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BASIC-TRANSLATIONAL REVIEW

# At The Bedside: Clinical review of chimeric antigen receptor (CAR) T cell therapy for B cell malignancies

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## ABSTRACT

**T cells kill microbial-infected and malignant cells by detection of nonself antigens with the TCR. Tumor reactivity can be encoded genetically by introducing a chimeric antigen receptor (CAR) into T cells. CARs are composed of an antigen-binding domain and an intracellular T cell activation domain. Early human trials evaluating CD19-targeted CAR T cells for chronic lymphocytic leukemia (CLL) showed limited responses until CARs included a costimulation domain, and conditioning chemotherapy was given before T cell infusion. Clinical trials evaluating CD19-targeted CAR T cells for B cell acute lymphoblastic leukemia (B-ALL) are demonstrating response rates up to 90%. However, these clinical outcomes are associated with a cytokine release syndrome (CRS), which is caused by T cell activation and manifests as high-grade fever, hypotension, and other cardiovascular complications. It is currently managed conservatively but can be treated with cytokine-directed therapy or with high-dose steroids. Current efforts are dedicated to confirming the clinical efficacy and managing toxicities in multicenter Phase II trials. We present a thorough overview of the preclinical and clinical development of CAR T cell therapy that will highlight important areas for the basic researcher to investigate in the laboratory and contribute to this exciting field.**
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## Introduction

The use of adoptive immunotherapy in the treatment of human disease is being evaluated in clinical trials. The novel nature of this therapy presents new challenges and brings with it a learning curve for physicians and researchers. In this review, we present a brief historical background of CAR T cell therapy and describe its biologic nature, as well as how it is used for B cell malignancies. We also review the toxicity profile of this novel approach to cancer therapy.

Abbreviations: ALL = acute lymphoblastic leukemia, allo-SCT = allogeneic stem cell transplant, B-ALL = B cell acute lymphoblastic leukemia, BBB = blood brain barrier, BiTE = bispecific T cell engager, BM = bone marrow, CAR = chimeric antigen receptor, CLL = chronic lymphocytic leukemia, CR = complete remission, CRS = cytokine release syndrome, DLBCL = diffuse large cell lymphoma, EFS = event-free survival, HSC = hematopoietic stem cell,

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## THE IMMUNE RESPONSE IN HEALTH AND DISEASE: ROLE OF T CELLS IN IMMUNE SURVEILLANCE FOR CANCER

Immune responses are triggered when T cells are presented an antigen by the MHC proteins. T Cells have been postulated to have a role in detecting and preventing cancer, a concept known as immune surveillance [1]. The incidence of cancer is higher in patients with compromised immune system, as was observed in patients with viral-induced immunosuppression, such as HIV and EBV. Higher rates of malignant lymphoma have also been observed in patients with abnormal immune function as a result of rheumatoid arthritis, although it is not clear whether the risk is only in those receiving immunosuppressive therapy [2]. Clinical responses have also been observed in patients with chronic myeloid leukemia, as a result of graft versus leukemia, which supports the argument that an immune-mediated approach can mediate disease responses for chemotherapy-refractory cancer [3, 4]. Furthermore, research suggests that tumor cells can evade or suppress anti-tumor responses [5–7]. Immunotherapy research is now focused on how to reverse cancer-mediated immune suppression, and one of the most powerful and exciting approaches involves the genetic retargeting of bulk, autologous T cells against a patient's cancer.

## ADVANTAGE OF CAR T CELLS OVER CONVENTIONAL CHEMOTHERAPY

Traditional adoptive T cell therapy involves the isolation of tumor-reactive T cells followed by ex vivo expansion until a sufficient number of T cells can be infused back into the patient.

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Whereas this therapy has generated anecdotal successes, the isolation and ex vivo expansion are laborious and time consuming [8, 9]. This constraint has limited the widespread adaption of adoptive T cell therapies. In contrast, the genetic retargeting of bulk T cells with a CAR allows the creation of a large number of tumor-reactive T cells in as short as 1 wk, although validating release criteria may extend the time the cells are ready for infusion. CARs are hybrid receptors composed of an antigen-binding domain derived from an antibody and intracellular activation domains derived from the TCR [10, 11]. Genetic modification of a T cell with a CAR endows the T cell with a new antigen specificity, and upon binding of its antigen, the CAR initiates signaling and activation of the T cell and killing of the target cell. CAR T cells have several advantages over conventional cytotoxic chemotherapy [10, 11]. First, the existing CAR T cells that target CD19 are not known to have off-target cytotoxicity. Secondly, the CAR T cell manufacturing process, in general, allows a mature, immunocompetent T cell to be redirected with new antigen specificity that can include proteins, peptides, LPS, DNA, and other biologic materials, which are not directly targetable with conventional cytotoxic chemotherapy. Furthermore, it is possible that memory cells can develop and confer long-term immunity to the host. The ideal CAR T cell will retain high specificity for the target and expand to achieve adequate numbers to engage a rapidly proliferating malignancy effectively. In addition, CAR T cells should persist in the host until all of the target malignancy is eradicated. However, CAR T cell persistence can be a double-edged sword: persistence is beneficial for immune-mediated eradication of disease, but persistent killing of on-target, off-tumor cells can lead to host toxicities.

## CAR CONSTRUCT DELIVERY INTO THE T CELL

To deliver the CAR requires transferring new gene(s) into the T cell, where it will confer a new antigen specificity and retain intracellular signaling capability. Several methods have been used to transfer genetic material into cells. Electroporation is a reliable and efficient means of gene transfer into cells, but its application in CAR T cells has been hampered by the time required to generate a sufficient number of T cells for infusion [12, 13]. Therefore, whereas electroporation is used, it is not the most frequently used gene-transfer system. The most common gene-transfer system for CAR T production is viral transduction with either lentivirus or gammaretrovirus. Gammaretrovirus production may be logistically simpler but has been associated with insertional leukemogenesis in HSCs [14]. Lentivirus production, however, is more laborious but potentially safer in regards to insertional oncogenesis. Fortunately, no insertional oncogenesis has been described in >100 CD19-targeted T cell productions, suggesting that genetic modification of mature, peripheral T cells is much safer than HSCs. Currently, both viral transduction methods are being used in early-phase clinical trials and have shown great promise.

**Research questions**

- *Does gammaretroviral transduction of mature T cells mediate insertion into genetic sites that have been previously identified as oncogenic?*
- *Are there methods of gene transfer other than viral transduction that can engineer a bulk product of CAR T cells within as short a period of time, such as 1 wk?*
- *What are the CAR T production failure rates in trials that use lentiviral versus gammaretroviral CAR transduction?*

## CLINICAL TRIALS IN B CELL MALIGNANCIES

### CD19 as a target for CAR T cells

CD19-targeted CAR T cells were the first CARs to be evaluated on a large scale in patients. CD19 is an attractive target, as its expression is limited to the B cell lineage, so there is limited potential for off target complications [10]. First-generation, CD19-targeted CAR T cells were safe but ineffective, as they had limited expansion and persistence in vivo [13, 15]. In contrast, second-generation CARs that include a costimulatory domain in tandem with the CD3ζ activation domain allow optimal T cell function, which is manifested by enhanced expansion and persistence [15–18]. The two types of second-generation, CD19-targeted CARs in clinical use include a 41BB costimulatory domain (19-BBz), which is used most commonly by investigators at UPENN (Philadelphia, PA, USA), or a CD28 costimulatory domain, which has been reported by investigators at the NCI (Bethesda, MD, USA) and at the MSKCC (New York, NY, USA). There are differences in a preclinical evaluation of second-generation CARs [19, 20], but their clinical activity against B cell malignancies has not been directly compared. Ongoing clinical trials using CD19-targeted CAR T cells for B cell malignancies are displayed in **Table 1**.

### Chronic lymphocytic leukemia

Some of the earliest clinical trials evaluating CAR T cells were performed in CLL patients (**Table 2**) [21–24]. MSKCC reported its experience with a Phase I dose-escalation study that included 8 CLL patients [24]. All patients had bulky and rapidly progressive disease, with the majority having adverse-risk cytogenetics. In the first cohort of 4 patients, no conditioning chemotherapy was given, and CAR T cells were infused at a dose of $1.2–3.0 \times 10^7$ 19-28z T cells/kg. No objective responses were seen, and the first 3 patients died of progressive disease [24]. The next patient to be enrolled died within 48 h after CAR T cell infusion [25]. The cause of death was believed a result of a preexisting but unrecognized microbial infection. The next cohort of patients was treated with cyclophosphamide, followed by CAR T cells ranging from $0.4–1.0 \times 10^7$ 19-28z T cells/kg [24]. The MSKCC investigators conditioned with cyclophosphamide chemotherapy to allow the CAR T cells to expand without competing with endogenous immune cells in the BM and blood. They noted

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MRD = minimal residual disease, MSKCC = Memorial Sloan Kettering Cancer Center, NCI = National Cancer Institute, NHL = non-Hodgkin lymphoma, ORR = objective response rate, OS = overall survival, PFS = progression-free survival, PR = partial remission, UPENN = University of Pennsylvania

**TABLE 1. Active clinical trials in the United States using autologous, viral-transduced, CD19-targeted CAR T cells**

| Clinical trial identifier/trial name | Disease type | Phase | CAR: target/signal domains | Gene vector | Eligible age (yr) | Conditioning therapy | Sponsor |
|---|---|---|---|---|---|---|---|
| NCT02535364 (ROCKET) | Relapsed/refractory adult B-ALL | 2 | 19-28z | γ-Retrovirus | ≥18 | ND | Juno |
| NCT01865617 | Relapsed/refractory NHL, ALL, CLL | 1/2 | 19-41-BBz | Lentivirus | ≥18 | ND | FHCRC |
| NCT01853631 (SAGAN) | Relapsed/refractory NHL, ALL, CLL | 1 | 19-28z vs. 19-28-BBz | γ-Retrovirus | ≤75 | Cyclophosphamide | BCM |
| NCT01860937 | Relapsed/refractory B-ALL | 1 | 19-28z | γ-Retrovirus | ≤26 | Cyclophosphamide-based regimens | MSKCC |
| NCT02614066 (ZUMA-3) | Relapsed/refractory adult B-ALL | 1/2 | 19-28z | γ-Retrovirus | ≥18 | Cyclophosphamide + fludarabine | Kite |
| NCT02625480 (ZUMA-4) | Relapsed/refractory B-ALL | 1/2 | 19-28z | γ-Retrovirus | 2–21 | Cyclophosphamide + fludarabine | Kite |
| NCT02030847 | Relapsed/refractory adult B-ALL | 2 | 19-41-BBz | Lentivirus | ≥18 | ND | UPENN |
| NCT02028455 (PLAT-02) | Relapsed/refractory B-ALL | 1/2 | 19-41-BBz (defined composition) | Lentivirus | 1–26 | Physician's choice | SCH |
| NCT01044069 | Relapsed/refractory B-ALL | 1 | 19-28z | γ-Retrovirus | ≥18 | Physician's choice | MSKCC |
| NCT02374333 | Relapsed/refractory B-ALL, NHL | 1 | 19-BBz | Lentivirus | 1–24 | ND | UPENN |

ND, Not described; Juno, Juno Therapeutics, Inc. (Seattle, WA, USA); FHCRC, Fred Hutchinson Cancer Research Center (Seattle, WA, USA); BCM, Baylor College of Medicine (Houston, TX, USA); Kite, Kite Pharma (Santa Monica, CA, USA); SCH, Seattle Children's Hospital (Seattle, WA, USA).

stabilization of disease in 3 patients, and duration of response ranged from 2 to 6 mo. Whereas no patients achieved a CR [24], the authors noted enhanced CAR T cell expansion in patients treated with conditioning chemotherapy before T cell infusion, arguing that CD19–CAR T cells should be infused after conditioning chemotherapy. Kochenderfer et al. [21, 26] also reported 2 cohorts of patients that included 7 with CLL treated with the 19-28z CAR. Three patients were induced into a CR (43%) and another 3 (43%) had PRs. All of the patients were conditioned with cyclophosphamide and fludarabine.

The UPENN group published results infusing CD19-targeted CAR T cells in 3 patients (aged 64–77 yr) with CLL [22]. These patients had advanced disease that no longer responded to chemotherapy. The BM was 40–95%, replaced by CLL after chemotherapy. The dose of CAR T cells ranged from $1.4 \times 10^5$ to $1.6 \times 10^7$/kg for all patients [22]. Upon treatment with the 19-BBz CAR T cells, 2 of the 3 patients achieved a CR, whereas the third patient had a PR that lasted >7 mo [22]. It was noted that a CRS occurred in all patients but was of relatively slow onset. One patient developed CRS on d 14, whereas the other 2 patients started having fever 1–3 d after infusion, followed by hypotension (d 11 in 1 patient) and transient cardiac dysfunction (d 15 in 1 patient) [22]. All patients responded to supportive care, but 1 required corticosteroids. In 2015, Porter et al. [27] published an updated report of 19-BBz CAR T cells infused in 14 patients with CLL, including the 3 patients discussed above. The cohort was heavily treated with a median of 5 chemotherapy regimens before CAR T cell therapy [27]. All received conditioning chemotherapy; 57% received cyclophosphamide either with pentostatin or fludarabine. Initial treatment response rates were 29% CR, 28% PR, and 57% ORR. Survival responses include a median PFS of 7 mo, median OS of 29 mo, and 18 mo PFS of 26.8% [27]. The determination by multiple groups that conditioning chemotherapy is required

**TABLE 2. Reported clinical trials of second-generation, CD19-targeted CAR T cells for CLL**

| Reference | Site | *n* | Mean age (yr) | Conditioning regimen | Gene transfer | Cell dose | Outcome |
|---|---|---|---|---|---|---|---|
| [24] | MSKCC | 8 | 65.5 | CTX 0–3 gm/m$^2$ | γ-Retrovirus | 0.4–3.0 $\times 10^7$/kg | PR/SD 38%<br>CR 0% |
| [21, 26] | NCI | 7 | 58 | CTX 60–120 mg/kg<br>Flu 25 mg/m$^2$ × 5 d | γ-Retrovirus | 0.3–2.8 $\times 10^7$/kg | CR 43%<br>PR 43% |
| [27] | UPENN | 14 | 67 | 21% FC<br>36% PC<br>43% B | Lentivirus | 0.14–11 $\times 10^{8a}$ | CR 28.5%<br>PR 28.5%<br>ORR 57%<br>NR 43% |

CTX, cyclophosphamide; SD, stable disease; Flu, Fludarabine; FC, fludarabine cyclophosphamide; PC, pentostatin cyclophosphamide; B, Bendamustine; NR, no response. [a]CAR T cell total dose since dose/kg was not reported.

and that second-generation CARs have improved function informed the development of the Phase I clinical trials evaluating CD19-targeted CAR T cells in patients with B-ALL.

- *UPENN, NCI, and MSKCC studies highlighted the potential of CAR T cells as a cancer therapy.*
- *Rapid, objective responses argued in favor of anti-tumor killing mediated by the CAR T cells.*
- *Other investigators are evaluating what is the optimal conditioning regimen before CAR T cells [28].*

## Clinical trials in B-ALL

Patients with B-ALL who relapse have a very poor prognosis [29–32]. The CR rate for adults with relapsed B-ALL, treated with salvage chemotherapy, is low (<30%), and the 5 yr survival rate is <10% in the absence of an allo-SCT [29–32]. Allo-SCT remains the only curative option with durable remissions in up to 40–50% of patients [29–32]. Unfortunately, most patients are ineligible for an allo-SCT because of comorbidities, lack of donor, and persistent or residual disease [29–32]. Blinatumomab is a T cell-engaging, CD19/CD3-BiTE antibody that has been recently approved for relapsed B-ALL. It also targets CD19 and serves as a bridge to allo-SCT [33]. Even with this therapy, many patients relapse and have a poor prognosis. B-ALL in relapse is a disease that is not responsive to standard therapies, and novel approaches are needed to improve outcomes.

Several studies of CD19-targeted CAR T cells for B-ALL have been reported (**Table 3**) [34–38]. In a study at MSKCC and reported by Davila et al. [35], a total of 16 B-ALL patients was treated with 19-28z CAR T cells. The population consisted of adults, and the age range was 18–59 yr. Many of the patients had adverse risk factors, including 25% who were Philadelphia chromosome positive and another 25% who had relapsed after allo-SCT. Preparatory chemotherapy was given to reduce tumor burden and facilitate a milieu in the BM and blood for optimal CAR T cell function. Patients were infused with $3 \times 10^6$ CAR T cells/kg, and the treatment was efficacious, with 88% of patients achieving a CR and a median time; 75% of all treated patients had no detectable MRD, as determined by high-sensitivity assays, such as flow cytometry or deep sequencing [35]. The high level of MRD-negative responses suggests that these remissions are high quality and of potentially longer durability than would be expected with chemotherapy alone. There were 8 patients who had refractory disease at the end of conditioning and before CAR T cell infusion. Of these, 6 (75%) went on to achieve MRD-negative status after CAR T cell therapy [35]. There is no salvage chemotherapy that has this degree of efficacy for B-ALL.

Another single-arm Phase I trial for B-ALL, using a similar 19-28z CAR, was reported by the NCI in 2014 [37]. It treated a total of 21 children and young adults with relapsed/refractory disease, which included 8 patients (38%) with disease that relapsed after allo-SCT. Conditioning was fludarabine 25 mg/m$^2$ for 3 d and 1 dose of cyclophosphamide 900 mg/m$^2$ [37]. This was a dose-escalation study with patients treated with doses ranging from 1 to $3 \times 10^6$ CAR T cells/kg. The therapy was safe, and the CR rate was reported as 66.7%, 1 mo after CAR T cell infusion. In addition, 57% of patients were classified as MRD negative [37].

The UPENN group also reported its experience using CD19-targeted CAR T cells for children ($n = 25$) and adults ($n = 5$) with relapsed/refractory B-ALL [38]. The cohort was enriched with several high-risk patients, including 18 who had relapsed after allo-SCT and 3 whose disease was refractory to BiTE therapy. The T cells were retargeted to CD19 by the 19-BBz CAR. Conditioning chemotherapy was the investigator's choice, but most received fludarabine and cyclophosphamide (15 patients), cyclophosphamide and vincristine (5 patients), or cyclophosphamide alone (3 patients). The CR rate was 90%, with 79% of patients achieving MRD-negative status. The median time to CR was 1 mo, OS was 78%, and EFS was 67% at 6 mo, albeit with short follow-up. Furthermore, in contrast to the studies reported by MSKCC and the NCI, which reported CAR T cell persistence up to 2–3 mo, the UPENN group reported CAR T cell persistence up to 6 mo [38].

- *The independent trials at 3 academic medical centers treating B-ALL represent the first reproducible and significant anti-tumor benefit mediated by CAR T cells and will likely lead to the first clinical indication for a gene-modified, adoptive cell therapy.*
- *The anti-leukemia responses are detected across all age groups.*
- *When compared with historical cytotoxic chemotherapy data, CAR therapy in B-ALL is strikingly more efficacious, with morphologic CR rates of 67–90%.*
- *MRD-negative rates of 57–79% suggest the responses will be more durable than with standard salvage chemotherapy.*
- *The increased number of patients induced into a CR has made it possible for more patients to receive allo-SCT than historical controls.*
- *The pattern of responses, with higher remission rates in ALL compared with CLL, suggests that the lymph node may be inhospitable for CAR T cell function.*

**TABLE 3. Reported clinical trials of second-generation, CD19-targeted CAR T cells for B-ALL**

| Reference | Site | *n* | Phase | CAR design | Median age (yr) | Conditioning chemotherapy | CAR dose | CR | MRD-negative | Bridge to allo-SCT | EFS | OS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [35] | MSKCC | 16 | 1 | 19-28z | 50 | CTX | $3 \times 10^6$/kg | 88% | 75% | 44% | ND | ND |
| [38] | UPENN | 30 | 1 | 19-BBz | 14 | Physician's choice | 0.8–21 $\times 10^6$/kg | 90% | 79% | 10% | 67% at 6 mo | 78% at 6 mo |
| [37] | NCI | 21 | 1 | 19-28z | 13 | Flu 25 mg/m$^2$ $\times$ 3 CTX 900 mg/m$^2$ | 0.03–3 $\times 10^6$/kg | 67% | 57% | 48% | ND | 51.6% at 10 mo |

### Other B cell malignancies

The largest cohort of autologous CD19-targeted CAR therapy in NHL, other than CLL, has been reported by the NCI. It treated a total of 15 patients with chemotherapy refractory disease, of whom 9 had DLBCL. Four of 7 evaluable patients (57%) with DLBCL achieved CR, and the duration of response was 9–22 mo [26]. This suggests that DLBCL may be susceptible to CD19-targeted CAR T cells and serves as a rationale for expanded Phase II trials focusing on this disease. Another malignancy in which CAR therapy has recently demonstrated efficacy is multiple myeloma. For example, a case report of 1 patient with relapse refractory multiple myeloma was treated with CD19 CAR T cell therapy and achieved a CR that was durable for >1 yr [39]. This suggests that a disease other than NHL or B-ALL may warrant further investigation with CD19-targeted therapy.

**Research questions**

- *Are there antigens other than CD19 that can be targeted with a CAR to mediate reproducible, efficacious, and safe outcomes in patients with cancer?*
- *Why is B-ALL so much more sensitive to CD19-targeted CAR T cells than CLL or other NHL? Might it be tumor microenvironment or trafficking?*
- *Do CAR T cells produced from patients with CLL or B-ALL differ in function?*
- *Can conditioning chemotherapy be further optimized? Is chemotherapy the only option for conditioning before CAR T cell infusion?*
- *With similar CR rates, is CAR T persistence required for durable remissions?*
- *Why does 41BB-containing CAR T cells persist longer than CD28-containing CAR T cells?*

## TOXICITIES ASSOCIATED WITH CAR T CELL THERAPY

### CRS

The CRS is an immune-mediated disorder that occurs after CAR T cells are infused into patients and is initiated by bulk T cell activation, leading to secretion of large amounts of inflammatory cytokines and the recruitment and activation of other immune cells [21, 22, 26, 34–38]. CRS spans the spectrum from mild to life threatening, and not all patients experience this toxicity. The syndrome typically begins within 1–5 d of CAR T cell infusion with fevers (often high grade, >40°C) [21, 35, 37, 38]. Up-regulated cytokines include but are not limited to IL-2, IFN-γ, TNF, and IL-6 [21, 35, 37, 38]. Studies suggest a strong correlation between CRS and tumor burden [35, 37, 38]. In addition to fevers, patients experience respiratory distress, hypotension, and neurologic disorders. These toxicities require intensive management, including pressors and intubation with mechanical ventilation. To normalize reporting across numerous CAR T cell studies, a grading system has been developed by Lee et al. [40].

In a study by Lee et al. [37], CRS was noted in 16 (76%) patients, of whom 14 (68%) achieved a CR. Most CRS were Grades 1–2, and the rates of Grades 3 and 4 CRS were 14.3% each. In the study by Davila et al. [35], Grade 4 CRS was noted in 25% of patients, and all recovered with supportive care and/or pharmacologic intervention. Maude et al. [38] also reported Grade 3–4 CRS in 27% of patients. A summary of CRS events in clinical trials is displayed in **Table 4**. Despite these toxicities, it is reassuring that they resolved with conservative medical management. Steroids and/or tocilizumab, which is a mAb that binds the IL-6R, has been used to treat CRS in patients. Tocilizumab, which has been demonstrated to moderate CRS toxicities, was evaluated as IL-6 is often elevated during CRS [36]. In the study by Lee et al. [37], tocilizumab was infused in 4 patients (19%), 2 of whom also required corticosteroids. In the study by Davila et al. [35], corticosteroids were used in the first 3 patients who developed severe CRS, and they noted rapid elimination of CAR T cells. Subsequent patients were treated with tocilizumab, leading to a resolution of symptoms but without any adverse effects on CAR T cell expansion and/or persistence. In the Maude et al. [38] report, 9 patients (30%) received tocilizumab, and 6 also received corticosteroids. In all of these studies, CRS was reversible, and no patient had Grade 5 toxicity or death, signifying that tocilizumab and/or corticosteroids enhance safety. However, Davila et al. [35] report that steroids are associated with early relapse after CAR T cell infusion, a complication not reported with tocilizumab. Therefore, tocilizumab is the preferred first-line agent with steroids reserved for CRS not responding to management.

### Neurotoxicity

CAR T cells also induce neurotoxicity in 29–43% of patients [27, 35, 37, 38]. A clear mechanism has not been identified at

**TABLE 4. CRS after infusing CD19-targeted CAR T cells in patients with B-ALL**

| | | | | | | | CRS grade | | | Neurotoxicity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference | Site | Disease | *n* | Phase | CAR T dose/kg | % with CRS | Grade | *n* | % | *n* | % | Reversible | TRM |
| [37] | NCI | B-ALL | 21 | 1 | $0.3–3.6 \times 10^6$ | 76% | 0–2 | 15 | 72 | 6 | 29 | 100% | 0% |
| | | | | | | | 3–4 | 6 | 28 | | | | |
| [35] | MSKCC | B-ALL | 16 | 1 | $3 \times 10^6$ | 100% | 0–2 | 9 | 56 | 6 | 38 | 100% | 0% |
| | | | | | | | 3–4 | 7 | 44 | | | | |
| [38] | UPENN | B-ALL | 30 | 1 | $0.08–21 \times 10^6$ | 100% | 0–2 | 22 | 73 | 13 | 43 | 100% | 0% |
| | | | | | | | 3–4 | 8 | 27 | | | | |

TRM, Therapy-related mortality.

this time, but the presence of CAR T cells in the CNS suggests that CAR T cells may be involved, directly or indirectly, with this toxicity [35, 37, 38]. It manifests as a spectrum of neurologic symptoms, including tremors, aphasia, somnolence, and various degrees of encephalopathy [35, 37, 38]. It is unknown if the neurotoxicities are a part of the CRS or if it is a separate entity. Conservative medical management has been successful for most patients, but some require steroids, which readily cross the BBB. It is unknown if tocilizumab has any effect in the intervention, as it is not expected to cross the BBB. Despite the concerning nature of these toxicities, they are self limited, and most patients recover with no persistent neurologic defects.

### B cell aplasia

As CD19 is expressed by all B cells, including precursors, effective targeting will lead to B cell aplasia. In the study by Porter et al. [27], all responding patients developed B cell aplasia and required intravenous Ig repletion on a periodic basis. In fact, the B cell aplasia lasted up to 4 yr in certain individuals. The disappearance of B cells, including mature and immature forms, is an on-target, off-tumor effect of CAR T cell therapy and can be a cause for concern, given the essential role of mature B cells in preventing and eliminating infections. It has been detected by most clinical trials evaluating CD19-targeted CAR T cells for B-ALL and highlights a concern when similar adoptive CAR T cells will be applied to other cancers, especially instances where the target is shared by critical tissues, such as respiratory epithelium [41].

**Research questions**

- *What cells are involved with the CRS? What cytokines do the cells produce?*
- *Is there a cytokine or effector cell that contributes to CRS toxicity but not anti-tumor killing?*
- *Do CAR T cells migrate to CNS in response to antigen or inflammation?*
- *Does tocilizumab only work through inhibition of IL-6?*

## CONCLUSIONS

Several Phase I clinical trials have demonstrated the potential of CAR T cells as a cancer therapy. Now research is focusing on enhancing the clinical efficacy and safety of this powerful new cancer immunotherapy. The initial focus of many groups is to confirm the efficacy of CAR T cells for B-ALL in multicenter Phase II trials. This is an important step that will determine if the CRS toxicities can be managed outside of the few medical centers that are the major CAR T cell clinical research sites. Other issues to consider in clinical trial design include an intention to treat analysis, CAR T cell dose, and to refine or standardize further the composition of conditioning therapy.

Whereas CR rates have been high, relapse remains a concern. The most common source of relapse is CD19-negative B-ALL progression. A recent study suggests that CD19-negative variants exist at low levels at the time of treatment in some patients [42]. Therefore, treatment with CD19-targeted CAR T cells may enrich for CD19-negative B-ALL tumor variants. A potential solution to this conundrum is to combine CD19-targeted CAR T cells with another targeted therapy. For example, the targeting of CD19 and CD22, which is also commonly expressed on B-ALL, may prevent CD19-negative outgrowth and relapse. In fact, CD22-targeted CAR T cells have mediated responses in patients that progressed after treatment with CD19-targeted CAR T cells [43].

CAR T cell therapy is rapidly evolving as a potent cancer immunotherapy. Clinicians are leading trials to determine if CAR T efficacy can be reproduced in a greater number of patients and at other academic centers. However, clinical experiences to date with the therapy have identified major new questions that could limit the widespread adaptation of CAR T cells (**Table 5**). It will be the role of basic researchers to address these questions with the goal of further optimizing the safety and efficacy of CAR T cells and laying the foundation for it to be adapted to other cancers.

**TABLE 5. Summary of CAR T cell research questions**

| | |
|---|---|
| CAR construct delivery into the T cell | • Does gammaretroviral transduction of mature T cells mediate insertion into genetic sites that have been previously identified as oncogenic?<br>• Are there methods of gene transfer other than viral transduction that can engineer a bulk product of CAR T cells within as short a period of time, such as 1 wk?<br>• What are the CAR T production failure rates in trials that use lentiviral versus gammaretroviral CAR transduction? |
| Clinical trials in B cell malignancies | • Are there antigens other than CD19 that can be targeted with a CAR to mediate reproducible, efficacious, and safe outcomes in patients with cancer?<br>• Why is B-ALL so much more sensitive to CD19-targeted CAR T cells than CLL or other NHL? Might it be tumor microenvironment or trafficking?<br>• Do CAR T cells produced from patients with CLL or B-ALL differ in function?<br>• Can conditioning chemotherapy be further optimized? Is chemotherapy the only option for conditioning before CAR T cell infusion?<br>• With similar CR rates, is CAR T persistence required for durable remissions?<br>• Why does 41BB-containing CAR T cells persist longer than CD28-containing CAR T cells? |
| Toxicities associated with CAR T cell therapy | • What cells are involved with the CRS? What cytokines do the cells produce?<br>• Is there a cytokine or effector cell that contributes to CRS toxicity but not anti-tumor killing?<br>• Do CAR T cells migrate to CNS in response to antigen or inflammation?<br>• Does tocilizumab only work through inhibition of IL-6? |

## AUTHORSHIP

O.O.O. and M.L.D. designed the outline, as well as composed and edited the manuscript and tables.

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### DISCLOSURES

The authors declare no conflicts of interest.

### REFERENCES

1. Roeser, J. C., Leach, S. D., McAllister, F. (2015) Emerging strategies for cancer immunoprevention. *Oncogene* **34,** 6029–6039.
2. Thomas, E., Brewster, D. H., Black, R. J., Macfarlane, G. J. (2000) Risk of malignancy among patients with rheumatic conditions. *Int. J. Cancer* **88,** 497–502.
3. Castro, F. A., Palma, P. V., Morais, F. R., Voltarelli, J. C. (2004) Immunological effects of donor lymphocyte infusion in patients with chronic myelogenous leukemia relapsing after bone marrow transplantation. *Braz. J. Med. Biol. Res.* **37,** 201–206.
4. Clinical Trials Committee British Society of Blood & Marrow Transplantation (BSBMT). (2005) Management of chronic myeloid leukaemia in relapse following donor lymphocyte infusion induced remission: a retrospective study of the Clinical Trials Committee of the British Society of Blood & Marrow Transplantation (BSBMT). *Bone Marrow Transplant.* **36,** 1065–1069.
5. Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., Powderly, J. D., Carvajal, R. D., Sosman, J. A., Atkins, M. B., Leming, P. D., Spigel, D. R., Antonia, S. J., Horn, L., Drake, C. G., Pardoll, D. M., Chen, L., Sharfman, W. H., Anders, R. A., Taube, J. M., McMiller, T. L., Xu, H., Korman, A. J., Jure-Kunkel, M., Agrawal, S., McDonald, D., Kollia, G. D., Gupta, A., Wigginton, J. M., Sznol, M. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **366,** 2443–2454.
6. Tumeh, P. C., Harview, C. L., Yearley, J. H., Shintaku, I. P., Taylor, E. J., Robert, L., Chmielowski, B., Spasic, M., Henry, G., Ciobanu, V., West, A. N., Carmona, M., Kivork, C., Seja, E., Cherry, G., Gutierrez, A. J., Grogan, T. R., Mateus, C., Tomasic, G., Glaspy, J. A., Emerson, R. O., Robins, H., Pierce, R. H., Elashoff, D. A., Robert, C., Ribas, A. (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515,** 568–571.
7. Brahmer, J. R., Tykodi, S. S., Chow, L. Q., Hwu, W. J., Topalian, S. L., Hwu, P., Drake, C. G., Camacho, L. H., Kauh, J., Odunsi, K., Pitot, H. C., Hamid, O., Bhatia, S., Martins, R., Eaton, K., Chen, S., Salay, T. M., Alaparthy, S., Grosso, J. F., Korman, A. J., Parker, S. M., Agrawal, S., Goldberg, S. M., Pardoll, D. M., Gupta, A., Wigginton, J. M. (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* **366,** 2455–2465.
8. Dudley, M. E., Wunderlich, J. R., Shelton, T. E., Even, J., Rosenberg, S. A. (2003) Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. *J. Immunother.* **26,** 332–342.
9. Jin, J., Sabatino, M., Somerville, R., Wilson, J. R., Dudley, M. E., Stroncek, D. F., Rosenberg, S. A. (2012) Simplified method of the growth of human tumor infiltrating lymphocytes in gas-permeable flasks to numbers needed for patient treatment. *J. Immunother.* **35,** 283–292.
10. Sadelain, M., Rivière, I., Brentjens, R. (2003) Targeting tumours with genetically enhanced T lymphocytes. *Nat. Rev. Cancer* **3,** 35–45.
11. Bouhassira, D. C., Thompson, J. J., Davila, M. L. (2015) Using gene therapy to manipulate the immune system in the fight against B-cell leukemias. *Expert Opin. Biol. Ther.* **15,** 403–416.
12. Choi, Y., Yuen, C., Maiti, S. N., Olivares, S., Gibbons, H., Huls, H., Raphael, R., Killian, T. C., Stark, D. J., Lee, D. A., Torikai, H., Monticello, D., Kelly, S. S., Kebriaei, P., Champlin, R. E., Biswal, S. L., Cooper, L. J. (2010) A high throughput microelectroporation device to introduce a chimeric antigen receptor to redirect the specificity of human T cells. *Biomed. Microdevices* **12,** 855–863.
13. Jensen, M. C., Popplewell, L., Cooper, L. J., DiGiusto, D., Kalos, M., Ostberg, J. R., Forman, S. J. (2010) Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol. Blood Marrow Transplant.* **16,** 1245–1256.
14. Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Le Deist, F., Fischer, A., Cavazzana-Calvo, M. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* **302,** 415–419.
15. Savoldo, B., Ramos, C. A., Liu, E., Mims, M. P., Keating, M. J., Carrum, G., Kamble, R. T., Bollard, C. M., Gee, A. P., Mei, Z., Liu, H., Grilley, B., Rooney, C. M., Heslop, H. E., Brenner, M. K., Dotti, G. (2011) CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J. Clin. Invest.* **121,** 1822–1826.
16. Imai, C., Mihara, K., Andreansky, M., Nicholson, I. C., Pui, C. H., Geiger, T. L., Campana, D. (2004) Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia* **18,** 676–684.
17. Kowolik, C. M., Topp, M. S., Gonzalez, S., Pfeiffer, T., Olivares, S., Gonzalez, N., Smith, D. D., Forman, S. J., Jensen, M. C., Cooper, L. J. (2006) CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res.* **66,** 10995–11004.
18. Finney, H. M., Lawson, A. D., Bebbington, C. R., Weir, A. N. (1998) Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J. Immunol.* **161,** 2791–2797.
19. Brentjens, R. J., Santos, E., Nikhamin, Y., Yeh, R., Matsushita, M., La Perle, K., Quintás-Cardama, A., Larson, S. M., Sadelain, M. (2007) Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. *Clin. Cancer Res.* **13,** 5426–5435.
20. Milone, M. C., Fish, J. D., Carpenito, C., Carroll, R. G., Binder, G. K., Teachey, D., Samanta, M., Lakhal, M., Gloss, B., Danet-Desnoyers, G., Campana, D., Riley, J. L., Grupp, S. A., June, C. H. (2009) Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol. Ther.* **17,** 1453–1464.
21. Kochenderfer, J. N., Dudley, M. E., Feldman, S. A., Wilson, W. H., Spaner, D. E., Maric, I., Stetler-Stevenson, M., Phan, G. Q., Hughes, M. S., Sherry, R. M., Yang, J. C., Kammula, U. S., Devillier, L., Carpenter, R., Nathan, D. A., Morgan, R. A., Laurencot, C., Rosenberg, S. A. (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* **119,** 2709–2720.
22. Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., Bagg, A., June, C. H. (2011) T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci. Transl. Med.* **3,** 95ra73.
23. Porter, D. L., Levine, B. L., Kalos, M., Bagg, A., June, C. H. (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* **365,** 725–733.
24. Brentjens, R. J., Rivière, I., Park, J. H., Davila, M. L., Wang, X., Stefanski, J., Taylor, C., Yeh, R., Bartido, S., Borquez-Ojeda, O., Olszewska, M., Bernal, Y., Pegram, H., Przybylowski, M., Hollyman, D., Usachenko, Y., Pirraglia, D., Hosey, J., Santos, E., Halton, E., Maslak, P., Scheinberg, D., Jurcic, J., Heaney, M., Heller, G., Frattini, M., Sadelain, M. (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* **118,** 4817–4828.
25. Brentjens, R., Yeh, R., Bernal, Y., Riviere, I., Sadelain, M. (2010) Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a Phase I clinical trial. *Mol. Ther.* **18,** 666–668.
26. Kochenderfer, J. N., Dudley, M. E., Kassim, S. H., Somerville, R. P., Carpenter, R. O., Stetler-Stevenson, M., Yang, J. C., Phan, G. Q., Hughes, M. S., Sherry, R. M., Raffeld, M., Feldman, S., Lu, L., Li, Y. F., Ngo, L. T., Goy, A., Feldman, T., Spaner, D. E., Wang, M. L., Chen, C. C., Kranick, S. M., Nath, A., Nathan, D. A., Morton, K. E., Toomey, M. A., Rosenberg, S. A. (2015) Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J. Clin. Oncol.* **33,** 540–549.
27. Porter, D. L., Hwang, W. T., Frey, N. V., Lacey, S. F., Shaw, P. A., Loren, A. W., Bagg, A., Marcucci, K. T., Shen, A., Gonzalez, V., Ambrose, D., Grupp, S. A., Chew, A., Zheng, Z., Milone, M. C., Levine, B. L., Melenhorst, J. J., June, C. H. (2015) Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci. Transl. Med.* **7,** 303ra139.

28. Turtle, C. J., Berger, C., Sommermeyer, D., Budiarto, T., Hanafi, L.-A., Melville, K., Pender, B., Steevens, N., Chaney, C., Heimfeld, S., Cherian, S., Wood, B. L., Soma, L., Chen, X., Jensen, M., Riddell, S. R., Maloney, D. G. (2015) Immunotherapy with CD19-specific chimeric antigen receptor (CAR)-modified T cells of defined subset composition. *2015 ASCO Annual Meeting*, American Society of Clinical Oncology, Alexandria, VA (abs.).
29. Medical Research Council of the United Kingdom Adult ALL Working Party; Eastern Cooperative Oncology Group. (2007) Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* **109,** 944–950.
30. Forman, S. J., Rowe, J. M. (2013) The myth of the second remission of acute leukemia in the adult. *Blood* **121,** 1077–1082.
31. German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia. (2012) Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood* **120,** 2032–2041.
32. Programa Español de Tratamiento en Hematologia Group. (2010) Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA Study Group. *Haematologica* **95,** 589–596.
33. Topp, M. S., Gökbuget, N., Stein, A. S., Zugmaier, G., O'Brien, S., Bargou, R. C., Dombret, H., Fielding, A. K., Heffner, L., Larson, R. A., Neumann, S., Foà, R., Litzow, M., Ribera, J. M., Rambaldi, A., Schiller, G., Brüggemann, M., Horst, H. A., Holland, C., Jia, C., Maniar, T., Huber, B., Nagorsen, D., Forman, S. J., Kantarjian, H. M. (2015) Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, Phase 2 study. *Lancet Oncol.* **16,** 57–66.
34. Brentjens, R. J., Davila, M. L., Riviere, I., Park, J., Wang, X., Cowell, L. G., Bartido, S., Stefanski, J., Taylor, C., Olszewska, M., Borquez-Ojeda, O., Qu, J., Wasielewska, T., He, Q., Bernal, Y., Rijo, I. V., Hedvat, C., Kobos, R., Curran, K., Steinherz, P., Jurcic, J., Rosenblat, T., Maslak, P., Frattini, M., Sadelain, M. (2013) CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci. Transl. Med.* **5,** 177ra38.
35. Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., Chung, S. S., Stefanski, J., Borquez-Ojeda, O., Olszewska, M., Qu, J., Wasielewska, T., He, Q., Fink, M., Shinglot, H., Youssif, M., Satter, M., Wang, Y., Hosey, J., Quintanilla, H., Halton, E., Bernal, Y., Bouhassira, D. C., Arcila, M. E., Gonen, M., Roboz, G. J., Maslak, P., Douer, D., Frattini, M. G., Giralt, S., Sadelain, M., Brentjens, R. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci. Transl. Med.* **6,** 224ra25.
36. Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., Porter, D. L., Rheingold, S. R., Teachey, D. T., Chew, A., Hauck, B., Wright, J. F., Milone, M. C., Levine, B. L., June, C. H. (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* **368,** 1509–1518.
37. Lee, D. W., Kochenderfer, J. N., Stetler-Stevenson, M., Cui, Y. K., Delbrook, C., Feldman, S. A., Fry, T. J., Orentas, R., Sabatino, M., Shah, N. N., Steinberg, S. M., Stroncek, D., Tschernia, N., Yuan, C., Zhang, H., Zhang, L., Rosenberg, S. A., Wayne, A. S., Mackall, C. L. (2015) T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a Phase 1 dose-escalation trial. *Lancet* **385,** 517–528.
38. Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., Chew, A., Gonzalez, V. E., Zheng, Z., Lacey, S. F., Mahnke, Y. D., Melenhorst, J. J., Rheingold, S. R., Shen, A., Teachey, D. T., Levine, B. L., June, C. H., Porter, D. L., Grupp, S. A. (2014) Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371,** 1507–1517.
39. Garfall, A. L., Maus, M. V., Hwang, W. T., Lacey, S. F., Mahnke, Y. D., Melenhorst, J. J., Zheng, Z., Vogl, D. T., Cohen, A. D., Weiss, B. M., Dengel, K., Kerr, N. D., Bagg, A., Levine, B. L., June, C. H., Stadtmauer, E. A. (2015) Chimeric antigen receptor T cells against CD19 for multiple myeloma. *N. Engl. J. Med.* **373,** 1040–1047.
40. Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., Grupp, S. A., Mackall, C. L. (2014) Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* **124,** 188–195.
41. Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., Rosenberg, S. A. (2010) Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther.* **18,** 843–851.
42. Sotillo, E., Barrett, D. M., Black, K. L., Bagashev, A., Oldridge, D., Wu, G., Sussman, R., Lanauze, C., Ruella, M., Gazzara, M. R., Martinez, N. M., Harrington, C. T., Chung, E. Y., Perazzelli, J., Hofmann, T. J., Maude, S. L., Raman, P., Barrera, A., Gill, S., Lacey, S. F., Melenhorst, J. J., Allman, D., Jacoby, E., Fry, T., Mackall, C., Barash, Y., Lynch, K. W., Maris, J. M., Grupp, S. A., Thomas-Tikhonenko, A. (2015) Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov.* **5,** 1282–1295.
43. Fry, T. J., Stetler-Stevenson, M., Shah, N. N., Yuan, C. M., Yates, B., Delbrook, C., Zhang, L., Lee, D. W., Stroncek, D., Mackall, C. L. (2015) Clinical activity and persistence of anti-CD22 chimeric antigen receptor in children and young adults with relapsed/refractory acute lymphoblastic leukemia (ALL). *57th Annual Meeting & Exposition*, American Society of Hematology, Washington, DC (abs.).


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