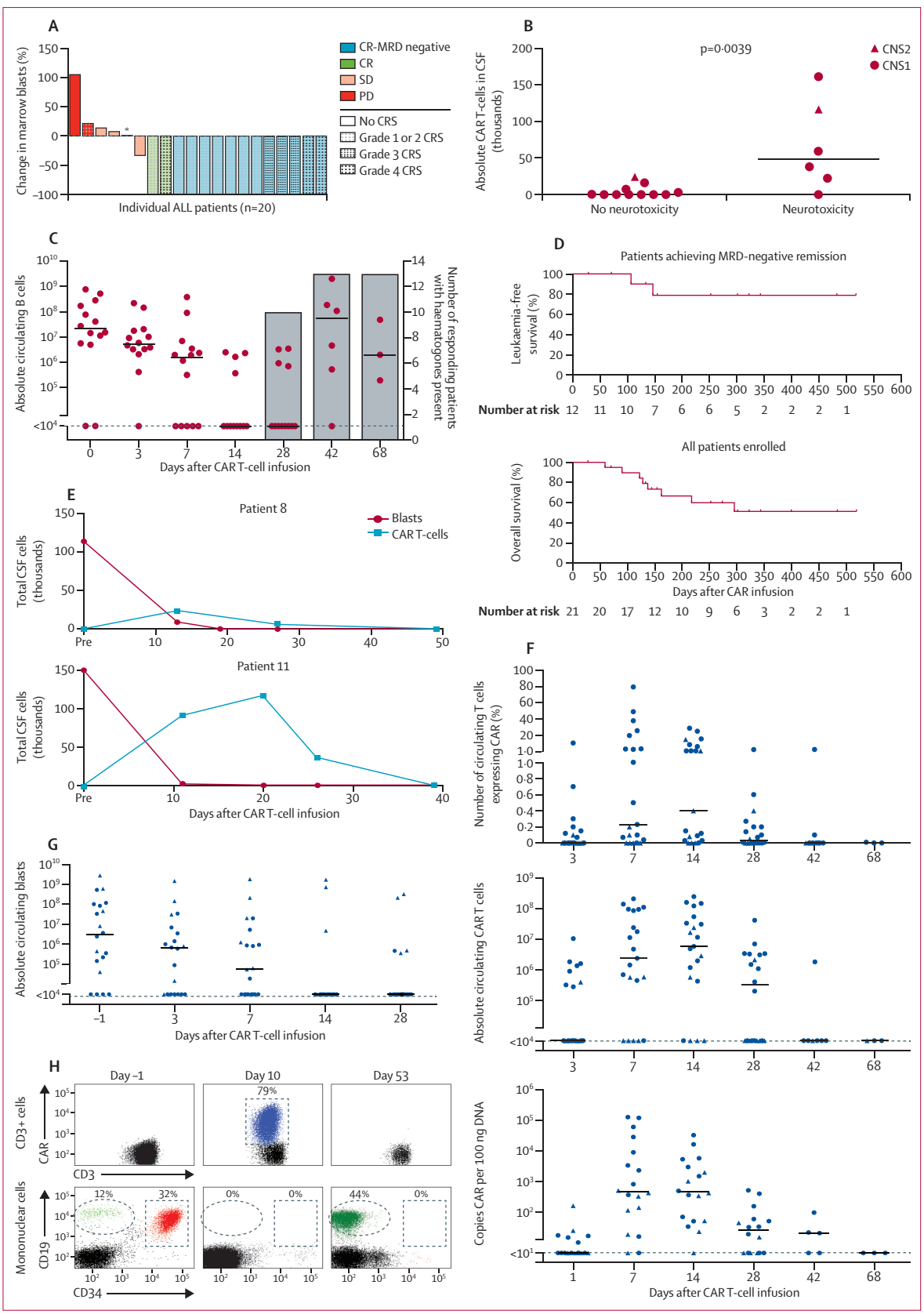
A
Change in marrow blasts (%)
CR-MRD negative
CR
SD
PD
No CRS
Grade 1 or 2 CRS
Grade 3 CRS
Grade 4 CRS
Individual ALL patients (n=20)
B
Absolute CAR T-cells in CSF (thousands)
p=0·0039
CNS2
CNS1
No neurotoxicity
Neurotoxicity
C
Absolute circulating B cells
Number of responding patients with haematogones present
Days after CAR T-cell infusion
D
Patients achieving MRD-negative remission
Leukaemia-free survival (%)
Number at risk 12 11 10 7 6 6 5 2 2 2 1
All patients enrolled
Overall survival (%)
Days after CAR infusion
Number at risk 21 20 17 12 10 9 6 3 2 2 1
E
Patient 8
Blasts
CAR T-cells
Total CSF cells (thousands)
Patient 11
Days after CAR T-cell infusion
F
Number of circulating T cells expressing CAR (%)
Absolute circulating CAR T cells
Copies CAR per 100 ng DNA
Days after CAR T-cell infusion
G
Absolute circulating blasts
Days after CAR T-cell infusion
H
Day –1
Day 10
Day 53
CD3+ cells
CAR
CD3
Mononuclear cells
CD19
CD34
12%
32%
79%
0%
0%
44%
0%

Figure 1: **Clinical activity and expansion of CD19-chimeric antigen receptor (CAR) T cells**
(A) Waterfall plot of the percent change in bone marrow blast frequency from baseline to day 28, response, and cytokine release syndrome (CRS) grading in all 20 patients with B-precursor acute lymphoblastic leukaemia (B-ALL) treated. *One patient with progressive disease (PD) because of a greater than 50% increase in circulating blasts. (B) CAR T cells in the CSF of 17 patients with B-ALL who underwent lumbar puncture within 1 month of CAR infusion annotated according to CNS leukaemia status (table 1 and appendix) and neurotoxicity. Patients who developed neurotoxicity had significantly higher concentrations of CSF CAR T cells (p=0·0039). Three additional patients did not have samples sufficient for analysis. (C) B-cell depletion and rapid recovery in peripheral blood in responding patients (n=14). Patients were followed up until recovery of circulating B cells or day 28, whichever occurred later. Circles designate circulating B cells in responding patients (left axis). Grey bars designate number of patients with evidence for normal B-cell progenitors in the marrow (eg, haematogones) at the designated time point (right axis). (D) Kaplan-Meier plot showing 51·6% overall survival probability after 9·7 months for all patients enrolled (top, n=21, median follow-up 10 months) and 78·8% leukaemia-free survival beginning at 4·8 months in patients with B-ALL who had MRD-negative remission (bottom, n=12). Ten of these 12 patients had a subsequent HSCT and all remain leukaemia free. (E) Disappearance of CSF leukaemia in two patients coincident with CAR T-cell migration to the CSF. (F) Percent (top) and absolute number (middle) of circulating CD19-CAR T cells by flow cytometry (n=21) and qPCR (n=18; bottom). Peripheral blood was analysed in each patient until CAR T cells were no longer detected or day 28, whichever occurred later. Horizontal solid lines show the median at each time point, and dashed lines indicate the lower limit of detection. Circles designate responding patients. Sample days were designated days 3 (range 1–5), 7 (5–9), 14 (12–16), 28 (25–31), 42 (45–49), and 68 (55–81). (G) Absolute number of circulating blasts in peripheral blood of all patients with B-ALL. Horizontal solid lines show the median at each time point, and dashed lines indicate the lower limit of detection. Circles designate responding patients and triangles designate patients who did not respond. Sample days were designated days 3 (range 1–5), 7 (5–9), 14 (12–16), and 28 (24–32). Patient 1 is the responding patient with blasts at day 28 by flow cytometry. (H) Time course of peripheral blood flow cytometry from a representative patient (patient 14) shows circulating leukaemia and low concentrations of non-malignant B cells at day –1, followed by CAR T-cell expansion coincident with clearance of leukaemia and non-malignant B cells by day 10, followed by disappearance of CD19-CAR T cells and B-cell recovery by day 53. Blue dots show CD19-CAR T cells; red dots show leukaemia blasts (CD19+CD34+); green dots represent normal B cells. CR=complete response. MRD=minimum residual disease. SD=stable disease.

	Age	Sex	Previous treatment	Number of relapses	Marrow blasts (% of mononuclear)		CNS status	CAR dose ($\times10^6$/kg)	Response (day 28)	CRS grade	Absolute circulating CAR T cells at day 28 ($\times10^6$/kg)	Days until HSCT after CAR
					Pre-treatment	Post-CAR						
1	13	M	C, R, I, T	8	30%	1%	1	1	CRi	2	1	··
2	16	F	C, R, T	2	35%	40%	1	0·03	SD	0	1·9	··
3	10	F*	C, R, I, T	Primary refractory	··	··	··	1	PD	1	0	··
4	11	F	C	Primary refractory	58%	<0·01%	1	1	CR, MRD Neg	1	2·8	47
5	10	M	C	1	10%	<0·01%	1	0·48	CR, MRD Neg	2	0·4	82
6	10	M	C	3	81%	99%	1	3†	PD	1	0	··
7	25	M	C, I	1	50%	0·03%	1	3	CR	4‡	0	··
8	18	M	C, R, T	1	0·2%	<0·01%	2	1	CR, MRD Neg	1	3	··
9	13	M	C, R, I, T, CAR	3	0·56%	<0·01%	1	3	CR, MRD Neg	1	3·1	45
10	5	M	C	Primary refractory	5%	<0·01%	1	3	CR, MRD Neg	3	0	54
11	23	M	C, R	1	84%	<0·01%	2	1	CR, MRD Neg	3	6·5	54
12	9	M	C	1	95%	96%	1	1	PD	0	0	··
13	27	F	C	3	21%	43%	1	1	PD	0	0	··
14	15	M	C, R, T	3	96%	<0·01%	1	1	CR, MRD Neg	4‡§	1·4	··
15	5	F	C, R, T	1	15%	10%	1	1	SD	0	0	··
16	25	M	C	Primary refractory	50%	<0·01%	1	1	CR, MRD Neg	4‡§	3·1	63
17	18	F	C	Primary refractory	0·03%	<0·01%	1	1	CR, MRD Neg	1	0·2	48
18	13	M	C, R, T	2	90%	97%	1	1	SD	0	0	··
19	21	M	C	2	7·7%	<0·01%	1	1	CR, MRD Neg	3‡	40·3	55
20	16	F	C	Primary refractory	0·7%	<0·01%	1	1	CR, MRD Neg	1	0	46
21	6	M	C	Primary refractory	0·56%	<0·01%	1	1	CR, MRD Neg	1	0	46

M=male. F=female. C=chemotherapy. R=radiation therapy. I=immunotherapy. T=allogeneic haemopoietic stem-cell transplant. CAR=CD19 chimeric antigen receptor. CRS=cytokine release syndrome. CR=complete response. MRD Neg=no minimal residual disease detected. CRi=CR with incomplete count recovery. SD=stable disease. PD=progressive disease. HSCT=haemopoietic stem-cell transplant. *Diffuse large B-cell lymphoma. †Actual dose received was $3{\cdot}6\times10^6$ CAR T cells per kg. ‡Tocilizumab. §Corticosteroid.

Table 1: **Patient demographic characteristics, response, and toxicity**

	Grade 2	Grade 3	Grade 4
Adverse event			
Acute kidney injury	..	1 (5%)	0
Cardiac arrest	..	0	1 (5%)
Cytokine release syndrome	..	3 (16%)	3 (16%)
QTc prolongation	..	1 (5%)	0
Febrile neutropenia	..	7 (37%)	0
Fever	..	9 (47%)	0
Hypertension	..	1 (5%)	0
Hypotension	..	2 (11%)	2 (11%)
Hypoxia	..	1 (5%)	1 (5%)
Left ventricular systolic dysfunction	..	0	1 (5%)
Multi-organ failure	..	1 (5%)	0
Pulmonary oedema	..	0	1 (5%)
Respiratory failure	..	0	1 (5%)
Haematological adverse event			
Prolonged activated partial thromboplastin time	..	1 (5%)	0
Anaemia	..	13 (68%)	0
Lymphocyte count decreased	..	1 (5%)	7 (37%)
Neutrophil count decreased	..	0	17 (89%)
Platelet count decreased	..	3 (16%)	7 (37%)
White blood cell decreased	..	4 (21%)	13 (68%)
Chemical laboratory abnormalities			
ALT increased	..	1 (5%)	0
AST increased	..	2 (11%)	0
Blood bilirubin increased	..	1 (5%)	0
CPK increased	..	1 (5%)	0
Hyperglycaemia	..	1 (5%)	0
Hypokalaemia	..	9 (47%)	0
Hyponatraemia	..	1 (5%)	0
Hypophosphataemia	..	7 (37%)	1 (5%)
Nervous system event			
Ataxia	1 (5%)	0	0
Dysphasia	0	1 (5%)	0
Headache	1 (5%)	0	0
Tremor	1 (5%)	0	0

Data are n (%). ALT=alanine aminotransferase. AST=aspartate aminotransferase. CPK=creatine phosphokinase.

Table 2: **Grade 3 and 4 toxicities, and grade 2 neurotoxicities possibly related to CD19-CAR T-cell therapy**

During subsequent expansion of the 1×10^6 cells per kg cohort (patients 11–21), grade 3 or 4 cytokine release syndrome occurred in four patients. Cytokine release syndrome of any grade occurred in 16 patients (figure 1, table 1), began a median of 4 days after cell infusion (range 1–7 days), and lasted a mean of 4·8 days (range 1–9 days). Grade 4 syndrome occurred in three of 21 patients (14·3%; 95% CI 3·0–36·3) and grade 3 syndrome occurred in three patients (14·3%; 95% CI 3·0–36·3%). The syndrome was fully reversible in all patients and was managed with supportive care alone (n=12), supportive care plus the anti-interleukin-6 receptor monoclonal antibody, tocilizumab (n=2), and supportive care plus tocilizumab and corticosteroids (n=2) (table 1). Patient 14 was successfully resuscitated after cardiac arrest on day 7 after 4 days of cytokine release syndrome, associated with a drop in his cardiac ejection fraction from a baseline of 65% to less than 25%.

Reversible neurotoxicity was seen in six patients and included grade 1 visual hallucinations (n=5) and transient dysphasia (n=1). All patients with neurotoxicity and for which CSF was assessable had evidence for CNS trafficking of CD19-CAR T cells and concentrations of CSF CD19-CAR T cells were higher in patients who developed neurotoxicity than in those who did not (p=0·0039; figure 1B). In one patient, neurological toxicity was associated with an abnormal MRI, consistent with mild encephalopathy with reversible splenial lesion syndrome that resolved within 2 weeks. No patient had evidence of seizure activity.

As expected, most patients had grade 3 or 4 cytopenia attributed to lympho-depleting chemotherapy. Median duration of absolute neutrophil count less than 500 was 8 days (0–38 days in responding patients), but prolonged (≥14 days) grade 4 neutropenia was noted in seven of 21 patients (33·3%; 95% CI 14·6–57·0). One patient had delayed-onset grade 4 neutropenia occurring on day 47, after resolution of the initial post-chemotherapy neutropenia. Anti-neutrophil antibodies were absent, and bone marrow showed a maturational block at the promyelocyte stage. Neutropenia resolved with filgrastim. Whether CD19-CAR T cells contributed to this event is unknown because trimethoprim-sulfamethoxazole was initiated after CD19-CAR therapy and is potentially myelosuppressive.

Peripheral blood from all patients was analysed at regular timepoints for non-malignant B cells, blasts, CAR T-cell number and T-cell subset distribution until day 28, recovery of normal B cells, or disappearance of CAR T cells, whichever occurred later. Of the 14 responding patients, 12 had undetectable circulating B cells after treatment with lympho-depleting chemotherapy and CD19-CAR T cells which nadired between days 14 and 28. However, B-cell recovery as evidenced by non-malignant CD19+ progenitors on marrow examination (eg, haematogones) was recorded in 13 patients at day 28 or shortly thereafter (figure 1C). Although haematogones were not seen in the marrow of patient 1 on day 28, he had residual leukaemia. Because both the lympho-depleting chemotherapy and the CD19-CAR T cells are expected to induce B-cell lymphopenia, the role of each in the B-cell lymphopenia seen in this population could not be identified. All grade 3 and 4 toxicities and all grade 2 or higher neurotoxicities at least possibly related to the regimen are shown in table 2. All toxicities resolved to normal or baseline. No evidence of GVHD was seen.

Intention-to-treat analysis shows a 66·7% (14/21) complete response rate (95% CI 43·0–85·4; table 1). Of 20 patients with B-ALL, the complete response rate was

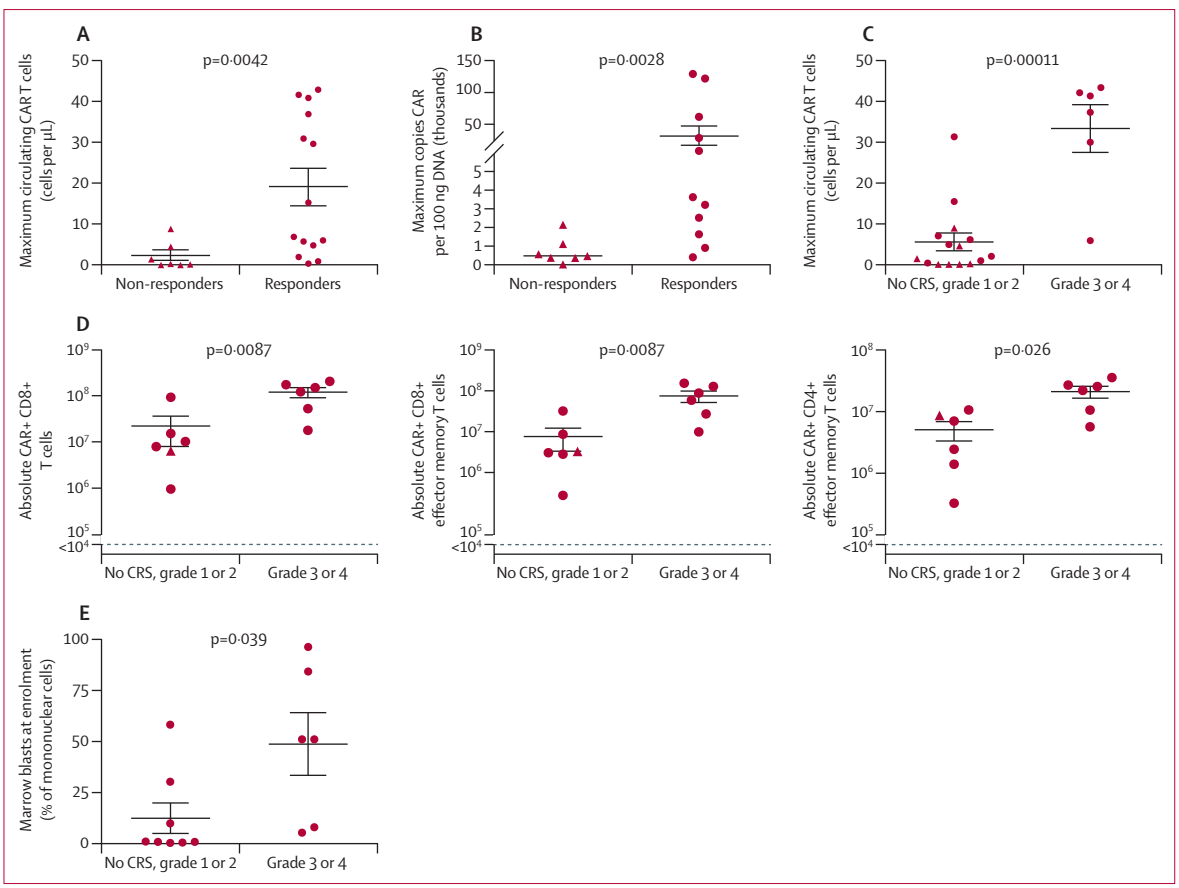

Figure 2: **Circulating chimeric antigen receptor (CAR) T-cell numbers are biomarkers of response and cytokine release syndrome (CRS) severity**
Responding patients had significantly higher peak circulating CAR T cells measured by (A) flow cytometry or (B) qPCR than non-responders. (C) Peak circulating CAR T cells correlated with CRS severity. Triangles show non-responding patients. (D) Higher absolute numbers of circulating CD8+ CAR+ T cells (p=0·0087), CD8+ effector memory CAR+ T cells (p=0·0087), and CD4+ effector memory CAR+ T cells (p=0·026) were seen in patients with grade 3 or 4 CRS than those without CRS or grade 1 or 2 CRS. Assessable circulating CAR T cells were not seen in non-responding patients except for one as shown by the triangle. Horizontal dashed lines indicate the lower limit of detection. (E) Responding patients with ALL with higher disease burden were significantly more likely to have grade 3 or 4 CRS than patients with lower disease burdens (p=0·039).

70% (95% CI 45·7–88·1), with 12 of 20 patients with B-ALL achieving MRD-negative complete response (60%; 95% CI 36·1–80·9; figure 1A). Overall survival at a median follow-up of 10 months was 51·6% at 9·7 months and beyond. Leukaemia-free survival of 12 patients who achieved an MRD-negative complete response was 78·8% beginning at 4·8 months (figure 1D). All ten patients who underwent HSCT and who had a CAR-induced MRD-negative complete response remain disease-free and no unexpected peritransplant toxicities were noted. Two patients who achieved MRD-negative complete response (patients 8 and 14) were judged ineligible by their treating physicians to undergo HSCT and both relapsed with CD19-negative leukaemia at 3 and 5 months, respectively. We saw no evidence for loss of CD19 expression in non-responding patients. Three patients (patients 1, 7, and 14) received second infusions of CD19-CAR cells 2–5·5 months after the first infusion for residual or recurrent B-ALL and none showed objective response.

11 (65%) of 17 patients with B-ALL with CSF specimens adequate for analysis had detectable CSF CAR T cells (median 2790 absolute CAR T cells (IQR 0–23 715). Two patients had evidence of CNS leukaemia at the time of cell infusion that disappeared coincident with a rise in CSF CD19-CAR T cells (figure 1E). No patient with a response in the bone marrow had CSF blasts by flow cytometry at day 28 restaging.

CD19-CAR T-cell expansion measured by flow cytometry and qPCR were highly correlated at days 7 (Spearman r=0·79; 95% CI 0·50–0·92) and 14 (r=0·89; 0·71–0·96). 18 (86%) of 21 patients had detectable circulating CAR T cells by flow cytometry, with peak expansion occurring around day 14 (figure 1F). This coincided with disappearance of circulating blasts in responding patients (figure 1G). In one representative patient (patient 14, figure 1H), circulating leukaemia cells are present on day –1, dramatic CAR T-cell expansion is seen on day 10 coincident with elimination of normal B cells and malignant blasts, followed by disappearance

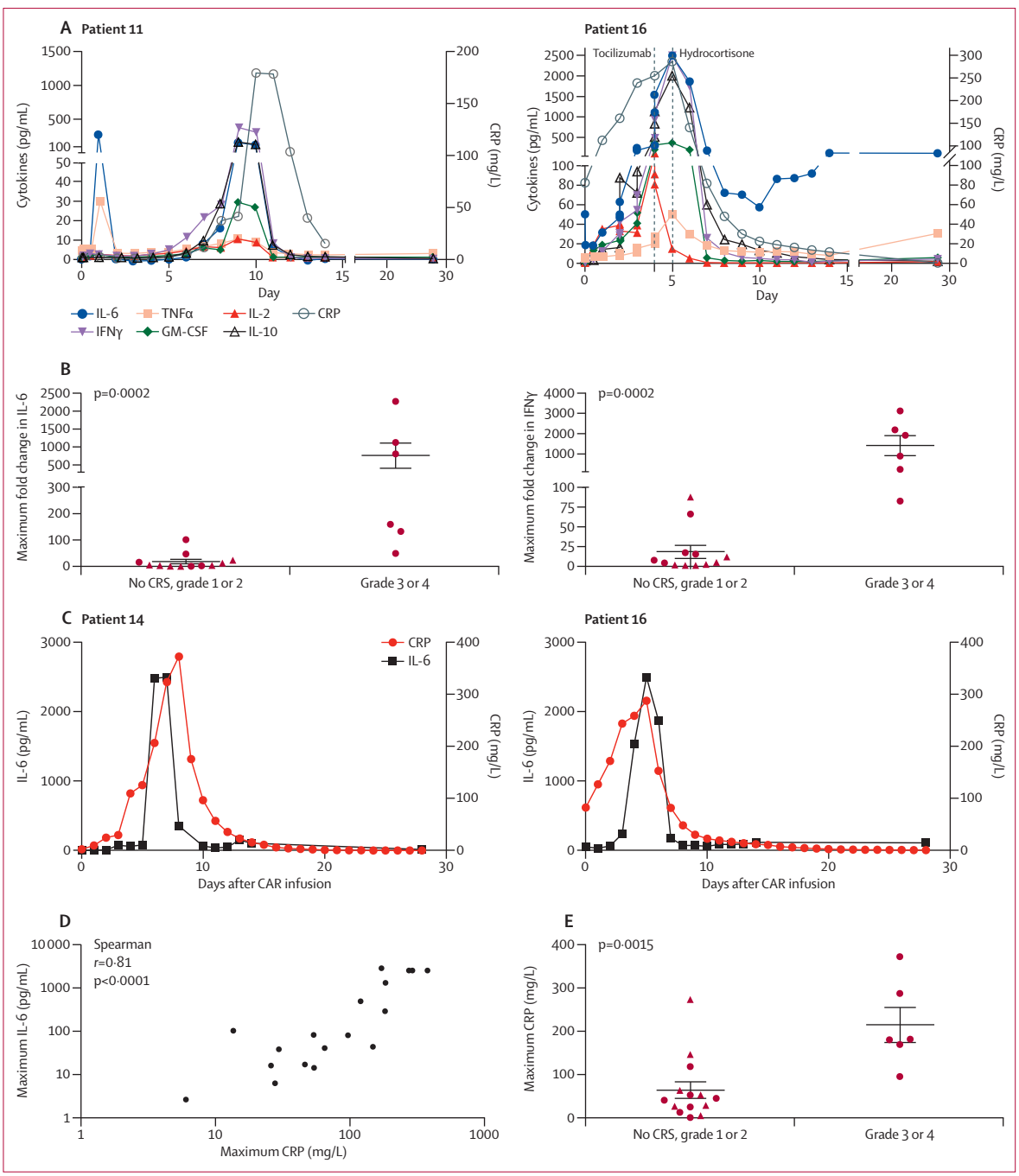

Figure 3: **Interleukin 6 (IL-6), interferon γ (IFNγ), and C-reactive protein (CRP) concentrations are biomarkers of cytokine release syndrome severity**
(A) Timecourse of rises in circulating inflammatory cytokines and C-reactive protein in one representative patient with grade 3 cytokine release syndrome (CRS) who did not receive tocilizumab (left) and another patient with grade 4 CRS who was treated with tocilizumab plus hydrocortisone (right). Normal C-reactive protein is <3 mg/L. (B) Maximum fold change in circulating interleukin 6 and interferon γ concentrations correlated with severe CRS (data available only for patients 1–19). (C) Temporal correlation between interleukin 6 and C-reactive protein increase in two representative patients. (D) Paired measurements of interleukin 6 and C-reactive protein shows a strong correlation (Spearman r=0·81, 95% CI 0·54–0·92; p<0·0001). Data are available only for patients 1–19. (E) Higher peak C-reactive protein concentrations are associated with increased CRS severity. CAR=chimeric antigen receptor. TNF=tumour necrosis factor. GM-CSF=granulocyte-macrophage colony-stimulating factor.

of CD19-CAR T cells by day 53 with recovery of normal B cells to concentrations four times higher than day –1 but continued absence of blasts. No CAR T cells were detected after day 68 in any patient, although several underwent HSCT (table 1) restricting the period of follow-up (median time to HSCT 51 days (IQR 46–55).

Four patients with detectable CD19-CAR T cells on the days immediately before initiation of the HSCT preparative regimen showed no evidence for CD19-CAR T cells at the time of first restaging after HSCT. In post-hoc analyses, we tested serum samples from all patients treated for the development of human anti-mouse antibodies that could affect CAR persistence but recorded none in any patient. Using 22 samples from 11 patients obtained before CD19-CAR therapy (n=11) and post-CD19-CAR infusion (n=11), we tested for T-cell-mediated anti-CAR responses as measured by proliferation to autologous CD19-CAR transduced versus non-transduced cells and saw increased proliferation to the autologous CD19-CAR transduced cells (p=0·036, appendix) raising the prospect that anti-CAR immune responses could diminish CD19-CAR persistence. Separate assessment of the differences within the 11 samples obtained before CD19-CAR therapy yielded a p value of 0·32, whereas the 11 samples obtained post-CD19-CAR infusion yielded a p value of 0·067. Owing to the small numbers of patients and samples for the study, we could be underpowered to detect differences in the post-CD19-CAR infusion group.

CD19-CAR T-cell expansion correlated both with response and toxicity because responding patients had significantly higher circulating CD19-CAR T cells (p=0·0042, figure 2A; p=0·0028, figure 2B) and higher CD19-CAR T-cell expansion correlated with greater cytokine release syndrome severity (p=0·00011, figure 2C). CAR-transduced T-cell subsets were analysed from peripheral blood of patients to establish whether any T-cell phenotypes correlated with severity of cytokine release syndrome. Patients with grade 3 or 4 syndrome had significantly higher concentrations of circulating CD8+CAR+ T cells, CD8+ effector memory CAR+ T cells, and CD4+ effector memory CAR+ T cells than those with no syndrome or grade 1 or 2 syndrome (figure 2D). In responding patients with ALL, the extent of disease burden, as measured by the percent blasts in the bone marrow immediately before enrolment on this protocol, correlated with severity of cytokine release syndrome, with patients with higher disease burden having more severe syndrome (p=0·039, figure 2E).

Cytokine concentrations and clinical symptoms in two representative patients who developed cytokine release syndrome are shown in figure 3A. Elevations and declines in interleukin 6, interferon γ, GM-CSF, and interleukin 10 temporally coincided with onset of the syndrome and resolution, respectively. Patient 11 was treated with supportive care alone for grade 3 cytokine release syndrome, whereas patient 16 received tocilizumab and corticosteroids for grade 4 disorder. Patients with grade 3 or 4 cytokine release syndrome had significantly higher changes in plasma interleukin 6 (Hodges-Lehmann estimator of difference 434·2, 95% CI 85·3–1127·3, p=0·0002) and interferon γ (1367·8, 226·7–2195·5, p=0·0002) concentrations than patients with grade 1 or 2 or no syndrome (figure 3B). In this small sample size, we did not record associations between rises in other cytokines tested and syndrome severity.

We also saw correlations between C-reactive protein and interleukin 6 concentrations as previously described.[22,23] The temporal relation of these correlations is shown in two representative patients (figure 3C) and the strong correlation between peak interleukin 6 concentrations and peak C-reactive protein concentrations is shown in figure 3D (Spearman r=0·81; 95% CI 0·54–0·92). Patients who had severe cytokine release syndrome had significantly higher peak C-reactive protein than those who had mild or no cytokine release syndrome (Hodges-Lehmann estimator of difference 141·7, 95% CI 63·4–254·0, p=0·0015; figure 3E).

Discussion

CARs provide a potent new approach for cancer immunotherapy. This first intention-to-treat analysis of consecutively enrolled patients on a clinical trial of CD19-CAR T cells for refractory B-cell malignancies shows that CD19-CAR therapy is feasible for a high proportion of patients with refractory B-ALL, induced a complete response in 70% of patients with B-ALL and an MRD-negative complete response in 60%, and a 78·8% probability of those rendered into an MRD-negative complete response remain leukaemia free beginning at 4·8 months (figure 1D; panel). Because the standard of care for refractory paediatric patients with B-ALL in MRD-negative remission is to proceed to HSCT when medically eligible, ten of 12 patients who became MRD-negative in our trial went on to HSCT and all remain disease free (median follow-up 10 months). We conclude that CD19-CAR T-cell therapy is an effective bridge to HSCT in patients with chemorefractory B-ALL. Because most patients who entered remission went on to HSCT, this study cannot assess the durability of response to CD19-CAR.

All toxicities associated with the therapy were reversible, with cytokine release syndrome the most common and severe. Our results are consistent with an emerging paradigm wherein severe cytokine release syndrome is associated with raised interleukin 6 and interferon γ concentrations, and treatment with anti-interleukin-6 receptor antibody, tocilizumab with or without corticosteroids, reverses the syndrome. We noted strong correlations between interleukin-6 and C-reactive protein concentrations, as previously described.[13,22] C-reactive protein might have use as a predictive biomarker of severe cytokine release syndrome. Improved understanding of the biology of this syndrome and optimisation of treatment algorithms for early intervention will probably enhance the safety and tolerability of this therapy. We recently proposed such an algorithm wherein tocilizumab is the preferred first-line agent for the treatment of severe cytokine release syndrome because response is rapid and high-dose corticosteroids might ablate the CAR T cells.[13,24]

Panel: **Research in context**

Systematic review

We searched Medline on Aug, 15, 2013, with the search terms "CD19 chimeric antigen receptor", "clinical trial", and "acute lymphoblastic leukemia", and did not restrict by date or language. No complete clinical trials investigating CD19-chimeric antigen receptor (CAR) T cells for acute lymphoblastic leukaemia (ALL) have been published. Several case reports or small series have been reported. One group reported on one adult patient with ALL[12] followed by four more,[15] then 11 more,[24] for a total of 16, but two of these had no detectable ALL, specifics on disease burdens and dosage were not included, and systematic reports of toxicities were absent. Another group reported a transient response in one of two patients with ALL who had disease at the time of treatment with a combination chemotherapy and CD19-CAR in donor-derived Epstein-Barr virus-specific T cells after haemopoietic stem-cell transplant[25] but cell dose was based on total T-cell number, not CAR T cells, which makes interpretation of toxicity and response difficult. Finally, Grupp and colleagues[14] reported on two children with ALL who received CD19-CAR. In total, there are reports of 20 children and adults with ALL treated with CD19-CAR T cells. This anecdotal evidence is promising but does not have the rigour of a well-designed clinical trial with established eligibility criteria, uniform treatment of patients, and measurements of toxicity and response.

Interpretation

Our study shows that CD19-CAR T-cell therapy mediates a complete response rate in refractory paediatric ALL that is substantially higher than that reported with the most recent US Federal Food and Drug Administration-approved agent for refractory paediatric ALL. The complete responses in six of six patients with primary chemorefractory ALL provide evidence that this therapy can eradicate chemoresistant leukaemia. The study shows that CD19-CAR therapy is feasible in a very high proportion of patients with ALL, with an acceptable toxicity profile. Furthermore, CD19-CAR constructs that do not persist long term can mediate potent anti-leukaemic effects with recovery of normal B-cell lymphopoiesis.

Our data show that robust CAR T-cell expansion correlates with both anti-leukaemic efficacy and cytokine release syndrome severity, and that CSF penetration of CAR T cells correlates with development of neurotoxicity. We used standard estimates of the number of total body T cells extrapolated from the peripheral blood lymphocyte counts[26] to calculate that anti-leukaemic effects are associated with 1–2·5 log expansion of CAR T cells during the first 1–2 weeks after infusion, followed by rapid CAR T-cell contraction. Prolonged persistence in patients treated with CD19-CAR incorporating a 4-1BB endodomain has been reported.[11,14] On the basis of unpublished studies from our group, we hypothesise that differential persistence might relate to differential susceptibility of T cells to exhaustion when CD28 versus 4-1BB endodomains are signalled. However, clearance of CD19-CAR T cells could also occur via immunological mechanisms because in post-hoc analyses we recorded T-cell proliferative responses to autologous CD19-CAR T cells in some patients (appendix), although the clinical significance of these findings is unknown because several patients with measurable T-cell-mediated anti-CAR reactivity sustained meaningful anti-leukaemic effects. Irrespective of the mechanism responsible for the shorter persistence of the CD19-CAR cells in this series, our results show that long-term persistence is not necessary to induce meaningful anti-tumour effects and shorter persistence could have potential benefits, because patients treated with this approach do not have severe, prolonged B-cell aplasia.

The newest drug to gain FDA approval for ALL is clofarabine, which was approved in 2004 for paediatric ALL and showed complete response ranging from 8% to 20% in published series.[27,28] The complete response rate in our study of 70% (95% CI 45·7–88·1) in patients with ALL is superior to that seen with clofarabine regimens and compares favourably with the 88% complete response rate reported by Davila and colleagues[13] in adult patients with ALL after CD19-CAR therapy. Importantly, the report of Davila and colleagues does not provide an intention-to-treat analysis of all patients apheresed for enrolment on the study, varying chemotherapy regimens were used and two patients already had been rendered MRD negative by chemotherapy at the time of CAR therapy, potentially confounding the role of CD19-CAR therapy in those patients. Our intention-to-treat study is the first to provide an accurate response rate in a homogenously treated patient population with a standardised treatment protocol.

This work also provides the first evidence that CD19-CAR T cells can eradicate leukaemia in CSF. CNS relapse, even with prophylactic intrathecal chemotherapy, remains a problem, and present therapies for CNS leukaemia have substantial short-term and long-term CNS toxicity, with no new therapies developed in decades.[29] Our results raise the prospect that CD19-CAR T cells could prevent or treat CNS leukaemia without long-term toxicity.

Eight patients were treated with donor-derived but autologously collected CAR T cells and no evidence of GVHD was noted. Although the study is not sufficiently powered to detect differences in response between post-HSCT and HSCT-naive patients, three (43%) of seven post-HSCT patients with ALL had an MRD-negative complete response versus nine (69%) of 13 HSCT-naïve patients. Of six patients with grade 3 or 4 CRS recorded in this study, only one was in a post-HSCT patient, which might point to diminished T-cell functionality in cells harvested from patients post-HSCT, and if confirmed could warrant different dosing in HSCT patients.

Several reasons might explain why CD19-CAR T-cell therapy did not successfully treat some patients. Inadequate T-cell function related to a previous clofarabine-based regimen administered 30 days before leukapheresis was probably the reason in patient 2. We postulate that overwhelming disease burden might have contributed in two patients (patients 6 and 12) who had high marrow blast content and circulating blasts and hepatosplenomegaly. CD19 antigen loss occurred in two patients (patients 7 and 14). The experience in this series, and the CD19–relapse in other series after CD19-CAR[14] and after treatment with blinatumumab,[30] suggest that loss of CD19 expression is a potential

Achilles heel of CD19-directed therapies for B-ALL. The CD19– B-ALL that emerged in the two patients treated here retained expression of CD22, and on this basis we have created a CD22-CAR that could address this problem.[31] Future studies could use bivalent CARs targeting both CD19 and CD22 to prevent escape due to antigen loss, as has been reported in preclinical models targeting other antigens.[32]

Maximum tolerated dose identified in this study might vary depending on leukaemic burden and T-cell functionality. We recognise that establishing one maximum tolerated dose for all patients for CD19-CAR T cells, which undergo variable expansion in vivo, might be prone to error. However, because very high doses of CARs might be associated with increased toxicity,[33] and the dose of CD19-CAR cells established as the maximum tolerated dose in this study is similar to the dose used in several other continuing or recently completed clinical trials,[13,34] our next study will maintain the CD19-CAR dose established as the maximum tolerated dose here and incorporate a more intensive chemotherapy regimen in patients with extensive disease, in an attempt to increase the response rate, while potentially diminishing the risk for severe cytokine release syndrome.

In summary, this intention-to-treat analysis of patients treated on a uniform protocol with anti-CD19-CAR T cells for refractory B-cell malignancies in children and young adults shows that the therapy is feasible and yields a high response rate after one infusion of cells manufactured within 11 days. CD19-CAR T cells trafficked to the CNS and mediated clearance of CNS disease in two cases. Activity in six cases with primary, chemorefractory B-ALL shows the promise of this approach for treatment of chemoresistant disease. Anti-leukaemic effects are associated with transient, substantial expansion of CD19-CAR T cells and correlations are seen between the magnitude of CD19-CAR T-cell expansion and response and toxicity associated with cytokine release syndrome. The safety of this therapy will probably improve with the adoption of standardised treatment algorithms aimed at preventing life-threatening cytokine release syndrome[24] and incorporating this therapy into the treatment of patients with low disease burdens. Therapy with CD19-CAR T cells can be an effective bridge to transplant for a sizeable percentage of patients with refractory B-ALL and is associated with a favourable long-term survival in this series.

Contributors

DWL and CLM contributed to study design, CAR T-cell manufacturing design, data collection, data interpretation, writing the report, literature search, and figures. ASW contributed to study design, data collection, data interpretation, and reviewed and edited the report. MS and DS contributed to CAR T-cell manufacturing design and supervised GMP production of CAR T cells. MS-S, CY, YKC, LZ, NT, and HZ contributed to data collection and data interpretation. JNK, SAF, and SAR contributed to retroviral vector design and production. SMS provided statistical analysis and edited the report. TJF, RO, and NNS contributed to study design and reviewed and edited the report. All authors take responsibility for the accuracy and completeness of the data and for the analyses.

Declaration of interests

We declare no competing interests.

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