ADVANCES IN CELL-BASED IMMUNE THERAPEUTICS IN HEMATOLOGY

CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date

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Adoptive transfer of T cells genetically modified to express chimeric antigen receptors (CARs) targeting CD19 has produced impressive results in treating patients with B-cell malignancies. Although these CAR-modified T cells target the same antigen, the designs of CARs vary as well as several key aspects of the clinical trials in which these CARs have been studied. It is unclear whether these differences have any impact on clinical outcome and treatment-related toxicities. Herein, we review clinical results reflecting the investigational use of CD19-targeted CAR T-cell therapeutics in patients with B-cell hematologic malignancies, in light of differences in CAR design and production, and outline the limitations inherent in comparing outcomes between studies. (*Blood.* 2016;127(26): 3312-3320)

Introduction

Autologous T cells modified to express a chimeric antigen receptor (CAR) targeted to CD19 induce high rates of remission in patients with refractory B-cell hematologic malignancies.[1] A CAR is a recombinant receptor construct composed of an antibody-derived extracellular single-chain variable fragment (scFv), linked to intracellular T-cell signaling domains of the T-cell receptor (TCR), thereby redirecting T-cell specificity to the tumor in an HLA-independent manner.[2] Expansion and differentiation of naïve T cells requires both antigen-specific interaction of the peptide:MHC complex with the TCR (signal 1) and costimulatory signaling via interaction of costimulatory receptors on the T-cell surface with cognate ligands on target cells or professional antigen-presenting cells (signal 2).

Multiple iterations of CARs have been developed and investigated in clinical studies. First-generation CARs consisted of target-specific scFv fused to the CD3ζ endodomain of the TCR/CD3 complex. As first-generation CAR T cells exhibited limited persistence, expansion, and antitumor efficacy in preclinical and clinical studies, second-generation CARs incorporated cytoplasmic signaling domains of T-cell costimulatory receptors (eg, CD28, 4-1BB) to provide "signal 2." A third-generation CAR places multiple costimulatory domains in tandem.

CD19-targeted CAR constructs from several different institutions have demonstrated consistently high antitumor efficacy in children and adults with relapsed B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), and B-cell non-Hodgkin lymphoma (B-NHL). CAR T-cell products used by each institution differ in several respects, including CAR design, T-cell activation and transduction methods, and cell doses (Table 1). Furthermore, heterogeneous patient populations, infused CAR T-cell doses, and lymphodepleting chemotherapy regimens, along with limited published reports, complicate direct comparison of clinical outcomes associated with different CAR T-cell products. In this review, we highlight several mature and preliminary clinical investigations of CD19-targeted CAR T cells in hematologic malignancies, focusing on clinical outcomes, associated toxicities, in vivo T-cell persistence, and examine these observations in light of differences between therapeutic strategies.

Clinical outcome of CD19-targeted CAR T cells in B-cell hematologic malignancies

Characteristics of CD19-targeted CAR T-cell products, lymphodepleting chemotherapy, and associated clinical outcomes in the largest reported clinical studies are summarized in Table 1.

CD19-targeted CAR T cells in adult B-ALL

MSKCC and FHCRC have presented the largest bodies of data reflecting CD19-targeted CAR T-cell therapy in adults with relapsed B-ALL. The MSKCC team, using a CAR construct containing CD28 costimulatory domain (19-28z), presented updated results of their phase 1 trial in 46 adult patients at the 2015 annual meeting of the American Society of Hematology (ASH).[8-10] Patients' high-risk features included ≥3 prior lines of treatment (n = 26), prior allogeneic hematopoietic stem cell transplantation (allo-HSCT, n = 18), and Philadelphia chromosome positivity (n = 14). Immediately prior to T-cell infusion, 25 patients had morphologic disease (≥5% blasts in bone marrow [BM] or measurable extramedullary disease), and 21 patients had minimal disease (<5% blasts in BM). Thirty-seven of 45 evaluable patients achieved/maintained morphologic CR, which was MRD− by flow cytometry in 30 of 36 evaluable patients. Similar CR rates were observed regardless of age, pre-T-cell disease burden, number of prior therapies, and prior allo-HSCT status. Thirteen of 37 CR patients underwent allo-HSCT, but 6-month OS did not differ significantly between patients who underwent allo-HSCT (79%) and those who did not (80%). Six-month OS of patients who achieved MRD− CR was 80%. Eighteen patients relapsed during follow-up, including 3 patients relapsing with undetectable CD19 expression.

Table 1. Summary of CD19-CAR constructs in clinical trials

Institution	Gene transfer method	ScFv*	Costimulatory domain	Patient populations	Lymphodepleting chemotherapy	Infused cell doses	Responses observed
CHOP[3,4]	Lentivirus	FMC63	4-1BB	Pediatric ALL; n = 53[4]	Investigator's choice	1.07×10^6 to 17.36×10^6 CAR$^+$ T cells/kg	CR: 50/53 (MRD− in 45/50) 12 mo RFS: 45% 12 mo OS: 78%
NCI[5]	γ-retrovirus	FMC63	CD28	Pediatric ALL; n = 20	Cy 900 mg/m² ×1 + Flu 25 mg/m² ×3 d	1×10^6 (N = 16) vs 3×10^6 (N = 4) CAR$^+$ T cells/kg	CR: 14/20 (MRD− in 12/14) LFS: 79% at 4.8 mo (MRD− CR patients) OS: 52% at 7.8 mo (all)
NCI[6]	γ-retrovirus	FMC63	CD28	Adult CLL and B-NHL; n = 8	Cy 60 mg/kg ×2 d + Flu 25 mg/m² ×5 d	0.3×10^7 to 3.0×10^7 CAR$^+$ T cells/kg	ORR: 6/8 (CLL, 3/4; FL, 2/3); CR, n = 1 (CLL) and PR, n = 5
NCI[7]	γ-retrovirus	FMC63	CD28	Adult B-NHL; n = 15	Cy 60 mg/kg ×1-2 d + Flu 25 mg/m² ×5 d	1×10^6 to 5×10^6 CAR$^+$ T cells/kg	CR: 4/7 (refractory DLBCL, 4/6 (indolent B-NHL)
MSKCC[8-10]	γ-retrovirus	SJ25C1	CD28	Adult ALL; n = 46[9]	Cy or Cy + Flu	1×10^6 vs 3×10^6 CAR$^+$ T cells/kg	CR: 37/45 (MRD− in 30/36) among evaluable patients 6 mo OS: 65% (all) and 80% (MRD− CR patients)
FHCRC[11]	Lentivirus	FMC63	4-1BB	Adult ALL; n = 29	Cy or Cy 60 mg/kg ×1 + Flu 25 mg/m² ×3 d	2×10^5, 2×10^6, and 2×10^7 CAR$^+$ T cells/kg; 1:1 CD4$^+$:CD8$^+$	CR: 10/12 (Cy only) and 14/14 (Cy + fludarabine); MRD status unspecified
FHCRC[12]	Lentivirus	FMC63	4-1BB	Adult B-NHL; n = 28 Adult CLL; n = 6	Cy 60 mg/kg ×1 ± etoposide or Cy 60 mg/kg ×1 + Flu 25 mg/m² ×3 d	2×10^5, 2×10^6, and 2×10^7 CAR$^+$ T cells/kg 1:1 CD4$^+$:CD8$^+$	ORR (B-NHL): 6/12 (CR, n = 1, PR, n = 5 in Cy cohort) and 8/12 (CR, n = 5, PR, n = 3 in Cy + Flu cohort) ORR (CLL): 5/6 (CR, n = 3, PR, n = 2)
UPenn[13]	Lentivirus	FMC63	4-1BB	Adult CLL; n = 14	Investigator's choice	0.14×10^8 to 11×10^8 CAR$^+$ T cells	ORR: 8/14 (MRD− CR, n = 4, PR, n = 4) Median PFS: 7 mo Median OS: 29 mo
UPenn[14]	Lentivirus	FMC63	4-1BB	Adult CLL; n = 26	Investigator's choice	5×10^7 vs 5×10^8 CAR$^+$ T cells	ORR: 9/23 (CR, n = 5, PR, n = 4)
UPenn[15]	Lentivirus	FMC63	4-1BB	Adult B-NHL; n = 24†	Investigator's choice	3.08×10^6 to 8.87×10^6 CAR$^+$ cells/kg	ORR: 15/22 (DLBCL, 7/13; FL, 7/7, MCL, 1/2) PFS: 62% at 11.7 mo
UPenn[14]	Lentivirus	FMC63	4-1BB	Adult ALL; n = 12	Investigator's choice	6.5×10^6 to 8.45×10^6 CAR$^+$ cells/kg	CR: 8/9 (all MRD− in all)

CHOP, Children's Hospital of Philadelphia; CR, complete response; Cy, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; FHCRC, Fred Hutchinson Cancer Research Center; FL, follicular lymphoma; Flu, fludarabine; LFS, leukemia-free survival; MCL, mantle cell lymphoma; MRD−, minimal residual disease negative; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; ORR, objective response rate (in evaluable patients); OS, overall survival; PFS, progression-free survival; PR, partial response; RFS, relapse-free survival; UPenn, University of Pennsylvania.

*ScFv from which CAR antigen-recognition moiety was derived.

†Of 38 enrolled, 24 received the protocol-specified dose and were included in the analysis.

Investigators from the FHCRC presented updated results of their phase 1 trial of CD19-specific CAR T cells in 29 adult patients at the 2015 ASH meeting.[11] Key differences compared with the MSKCC trial (see Table 1) include incorporation of the 4-1BB costimulatory domain (vs CD28) and separate expansion and infusion of CD4:CD8 T-cell subsets in a 1:1 ratio. This group recently reported preclinical studies suggesting CD19-targeted CAR T-cell products of defined T-cell composition may provide synergistic activity from the most potent transduced T-cell subsets (ie, greater cytokine production from $CD4^+$ CAR T cells, particularly naïve $CD4^+$ CAR T cells, greater direct antitumor effects from $CD8^+$ CAR T cells with a central memory phenotype) with lower overall CAR T-cell dose and more uniform product composition between individual patients.[16] Ten patients had undergone prior allo-HSCT. Median BM blast percentage preinfusion was 17% (range, 0% to 97%). Although 10 of 12 evaluable patients who received Cy as lymphodepleting chemotherapy achieved BM CR, 7 of these 10 relapsed (median 66 days). Five patients were re-treated with CAR T cells with no response. Investigators postulated better lymphodepletion may lead to enhanced T-cell persistence and added Flu 25 mg/m^2 ×3 days to Cy 60 mg/kg in 14 subsequent patients. All 14 achieved BM CR, and patients who received Cy/Flu were noted to have longer disease-free survival than those who received Cy alone. MRD status of patients achieving/maintaining CR postinfusion was unspecified.

CD19-targeted CAR T cells in pediatric B-ALL

Investigators from CHOP/UPenn reported in detail on 25 children with relapsed B-ALL treated with CD19-targeted CAR T cells containing a 4-1BB costimulatory domain (CTL019).[3] Twelve percent of patients were in first relapse, 88% were in second or subsequent relapse, and 72% had previously undergone allo-HSCT. After leukapheresis, patients received interim therapy at the discretion of treating physicians. Updated results note 62% of patients had morphologic disease, and 38% had minimal disease at CTL019 infusion.[4] Three of 6 patients in the initial report relapsed with undetectable CD19 expression by flow cytometry, with persisting CTL019 cells.[3] The investigators most recently updated their findings at the 2015 ASH annual meeting, reporting on 53 children/young adults with relapsed/refractory B-ALL treated with CTL019 (Table 1). Forty-one had detectable B-ALL, 12 of whom were MRD− at CTL019 infusion. Fifty of 53 patients achieved or maintained morphologic CR, which was MRD− by flow cytometry in 45 of 50 evaluable patients. Of 20 patients relapsing post-CTL019, 13 had CD19-negative blasts.[4] Preliminary reports from Seattle Children's Hospital using CD19-targeted CAR T cells incorporating a 4-1BB costimulatory domain (JCAR017) also suggest high rates of CR in pediatric patients with relapsed/refractory B-ALL, although mature data have not yet been reported (#NCT02028455).

Investigators from the NCI similarly investigated the safety and efficacy of CD19-CAR T cells in 20 pediatric patients with relapsed B-ALL.[5] In contrast to the CHOP trial, 65% of patients had not undergone prior allo-HSCT. Fourteen of 20 patients achieved/maintained CR, which was MRD− in 12 of 14 patients. Ten of 12 patients who achieved MRD− CR proceeded to allo-HSCT; all but 1 of these 10 had no prior allo-HSCT. Relapses occurred within 6 months of CD19-CAR T-cell infusion in 2 patients who achieved MRD+ CR and 2 who did not undergo allo-HSCT after achieving MRD− CR. Investigators treated 3 patients with another CAR T-cell infusion at relapse (2-2.5 months following first infusion); none showed objective response.

CD19-targeted CAR T cells in CLL and B-NHL

Although CD19-targeted CAR T cells were first explored in patients with CLL and B-NHL, few mature data have been reported.

Investigators from UPenn have treated >40 patients with relapsed/refractory CLL with CTL019.[14] In abstract form, they reported a phase 2 dose optimization study in which 26 patients with relapsed/refractory CLL were randomly assigned to receive 5×10^7 (n = 13) vs 5×10^8 (n = 13) CTL019 following lymphodepleting chemotherapy. High-risk features included abnormalities of p53/chromosome 17p (n = 10) and progression on ibrutinib (n = 2). Nine of 23 patients achieved objective response, including CR in 5 patients; MRD status was not reported among those attaining CR. A clear dose-response relationship was not evident. Three patients progressed after achieving response, in the setting of developing aggressive CD19-negative lymphoma (n = 2) or loss of CTL019 persistence (n = 1).[14] More recently, the investigators published a detailed follow-up report of the pilot trial of CTL019 in 14 adult patients with relapsed CLL.[13] Patients had a median of 5 prior therapies, and 6 patients had deletion of chromosome 17. Objective response was evident in 8 of 14 patients; 4 patients achieved CR post-CTL019 infusion; all patients attaining CR achieved MRD negativity by deep sequencing analysis of the immunoglobulin heavy chain locus by 3 months posttreatment. Although all patients with PR subsequently relapsed 5 to 13 months after treatment, no patient with CR has relapsed with a median duration of response of 40 months (range, 21-53 months).

Investigators from the NCI demonstrated the efficacy of CD19-targeted CAR T cells in 8 patients with relapsed CLL and B-NHL.[6] In addition to lymphodepleting chemotherapy, patients received interleukin-2 (IL-2) postinfusion until toxicity precluded further administration. Responses lasted 7 to 18+ months; 1 patient with CLL attained durable CR associated with B-cell aplasia.[6] This group subsequently reported on 15 patients with chemotherapy-refractory DLBCL and other indolent B-NHL treated with a lower CAR T-cell dose and without exogenous IL-2.[7] Four of 7 evaluable patients with refractory DLBCL achieved CR, which appeared durable in 3 of 4 cases (9-22+ months), and 3 patients with CLL achieved durable CR confirmed by BM flow cytometry (14-23+ months).[7]

Investigators from UPenn and FHCRC presented preliminary data on adults with relapsed/refractory B-NHL treated with CD19-targeted CAR T cells at the 2015 ASH annual meeting (Table 1).[12,15] UPenn reported on 38 adults with relapsed/refractory B-NHL treated with CTL019 following investigators' choice of lymphodepleting chemotherapy. Twenty-four patients received the protocol-specified-dose of CTL019. Fifteen of 22 evaluable patients achieved objective response including 7 of 13 patients with DLBCL and 7 of 7 with FL; the proportion achieving CR was unspecified.[15] In the FHCRC trial, $CD4^+$ and $CD8^+$ T cells are expanded separately and infused in a 1:1 ratio, similar to their aforementioned study in B-ALL. Sixteen of 28 patients with B-NHL in FHCRC's report had undergone prior HSCT (autologous, n = 13; allogeneic, n = 3). CAR T-cell persistence was short in most of the 12 patients treated with Cy-based lymphodepleting chemotherapy, and similar to FHCRC's experience in adults with ALL, a cytotoxic T lymphocyte–mediated response to the murine component of the CAR transgene was observed. Objective responses were observed in 6 of 12 patients (CR, n = 1 [DLBCL]; PR, n = 5); retreatment of 5 patients led to no further clinical responses. As such, Flu was added to the lymphodepleting regimen in 16 subsequent patients, with an objective response rate of 67% (CR, 42%) observed, including responses in 6 of 8 patients with DLBCL (CR, n = 3) and CR in 2 of 3 patients with FL. Among 6 additional patients with relapsed/refractory CLL, 5 exhibited complete clearance of peripheral blood and BM by flow cytometry at 4 weeks postinfusion.[12]

Investigators from MSKCC have additionally reported preliminary results of patients with relapsed/refractory B-NHL with positron emission tomography positivity and/or BM involvement following salvage therapy treated with 5×10^6 to 1×10^7 19-28z CAR T cells per kg, administered

Table 2. Cytokine release syndrome and neurological toxicity in prominent CD19-targeted CAR T-cell therapeutic trials to date

Institution	Patient populations	CRS incidence and severity	CRS grading	CRS-specific management	Neurological toxicity*
CHOP[3,4]	Pediatric ALL	25/25 with any CRS (initial cohort); 8/25 required vasopressors 48/53 with ≥grade 1 CRS (most updated)	UPenn	Toci (28%) ± CS (n = 9); reversed in all cases	13/25 (initial cohort), ranging from delirium to global encephalopathy (aphasia, seizures, hallucinations); reversed in all cases
NCI[5]	Pediatric ALL	15/20 with ≥grade 1 CRS (grade 3, n = 3; grade 4, n = 3); 1 patient with cardiac arrest	NCI	Toci alone (n = 2); Toci + CS (n = 2)	6/20, including visual hallucinations (n = 5) and transient dysphasia (n = 1)
NCI[7]	Adult B-NHL	12/15 (fever); 4/15 (hypotension)	NFG	Toci (n = 2)	6/15, including confusion, obtundation, aphasia, encephalopathy
MSKCC[8-10]	Adult ALL	11/46 with severe CRS (requiring vasopressors or mechanical ventilation)	NFG	Not formally reported	13/46 with ≥grade 3 neurological toxicity
FHCRC[11]	Adult ALL	7/27 with severe CRS (fever/hypotension requiring ICU care); fatal in 2 patients	NFG	Not formally reported	13/27 with ≥grade 3 neurological toxicity
FHCRC[12]	Adult CLL and B-NHL	0/12 with severe CRS (Cy cohort) 2/16 with DLT (Cy + Flu cohort)	NFG	Not formally reported	Not formally reported
UPenn[13]	Adult CLL	9/14 with ≥grade 1 CRS (grade 3-4, n = 6; ICU admission, n = 4)	UPenn	Toci and/or CS (n = 5)	6/14, including ≤grade 2 hallucinations, confusion, delirium (n = 5) and grade 4 confusion (n = 1)
UPenn[14]	Adult CLL	14/26 with any CRS	NFG	Toci ± CS (n = 3)	Not formally reported
UPenn[15]	Adult B-NHL	16/24 with any CRS (grade 2, n = 14; grade 3, n = 1; grade 4, n = 1)	UPenn	Not formally specified	3/24, including delirium (grade 2, n = 1; grade 3, n = 1), and encephalitis (grade 5, n = 1)
UPenn[14]	Adult ALL	11/12 with ≥grade 3 CRS; 3 patients with refractory/fatal CRS	UPenn	Toci alone (n = 6); Toci + CS (n = 2); Toci + siltuximab + CS (n = 1)	Not formally reported

CS, corticosteroid; DLT, dose-limiting toxicity; ICU, intensive care unit; NFG, not formally graded; Toci, tocilizumab.
*Graded by Common Terminology Criteria for Adverse Events v4.0.

following high-dose chemotherapy and autologous HSCT. As of the 2015 American Society of Clinical Oncology annual meeting, 6 of 11 enrolled patients achieved CR, which were durable and ongoing in 4 of 6 patients (13-21+ months).[17]

Toxicities of CD19-targeted CAR T cells

All trials of CD19-targeted CAR T cells have reported similar treatment-related toxicities, particularly cytokine release syndrome (CRS), neurological toxicities, and B-cell aplasia, although severity of observed toxicities differs. CRS reflects a systemic inflammatory response syndrome in the hours to days following CAR T-cell infusion, characterized by elevations of proinflammatory cytokines and T-cell activation and expansion, with clinical features including fevers, myalgias, malaise, and, in more severe cases, a capillary leak syndrome associated with hypoxia, hypotension, and occasionally renal dysfunction and coagulopathy. Trials have used different definitions to grade CRS, as current Common Terminology Criteria for Adverse Events grading of CRS only describe infusion-related symptoms. Severe CRS may be treated with the IL-6 receptor inhibitor tocilizumab as anticytokine therapy or with lymphotoxic corticosteroids. The reported nature, incidence, severity, and treatment of CRS and neurological toxicity following CD19-targeted CAR T-cell administration in the largest series is summarized in Table 2. B-cell aplasia is a

Table 3. CRS grading systems

Institution	Grade 1	Grade 2	Grade 3	Grade 4
NCI[5]	Mild reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment; prophylactic medications indicated for ≤24 h	Severe symptoms including any 1 or more of the following: a drop in blood pressure ≥20% from baseline, not responsive to fluid within 24 h; grade 3 respiratory dysfunction; grade 3 creatinine indicative of renal dysfunction; grade 3 neurological dysfunction	Life-threatening consequences; vasopressor or ventilator support indicated
UPenn[13]	Mild reaction; treated with supportive care	Moderate: requiring IV therapies; some signs of organ dysfunction related to CRS; hospitalization for management of CRS-related symptoms including fevers with associated neutropenia	More severe reaction: hospitalization required for management of symptoms related to organ dysfunction including grade 4 liver function test or grade 3 creatinine elevation related to CRS; includes hypotension treated with IV fluids or low-dose pressors	Life-threatening complications such as hypotension requiring high-dose pressors, hypoxia requiring mechanical ventilation

toxicity and pharmacodynamic marker of CD19-targeted CAR T-cell administration, particularly in patients in whom long-term CAR T-cell persistence is observed.[4,13] Functional consequences of B-cell aplasia may be abrogated, in part, by administration of intravenous immune globulin.

Toxicity of CD19-targeted CAR T cells in pediatric B-ALL

Investigators at CHOP/UPenn observed CRS in all 25 pediatric patients treated with CTL019 in their published report, and as of most recent update, CRS of grade 1 or greater in all but 5 of 53 treated patients by their institution's grading scale (Table 3).[3,4,13] Severe CRS (requiring vasopressors) was associated with elevated C-reactive protein, ferritin, IL-6, interferon γ (IFN-γ), and soluble IL-2 receptor.[3] Severe CRS began earlier than mild/moderate CRS following CTL019 infusion (median 1 vs 4 days).[3] High disease burden was correlated with severity of CRS and associated with higher levels of $CTL019^+$ $CD8^+CD3^+$ cells.[3,4] Similarly, the NCI group reported CRS in 15 of 20 treated patients.[5] CRS began a median of 4 days (range, 1-7) following CAR T-cell infusion and lasted a mean of 4.8 days (range, 1-9). They used their own grading system to define CRS (Table 3).[5] At the initial dose of 3×10^6 CAR T cells per kg, 2 of 4 patients experienced grade 3 to 4 CRS, and maximum tolerated dose was determined to be 1×10^6 CD19-CAR T cells per kg. At the lower dose (1×10^6 CD19-CAR T cells per kg), 4 of 16 patients (25%) experienced grade 3 to 4 CRS, all in patients with high burden of disease at time of T-cell infusion. The small number of patients treated with a higher dose of T cells limits our ability to infer a relationship between T-cell dose and severity of CRS. However, similar to observations made in the MSKCC and CHOP studies, high pre-T-cell disease burden correlated with severe CRS.[3,8] All cases of CRS were fully reversible (managed per Table 2). Investigators also examined cerebrospinal fluid (CSF) samples from 17 patients for evidence of CAR T cells within 1 month of treatment, and reported a correlation between neurotoxicity and CSF CAR T cells. However, the sample size was small (ie, total events = 6), and not all patients with neurotoxicity had detectable CAR T cells in CSF. Furthermore, MSKCC's adult B-ALL study did not show a correlation between CSF CAR T cells and neurotoxicity.[8]

Toxicity of CD19-targeted CAR T cells in adult B-ALL

Among 46 adults with relapsed B-ALL treated with 19-28z CAR T cells at MSKCC, severe CRS (ie, requiring vasopressors or mechanical ventilation) was observed in 11 patients.[9] All cases of severe CRS occurred in patients with morphologic disease at CAR T-cell infusion. Furthermore, the study demonstrated a correlation between T-cell dose and severity of toxicity. Eleven patients with morphologic disease received 3×10^6 19-28z CAR T cells per kg, and treatment-related grade 5 toxicities (sepsis, multiorgan failure) occurred in 3 patients in this group. Cell dose was subsequently adjusted based on disease burden, such that patients with morphologic disease received 1×10^6 19-28z CAR T cells per kg and patients with minimal disease received 3×10^6 CAR T cells per kg. No grade 5 toxicity has been observed in 14 subsequent patients with morphologic disease and 21 with minimal disease. Patients experienced similar ranges of neurological side effects following CAR T cells to those reported in pediatric B-ALL series. Although severe CRS exclusively occurred in patients with high disease burden, grade 3 to 4 neurological toxicities occurred in 3 of 21 patients (14%) with minimal disease. Investigators from FHCRC similarly observed a correlation between pretreatment disease burden and the incidence of CRS following CD19-targeted CAR T-cell infusion in adults with B-ALL.[11]

Investigators at UPenn presented preliminary results of their phase 1 trial of CTL019 in adults with relapsed B-ALL at the 2014 ASH annual meeting.[14] Eleven of 12 patients experienced severe CRS. Eight of 11 patients with severe CRS were managed successfully with anti-

IL-6 directed therapy, but 3 developed refractory CRS despite tocilizumab and corticosteroid administration and died within 3 to 15 days of CTL019 infusion. All 3 patients had high disease burden at T-cell infusion and received Cy 300 mg/m^2 every 12 hours ×6 doses followed by 6.5×10^6 to 8.45×10^6 CTL019 cells per kg. These 3 patients received a higher T-cell dose compared with median CTL019 dose of 3.62×10^6 cells per kg in the other 9 patients, suggesting a possible relationship between the higher cell dose and increased toxicity, as observed in the MSKCC and FHCRC studies.[8,9]

Toxicity of CD19-targeted CAR T cells in adult CLL and NHL

Investigators at UPenn observed delayed CRS in adults with CLL treated with CTL019, with some patients requiring anticytokine therapy as late as 55 days after T-cell infusion (Table 2).[13,14] CRS was associated with higher peak T-cell expansion, but, unlike in B-ALL, higher pretreatment disease burden did not predict development of CRS. Despite a higher dose of T cells compared with the dose used in their adult B-ALL study, no grade 5 CRS was observed in the pilot study.[13] One patient with B-NHL, however, experienced grade 5 encephalitis considered possibly attributable to CTL019.[13-15] FHCRC reported no DLTs among patients with B-NHL receiving 2×10^5 to 2×10^7 CAR T cells per kg following Cy-based lymphodepletion, although noting DLT (unspecified further) among 2 of 7 patients treated with 2×10^7 CAR T cells per kg following Cy and Flu, with marked elevations in IL-6 within the first day of CAR T-cell infusion heralding severe toxicity in each of the affected patients.[12] The NCI group described grade 3 or greater toxicities in 13 of 15 patients with DLBCL or more indolent B-cell malignancies, largely within the 2 weeks following CAR T-cell infusion. Elevations in IL-6 and/or IFN-γ were observed contemporaneous with peak toxicity.[7] In MSKCC's study of 19-28z CAR T cells administered following high-dose chemotherapy and autologous stem cell rescue in patients with relapsed/refractory aggressive B-NHL, DLT was CRS at the 1×10^7 19-28z per kg dose level; 6 of 11 patients developed severe CRS, which was treated effectively with tocilizumab and/or corticosteroids.[17]

In summary, although greater pretreatment disease burden correlates with increased risk of severe CRS in B-ALL, such a relationship has not been conclusively demonstrated in CLL and B-NHL despite higher doses of CAR T cells administered in these patients. The mechanism of neurological toxicities associated with CD19-targeted CAR T cells similarly remains unknown. Although the presence of CAR T cells in CSF has been implicated as a potential etiology, CAR T cells are frequently found in the CSF of patients with or without neurological symptoms, suggesting they are unlikely to be direct mediators of neurological toxicity.[8]

In vivo expansion and persistence of CAR T cells

In vivo CAR T-cell persistence is often considered to be a surrogate marker of long-term clinical efficacy of CAR T-cell therapy. To an extent, the lack of standardized assays to quantify CAR T cells in blood or BM complicates cross-trial comparison of persistence data. The most extensive CAR T-cell persistence data come from studies conducted at CHOP/UPenn and the NCI, both using flow cytometry and quantitative polymerase chain reaction (qPCR) to measure in vivo expansion and persistence of CAR T cells. As CAR T-cell detection by flow cytometry depends on surface CAR expression, transduced T cells without surface CAR expression might be detected by qPCR but not flow cytometry. In

pediatric patients with B-ALL, investigators at CHOP reported a lower limit of quantification of 5 copies per μg DNA by qPCR, with report of a patient with detectable CTL019 cells by qPCR (but not flow cytometry) up to 2 years.[3] Interestingly, 3 patients with no response to CTL019 had high peak levels of CTL019, detected by qPCR (6066-178 481 copies per μg DNA), whereas flow cytometry did not detect circulating CTL019. Investigators at UPenn used qPCR to measure CAR T-cell persistence in adults with CLL, although specified a lower limit of detection for their qPCR assay (<25 copies per μg DNA).[13] They reported degree of expansion and duration of persistence correlated with response, and long-term persistence of CTL019 cells has been detected up to 14 to 49 months in 4 patients who achieved MRD− CR, with persistent B-cell aplasia sustained for 4 years in some patients. Similarly, in their dose optimization study for patients with CLL, the investigators observed in vivo expansion of CTL019 in all responding patients, with CTL019 generally representing a higher proportion of CD3$^+$ cells at peak expansion by flow cytometry in responders.[14] MSKCC has reported somewhat shorter persistence duration of 1 to 6 months following 19-28z CAR T-cell infusion in adults with B-ALL by flow cytometry and qPCR.[9,10]

The lower limit of quantification for qPCR assay used for pediatric patients with B-ALL at the NCI was 100 copies per μg DNA.[5] When measured by flow cytometry or qPCR, CAR T cells reached maximum in vivo expansion ~14 days after infusion and became undetectable in all patients after day 68, with contemporaneous reemergence of normal B cells. However, analysis of CAR T-cell persistence in this study was limited, as most patients achieving MRD− CR underwent allo-HSCT at a median time to transplant of 51 days (range, 45-82). In 4 patients who had detectable CAR T cells immediately prior to initiation of conditioning for allo-HSCT, CAR T cells were no longer detectable at first restaging following allo-HSCT.

Investigators at the NCI reported CAR T-cell persistence in peripheral blood up to 181 days postinfusion by qPCR in a subset of patients with B-NHL. Peak levels were noted prior to day 10 postinfusion and were widely variable.[6] In their more recent report, highest levels of CAR T cells (9-777 CAR$^+$ T cells per μL) were detected 7 to 17 days following infusion, with rapid decline observed thereafter; a clear correlation between peak expansion and response was not evident, and maximal persistence was not specified.[7]

It remains unclear whether inclusion of the 4-1BB costimulatory domain is responsible for longer CAR T-cell persistence (eg, potentially by ameliorating T-cell exhaustion) or whether limited CAR T-cell persistence in some series may be because of immunological clearance (eg, cytotoxic T lymphocyte–mediated anti-CAR responses reported in the NCI and FHCRC studies) and/or inadequate lymphodepletion.[5,11,12] Furthermore, as some patients have remained in durable molecular remissions despite undetectable circulating CAR T cells, the optimal length of CAR T-cell persistence remains unknown.[8] In fact, depth of response and potency of CAR T cells may be more critical than persistence in inducing durable remissions, as CD19-negative relapses have occurred despite persisting CAR T cells.[3]

Applying CD19-targeted CAR T-cell therapies: understanding differences

Alternative transduction methods

The studies described herein have employed a γ retrovirus or lentivirus for gene transfer. These viral transduction methods are associated with high transduction efficiency and reliably generate T-cell products of the desired dose. In contrast, investigators from MD Anderson Cancer Center have investigated nonviral gene transfer using the *Sleeping Beauty* system for cut-and-paste transposition.[18] Potential limitations of this system include decreased transduction efficiency and need for longer coculture to generate desired CAR T-cell products. Advantages include eliminating costs associated with viral transduction methods and risks of viral insertional oncogenesis. The MD Anderson Cancer Center group reported on 13 patients (ALL, n = 8; B-NHL, n = 3; CLL, n = 2) with active B-cell malignancies, some of whom had undergone prior allo-HSCT, treated with 1×10^6 to 5×10^7 CAR T cells per m^2, and noted 3 patients were alive and in remission at a median of 3 months postinfusion. Of 12 additional patients treated with 1×10^6 to 5×10^7 donor-derived CAR T cells per m^2 following allo-HSCT (ALL, n = 10; B-NHL, n = 2), 3 (all with B-ALL) remain alive and in remission at median 5 months postinfusion.[19] Although these early data suggest the possibility of lower activity of CAR T cells manufactured using the *Sleeping Beauty* system, other variables including the CAR costimulatory domain, lymphodepletion regimens, and clinical characteristics of treated patients may have contributed to the modest benefits observed. Review of more robust and mature data may help to clarify gene transfer efficiency, end-of-production CAR T-cell phenotype, expansion, persistence, and clinical efficacy associated with this strategy.

First- vs second-generation CARs

We and others have shown the importance of costimulation in relevant preclinical models.[20] Several small early clinical studies employed first-generation CAR T cells and demonstrated limited clinical efficacy and CAR T-cell persistence.[21-23] Additionally, investigators from the Baylor College of Medicine treated 6 patients with relapsed/refractory B-NHL with a first-generation CD19-targeted CAR T-cell product simultaneously with a second-generation CAR T-cell product containing a CD28 costimulatory domain. Using qPCR, they observed strikingly superior expansion and persistence of CAR T cells incorporating the CD28 endodomain, with molecular signals of second-generation (but not first-generation) CAR T cells showing greater than sixfold expansion in the 2 weeks postinfusion, with nadir by 4 to 6 weeks postinfusion and demonstrable capacity to expand when restimulated ex vivo by TCR engagement, highlighting the importance of CAR T-cell costimulation.[24] Clinical benefit appeared modest, with 2 patients experiencing 3 to 10 months of stable disease; all patients ultimately experienced disease progression.

4-1BB vs CD28 costimulatory domains: persistence and relapse

The second-generation CD19-targeted CARs with widest clinical experience incorporate either a CD28 costimulatory domain or a 4-1BB costimulatory domain (Table 1). Across several studies, CAR T-cell persistence appears to be more durable in recipients of CAR T cells bearing a 4-1BB domain.[3,13] To date, pediatric patients with B-ALL treated with CD19 CAR T cells incorporating a 4-1BB (vs CD28) costimulatory domain appear to exhibit a greater rate of MRD− CR (Table 1).[3-5] Notwithstanding this trend, whether the incorporation of either 4-1BB or CD28 will ultimately be associated with superior long-term OS and event-free survival among similar patient groups remains unknown. The incidence of relapse after achieving CR (18 of 37 following 19-28z CAR T-cell therapy in adults treated at MSKCC, 20 of 50 following CTL019 in pediatric patients treated at CHOP/UPenn) may be similar, although comparison may be further complicated by differences between adult vs pediatric patients with B-ALL.[4,9] Relapse of patients with B-ALL treated with CTL019 appears to occur in the setting of loss of B-cell aplasia, in which case relapse tends to be CD19$^+$,

or as CD19^{−} escape variants.[3,4] In contrast, CD19^{−} relapse appears somewhat less common following 19-28z CAR T-cell treatment.[9] Among adults with B-ALL, similar rates of morphologic CR following CD19-targeted CAR T-cell infusion were observed by the MSKCC and FHCRC groups; the proportion of patients in the FHCRC group achieving MRD[−] CR remains uncertain.[9,12] Although a high rate of relapse was observed among adults treated at FHCRC, this may reflect inadequate lymphodepletion in patients receiving Cy monotherapy, with rejection of the murine component of the scFv; early data suggest Flu may help to overcome this limitation.[11,12]

The heterogeneous populations of patients with CLL and B-NHL treated with CD19-targeted CAR T cells to date limits comparison on the basis of costimulatory domains. Among patients with B-NHL, impressive responses have been observed in patients with spectrum of indolent and aggressive histologic subtypes treated with CD19-targeted CAR T cells by the NCI, UPenn, and FHCRC groups, and a clear benefit to 1 costimulatory domain is not yet evident.[7,12,15,17]

Lymphodepletion

Lymphodepleting chemotherapy may enhance CAR T-cell responses by eradication of regulatory T cells, elimination of other immune cells that may compete for homeostatic cytokines, and enhancing antigen-presenting cell activation.[25,26] Antitumor efficacy clinical studies of first-generation CAR T cells may have been limited by ineffective lymphodepletion as well as lack of costimulation. Additionally, in an early cohort of patients with purine-analog refractory CLL and bulky lymphadenopathy treated with 19-28z CAR-modified T cells at MSKCC, no objective responses were observed among 3 patients who received 1.2×10^7 to 3.0×10^7 19-28z CAR T cells per kg without lymphodepleting chemotherapy. Four subsequent patients received Cy 1.5 to 3.0 g/m^2 followed by 0.4×10^7 to 1.0×10^7 CAR T cells per kg, with 1 patient exhibiting marked reduction of peripheral adenopathy and 2 others with stable disease.[27]

Investigators from Baylor College of Medicine similarly postulated inadequate lymphodepletion may have accounted for limited CAR T-cell persistence in their phase 1 trial of CD19-directed virus-specific T cells. In brief, they administered escalating doses of donor-derived virus-specific T cells (cytomegalovirus, Epstein-Barr virus, adenovirus) modified to express a CD19-targeted CAR to 8 patients with B-cell malignancies following allo-HSCT. No lymphodepleting chemotherapy was administered. They observed no increase in IL-6, tumor necrosis factor α, or IFN-γ levels in the first 6 weeks following infusion, as other groups have observed in the setting of CAR T-cell expansion; only 2 of 6 evaluable patients had objective responses evident at 6 weeks postinfusion. Low levels of transgene signals were detectable by qPCR from 1 to 12 weeks postinfusion, without an identifiable CAR[+] population by fluorescence-activated cell sorter; expansion appeared more prominent in 2 patients with Epstein-Barr virus reactivation.[28]

Adequate lymphodepletion may prevent transgene rejection, as observed by the FHCRC group in adults with B-ALL and B-NHL as previously described herein.[11,12] Higher CAR T-cell levels were in adults with B-ALL receiving Flu in addition to Cy (vs Cy alone) at 28 days following CAR T-cell-infusion (55.8 vs 0.10 CD8^{+} CAR T cells per mL and 2.1 vs 0.02 CD4^{+} CAR T cells per mL, respectively); a trend toward improved disease-free survival was also observed in the cohort receiving Flu + Cy.[11] Similarly, in their trial enrolling adults with B-NHL, significantly higher peak CAR T-cell levels were observed in recipients of Flu + Cy compared with Cy alone (31.9 vs 0.55 CD8^{+} CAR T cells per mL and 16.5 vs 0.31 CD4^{+} CAR T cells per mL, respectively).[12]

Investigators from the NCI recently reported on 20 adults with progressive B-cell malignancies following allo-HSCT (B-NHL, n = 10; B-ALL, n = 5; CLL, n = 5) treated with escalating doses of allogeneic CD19-targeted CAR T cells (0.4×10^6 to 8.2×10^6/kg) bearing a CD28 costimulatory domain.[29] Although no lymphodepleting chemotherapy was administered, objective responses were observed in 8 patients. Most notably, 4 of 5 patients with B-ALL attained MRD[−] CR with subsequent recovery of normal polyclonal B-cells. Higher peak levels of CAR T cells were observed by qPCR among patients achieving CR/PR.[29] Patients with non-ALL/DLBCL diagnoses were required to have received ≥1 donor lymphocyte infusion before enrollment and were eligible if they had grade 0-1 acute graft-versus-host disease (GVHD) and mild or no chronic GVHD; no patients developed new GVHD. The responses observed following CAR T-cell infusion without antecedent lymphodepletion in patients with relapsed B-ALL remains striking. Although natural allogeneic responses may have contributed to the observed activity, the absence of GVHD in responders and historically poor response rates to donor lymphocyte infusion among patients with B-ALL argues against targeting of allogeneic antigens as the primary antileukemic activity. Whether lymphodepletion might further enhance the activity of allogeneic CAR T cells in this setting remains unclear.

CAR T-cell dose

As described in Table 1, the largest series have employed wide ranges of infused CAR T-cell doses, sometimes in the context of dose escalation. Several reports have suggested a correlation between infused CAR T-cell dose and the incidence and severity of CRS; CRS has been the DLT in multiple studies.[4,5,8,9,11,12] As previously noted, the correlation between cell dose and CRS ultimately led our group to define a lower goal cell dose for patients with morphologic disease than for those with MRD (1×10^6 vs 3×10^6 19-28z CAR T cells per kg).[8,9] A higher CAR T-cell dose is well tolerated in patients with MRD. We have previously observed significantly less robust in vivo expansion of CAR T cells in patients with MRD vs morphologic B-ALL, suggesting these patients receive an effectively lower dose of CAR T cells per fixed infused dose.[8,10] Nonetheless, whether a higher dose is truly required for greater efficacy in patients with MRD remains uncertain. Several studies in pediatric B-ALL, adult B-ALL, CLL, and B-NHL have compared responses among dose levels as planned by dose escalation or randomization and have not identified a clear correlation between greater CAR T-cell dose and greater efficacy or CAR T-cell persistence.[5,11,12,14] Such reports are presently limited by small size, and the optimal CAR T-cell dose and product composition to balance toxicity and efficacy remains uncertain.

Conclusions

CD19-targeted CAR T cells have emerged as a highly effective therapy in patients with refractory B-cell malignancies. Despite the differences in costimulatory domain, T-cell transduction method, and doses of infused CAR T cells, these differences do not yet appear to have a consistent major impact on response rates and long-term outcome. All these studies have some common factors crucial for effectiveness: tumor-specific and universal target, lymphodepleting chemotherapy, a second-generation CAR, and T-cell expansion in short-term culture. However, in the absence of randomized trial

design, it will be difficult to make a fair comparison among the different CAR constructs.

In the meantime, several lessons can be learned from the current studies. First, instead of the "1-dose-for-all" approach often adopted in traditional chemotherapy and small-molecule inhibitors, the optimal effective and safe dose of CAR T cells might vary depending on pretreatment disease burden (at least in the case of B-ALL), lymphodepleting chemotherapy, and disease subtypes (ALL vs CLL/NHL). Forthcoming clinical trials should consider these factors when determining dose escalation schemes. Second, all CD19-targeted CARs demonstrate some degree of CRS and neurological side effects. However, before we can compare the toxicities of each treatment, it is imperative to create a uniform and standardized definition of CRS and neurological side effects specific and relevant to CAR-modified T-cell therapy. Third, there remains no standardized assay to measure CAR T-cell expansion and persistence. Although qPCR appears to be more sensitive, it does not always correlate with findings by flow cytometry, which appears to be more specific. Therefore, future trials should address optimal assays to measure CAR T cells as well as clinical relevance of CAR T-cell persistence.

Is 1 CAR best? Because of the differences of trial designs highlighted in this review, it is impossible to answer the question presently. Forthcoming clinical trials might compare different CARs in a homogenous patient population to choose the best performer. Furthermore, as CAR T cells directed to targets beyond CD19 are assessed clinically, optimizing CAR T-cell engineering to enhance T-cell persistence and to be resistant to hostile tumor microenvironment will be critical to reproduce the remarkable results observed with CD19-targeted CAR T cells in other tumors targeted by CARs to other antigens.

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Authorship

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