

# CD19-Targeted CAR T Cells: A New Tool in the Fight against B Cell Malignancies

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## Summary

Adoptive cell immunotherapy is a novel tool in the fight against cancer. Serving both effector and memory functions for the immune system, T cells make an obvious candidate for adoptive cell immunotherapy. By modifying native T cells with a chimeric antigen receptor (CAR), these cells can theoretically be targeted against any extracellular antigen. To date, the best-studied and clinically validated CAR T cells recognize CD19, a cell surface molecule on B cells and B cell malignancies. These CD19-directed T cells have shown clinical utility in chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas, with some patients achieving long-term disease remissions after treatment. This review will briefly summarize the current data supporting the use of adoptively transferred CAR T cells for the treatment of CD19-positive malignancies. Given these exciting results, the Food and Drug Administration has granted a 'breakthrough' designation for several variations of CD19-directed CAR T cells for treatment of adult and pediatric relapsed/refractory ALL.

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## Introduction

The ability of the immune system to attack cancer was first recognized in the late 1800s, when Dr. William Coley treated inoperable sarcomas with erysipelas [1]. Despite decades of research into immune-based strategies to treat cancer, until recently there have been few successes. These failures are likely due to immune toler-

ance resulting in an ineffective adaptive immune response. Adoptive T cell therapy is one way to break or bypass this immune tolerance.

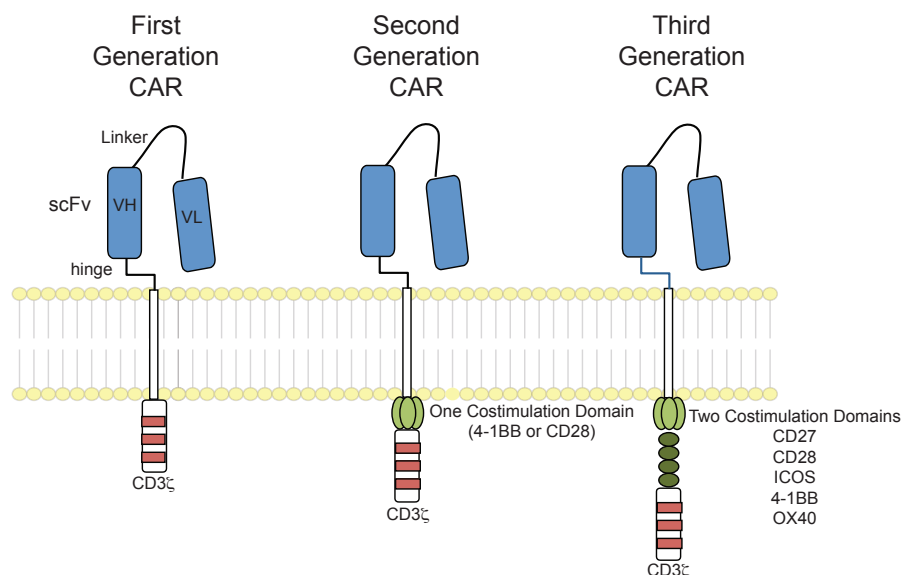
Chimeric antigen receptor (CAR) T cells are 'living drugs' in that they proliferate and retain the effector functions of activated T cells. CAR T cells are reprogrammed using synthetic biology and gene transfer techniques to attack cells expressing the target extracellular antigen, independent of major histocompatibility complex (MHC) presentation. In order to be effective, CAR T cells must traffic to tumor cells, bind the CAR's cognate antigen to activate the T cell, avoid inactivation/exhaustion by the local immunosuppressive microenvironment, proliferate, mount a tumor-directed cytotoxic response, and ideally form memory cells to prevent tumor recurrence.

The first true CAR T cell was produced in 1989, when the variable regions of the heavy and light chains from an antibody that bound to 2,4,6-trinitrophenyl were spliced onto the constant domains of the  $\alpha$  and  $\beta$  chains of the T cell receptor (TCR) [2]. Early CAR T cell trials in humans targeted human immunodeficiency virus (HIV), with studies showing an excellent safety profile but little clinical success [3, 4]. The first CAR trials in cancer occurred in solid tumors with little clinical efficacy [5, 6]. More recently, CAR T cells have been utilized in hematologic malignancies with improved outcomes, likely due to antigen selection, improvements in CAR design, and technological advances in gene transfer. This review will discuss CAR T cell design and generation, the results of clinical trials of CD19-directed CAR T cells in the treatment of chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas (NHL), and the toxicities associated with CD19 CAR T cell therapy.

## CAR T Cell Design and Generation

A number of variables in the manufacturing of CAR T cell therapies may affect their efficacy, including the design of the antigen

**Fig. 1.** Chimeric antigen receptor (CAR) design. CD19-directed CARs contain the variable heavy and light chains from an antibody that binds to CD19, a hinge region, transmembrane domain, and intracellular signaling domains. The different iterations of CAR design contain different intracellular signaling domains, with each successive generation adding additional costimulatory domains (original publication see [7], © American Society of Hematology).



receptor, the technique used for gene transfer, the ex vivo culturing system, and the types of T cells utilized. In addition, preparation of the host prior to infusion is likely important for engraftment. All of these variables are currently being studied to determine the optimal protocols to maximize efficacy while minimizing toxicity, cost, and time.

The CAR has 4 domains: the extracellular antigen binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain (fig. 1) [7]. The antigen-binding domain recognizes extracellular antigens in their native conformation and does not require presentation on an MHC molecule, allowing the CAR to function independently of patient haplotype and be targeted against many different extracellular ligands [8]. CD19-directed CARs have been generated from a single chain linked light and heavy chain variable region (scFv). Most groups use hinge and transmembrane domains derived from CD8 $\alpha$  or CD28 [7]. To transmit signals into the T cell, CARs were first designed with a single intracellular signaling domain (typically the CD3 $\zeta$  chain) [9, 10]. Future generations of CARs were designed to contain 1 or more costimulatory domains, which has resulted in improvements in T cell proliferation and persistence [11]. Most trials with CD19-directed CARs have used second generation CARs containing CD3 $\zeta$  with either CD28 or 4-1BB costimulatory domains. It is unclear which design, if either, is superior, although preclinical studies suggest that CARs containing the 4-1BB domain have improved persistence [11]. Many aspects of CAR design, such as epitope selection, affinity, length of the hinge region, and comparison of different combinations of intracellular signaling domains, are still under active investigation.

There are a number of strategies to introduce the CAR into primary human T cells, including transposon-based systems and transfection of plasmids [12], but most groups have used gammaretroviral or lentiviral transduction systems. Although these methods have a high efficiency and require short in vitro culture times, there are

concerns for insertional mutagenesis resulting in transformation of a T cell clone, as has been seen in stem cells [13]. To date, there have been no such cases of retrovirus-induced oncogenesis in CAR T cells (despite over 540 patient-years of clinical experience for the first HIV-targeted CAR T cells), potentially due to inherent resistance of mature human T cells to insertional transformation [4].

There is also variation in the ex vivo culturing of primary T cells that may affect clinical outcomes. First, the optimal starting population of T cells for genetic engineering and the best method to isolate or grow this population is unclear. Ideally, CAR T cells should be able to undergo self-renewal and effector differentiation in vivo upon antigen exposure, properties of T stem cell memory and T central memory cells [14]. In addition, harvesting T cells from individuals is variable from patient to patient and is also influenced by the underlying malignancy and prior therapies [15]. These problems may be overcome by using alternative sources of engineered T cells, such as lymphoid progenitors or pluripotent stem cells [16]. Second, groups are using different in vitro culturing conditions to expand and transduce their primary T cell populations, providing TCR stimulation through antibodies or antigen-presenting cells with or without various combinations of supporting cytokines [17]. These approaches can preferentially favor culturing of different subsets of T cells and have variable costs and time required for each, potentially affecting the efficacy and feasibility of generating the final product [18].

Successful transfer of genetically engineered T cells back into the human host is dependent on engraftment and persistence of the modified cells. Animal studies have shown that treatment of the host with lymphodepletion (via chemotherapy and/or radiation therapy) results in improved engraftment and tumor killing [19, 20]. Although no large-scale direct comparisons of lymphodepletion versus none in humans have been performed, small studies and historical comparisons suggest lymphodepletion is beneficial for engraftment [21–23]. Lymphodepletion likely facilitates en-

graftment by many mechanisms: killing resident T cells so that transferred T cells expand due to homeostatic mechanisms; reducing the population of immunosuppressive Treg cells; reducing tumor burden; and inducing inflammation that promotes T cell proliferation/maintenance. Most current protocols for CD19-directed CAR T cells now use a preconditioning regimen.

## CD19 CAR T Cells

CD19 is a member of the immunoglobulin superfamily of proteins expressed on the surface of B cells at most stages of their development, where it functions as a critical component of the B cell receptor signaling complex [24]. CD19 serves as an ideal target for CAR-directed therapies because it is expressed on most B cell malignancies (including CLL, B-ALL, and many NHL), it is not expressed on hematopoietic stem cells, and elimination of all CD19+ B cells in the body is a manageable on-target treatment effect [25]. One potential drawback of CD19 as a target is that its surface expression is not required for maintenance of the tumorigenic phenotype, and escape variants have been noted [26–28]. Although not the first target to be studied, CAR T cell therapies directed against CD19 are the most mature to date, with the majority of clinical trials led primarily by 3 research institutions – University of Pennsylvania (UPenn), the National Cancer Institute (NCI), and Memorial Sloan Kettering Cancer Center (MSKCC). While both the NCI and MSKCC are using a second-generation CAR with CD3 $\zeta$  and CD28 intracellular signaling domains that is retrovirally transduced into T cells [29, 30], UPenn has selected a second-generation CAR with CD3 $\zeta$  and 4–1BB stimulatory domains using a lentiviral transduction system [11]. All 3 groups have reported dramatic clinical responses.

## CLL

CLL is an indolent B cell leukemia of adults, with approximately 15,000 new diagnoses per year in the US. Although usually responsive to immunochemotherapy at first, resistance develops with time such that this disease is not curable, except by allogeneic stem cell transplant (SCT). The 5-year overall survival rate is 66% [31].

A number of publications by 4 different groups demonstrated that CD19-directed CAR T cells have efficacy against relapsed/refractory CLL. The group from UPenn published 2 reports of 3 patients with refractory CLL treated with their CD19-directed CAR T cell product, CTL019 [32, 33]. The patients were all pretreated with chemotherapy prior to infusion. These cells expanded at least 1,000-fold *in vivo*, trafficked to the bone marrow (an area of active residual disease), and persisted for at least 6 months. 2 patients achieved a complete response (CR), while the 3rd had a partial response (PR) to treatment. The outcomes of 14 patients with relapsed/refractory CLL on this trial have been reported, with 4 achieving a CR, 4 a PR, and 6 having progressive disease (PD), for an overall response rate of 57% [34]. Among the patients to have responded, some have evi-

dence of functional CAR T cell activity beyond 4 years. Most impressively, of those 4 patients who achieved CR, minimal residual disease (MRD) is not detectable as far out as 4 years, suggesting some patients may have disease eradication.

Brentjens et al. [21] from MSKCC published their results of the first 8 patients with CLL and 1 patient with relapsed B-ALL (discussed below) treated with CD19-directed CAR T cells. The first 3 CLL patients treated without preconditioning chemotherapy had no objective responses. Subject 4 passed from sepsis shortly after infusion [35]. The next 4 patients were pretreated with chemotherapy. Of these 4 patients, 1 had a PR, 2 had stable disease (SD), and 1 had PD. These patients had detectable CAR T cells in the blood as far as 30 days post infusion.

The group at NCI has published 3 papers documenting the response of patients with CLL (and other lymphomas, see below) receiving their CD19-directed CAR T cells [36–38]. In the first report, 8 patients (4 with CLL, 4 with NHL) were pretreated with chemotherapy, infused with CAR T cells, and then administered interleukin (IL)-2 until dose-limiting toxicity. Of the 4 patients with CLL, 1 had a CR, 2 had a PR, and 1 had SD. All responses lasted at least 6 months. All patients had detectable CAR T cells in the peripheral blood for at least 2 weeks, with the cells persisting up to 4 months in some patients [36]. In their second publication, Kochenderfer et al. [37] treated patients with CLL and NHL whose disease persisted after allogeneic stem cell transplant. The patients did not receive preconditioning chemotherapy nor IL-2. Of the 4 patients with CLL, 1 had a CR (lasting > 9 months), 1 had SD, and 2 had PD after infusions. CAR T cells were detectable in the blood and increased in numbers 7–14 days after infusion, suggesting proliferation; however, these cells were minimally detectable by 1 month post infusion. Finally, in their most recent publication, Kochenderfer et al. [38] reported the outcomes of 15 patients (4 with CLL, 11 with NHL) who were pretreated with chemotherapy and were not given exogenous IL-2. Of the 4 patients with CLL, 3 were in CR ongoing at 14–23 months after infusion and 1 had a PR for 4 months. Fine needle aspiration of a bulky lymph node 19 days after infusion of the CAR T cells revealed 70% of the cells were T cells, with 31% expressing the anti-CD19 CAR. CAR cells were also detected in the blood of all patients, peaking around 7–17 days after infusion, with variable persistence ranging from approximately 2 to 10 weeks.

In a creative twist on the classic CAR T cell design, a group at the Baylor College of Medicine (BCM) generated virus-specific T cells retrovirally transduced with a CD19-directed CAR with CD3 $\zeta$  and CD28 endodomains and infused them into patients who had relapsed or had high risk of relapse after allogeneic stem cell transplantation [39, 40]. The authors hypothesized that the viral specificity of the CAR T cells would allow the cells to control viral infection via their endogenous TCR, while the CD19-directed CAR would grant them cytolytic properties against the tumor cells. The infused donor-derived T cells were detectable by polymerase chain reaction (PCR) in the blood for 1–12 weeks post infusion, and PCR positivity was also found in disease sites (bone marrow and lymph nodes). Antitumor effects were seen in 2/4 patients with CLL

(1 with PR, 1 with SD), with PD in the remaining 2 patients. Interestingly, the authors did observe an increase in Epstein-Barr virus (EBV)-specific CAR cells in 2 patients with reactivated EBV, but no increase in 1 patient with adenovirus-specific CAR cells during an adenoviral infection.

In summary, 4 different groups (at UPenn, NCI, MSKCC, and BCM) have published clinical trials using CD19 CAR T cells in patients with CLL. All of these reported small patient numbers with variable efficacy (12.5–100% overall response rate). Encouragingly, the most recent studies show improved response rates, suggesting improvements in generation and culturing of CAR T cells may help with efficacy. Multiple groups are now reporting proliferative bursts of CAR T cells approximately 2 weeks after infusion, although the persistence of the cells varies between reports and is likely related to the CAR construct used (CD28 vs. 4–1BB costimulation). Of note, in the studies that assessed the immunophenotype of the CAR T cells after infusion, an increase in effector memory cells was seen early in the response, with a rise of central memory cells later [33]. This suggests that in some patients, CAR T cells can give rise to long-lasting memory cells against CD19-expressing tumor cells. Encouragingly, some patients who achieved a CR have remained in remission up to 4 years after treatment, raising the possibility of disease eradication [34].

## B-ALL

ALL is an aggressive leukemia with about 6,000 new cases per year, the majority of which are derived from B cells. This malignancy more commonly affects children, but can also occur in adults. Although 90% of children will survive past 5 years after diagnosis, historically adults have done much worse, with overall survival rates of 40–50% [41]. In adults who relapse after initial remission, the median survival is only approximately 6 months [42].

The group at MSKCC first reported CD19 CAR T cell treatment of an adult patient with B-ALL during his second remission [21]. The patient remained in remission for 8 weeks before undergoing an allogeneic SCT. Although impossible to know if the CAR T cells helped maintain his remission, he did have a persistent B cell aplasia prior to transplant, likely indicating activity of the CAR T cells. This same group published a case series of 5 adult patients with relapsed B-ALL after salvage chemotherapy (but prior to allogeneic SCT) [43]. Patients were pretreated with lymphodepleting chemotherapy. At the time of infusion, 2 patients had chemotherapy-refractory disease, 2 had responded to salvage chemotherapy but had evidence of MRD (MRD+), and 1 patient was MRD-. All 5 patients became MRD- within 8–59 days after infusion, with 4 proceeding to SCT (where they all remain in CR, although 1 passed from a suspected pulmonary embolus). The 5th patient was ineligible for SCT or additional CAR T cell therapy and relapsed after 90 days. CAR T cells proliferated in the blood within the first 2 weeks and were detectable in both the blood and bone marrow for up to 3–8 weeks after infusion. Of note, the patient who relapsed continued to express CD19 on his leukemic cells which were still susceptible

to CD19-directed CAR T cell killing in vitro. This suggests that his CAR T cells were not present in sufficient quantity after 90 days to be effective, although the patient did receive high-dose steroids for cytokine-release syndrome (CRS), which may have diminished CAR T cell persistence. The same group published their results from an additional 11 adult patients with relapsed/refractory B-ALL [44]. Patients were treated with salvage chemotherapy, followed by lymphodepleting chemotherapy and infusion of CAR T cells. Of the total 16 patients reported in this trial (5 from prior publication, 11 new patients), 88% of patients had a CR (75% MRD-). All patients had a peak of CAR T cell numbers within 1–2 weeks, with few to undetectable CAR T cells by 2–3 months after infusion. In their most recent update, this group has now reported that they have treated 33 adult patients with B-ALL (32 were available for response) with their CAR T cell product [45]. They reported 13/16 patients who had morphological disease and 16/16 patients with MRD+ disease at the time of infusion were in CR after infusion (91% CR, 82% MRD-). Interestingly, 2 patients relapsed with CD19-negative disease.

UPenn has also treated both children and adults with relapsed/refractory B-ALL with CTL019, first published as a case series of 2 treated children [26]. 1 child received prior lymphodepleting chemotherapy, the 2nd child did not. As with their previous studies in CLL, the CTL019 cells expanded > 1,000-fold and trafficked to sites of tumor (bone marrow). CAR T cells were also found in the cerebrospinal fluid of both patients up to 6 months post infusion, an important finding given that many lymphoid malignancies like B-ALL relapse in the central nervous system (CNS). After treatment, both patients went into CR (1 MRD-), with 1 ongoing at 11 months (now over 2 years) and 1 relapsed 2 months later with CD19-deficient leukemia cells. In their most recent publication, this group reported treating a total of 30 children and adults with relapsed/refractory ALL with CTL019 (fig. 2) [27]. All but 3 of the patients received lymphodepleting chemotherapy prior to infusion. 90% (27/30) of treated patients achieved a CR, with MRD negativity in 22/27. Of note, 2 patients had blasts in the CNS prior to treatment which cleared after infusion of CTL019 cells, and there were no CNS relapses. Of the 27 patients who achieved a CR, 7 had a relapse between 1.5 and 8.5 months following treatment. Of the patients who relapsed, 3 relapsed with CD19-negative disease. CTL019 cells remained detectable in the blood by flow cytometry for up to 11 months and detectable by more sensitive PCR methods for up to 2 years after infusion. Of note, B cell aplasia occurred in all patients who responded to treatment and persisted for up to 1 year after CTL019 cells were no longer detectable by flow cytometry, suggesting functional cells were still present.

The NCI treated 20 patients with relapsed/refractory ALL (and 1 patient with NHL, discussed below) with their CD19-directed CAR T cells [46]. Patients were pretreated with lymphodepleting chemotherapy. 14 patients had a CR (70%), with 12 of the 14 having an MRD- CR. 2 patients relapsed after 3 and 5 months with CD19-negative ALL. Of note, 65% of evaluated patients had evidence of CAR T cells within the cerebrospinal fluid, and 2 patients with CNS leukemia prior to therapy were cleared. CAR T cells had



peak expansion around day 14 and had disappeared from the circulation of every patient by day 68. Interestingly, CAR T cell expansion correlated both with response and toxicity.

Finally, in the series by Cruz et al. [40] using viral-specific T cells to generate CD19 CAR T cells, 4 of the 8 treated patients had B-ALL. 1 patient had a brief CR in response to infusion of CAR T cells but relapsed within 4 months. A 2nd patient had progressive disease. 2 patients were in remission at the time of infusion, and remained disease free 2–8 months after.

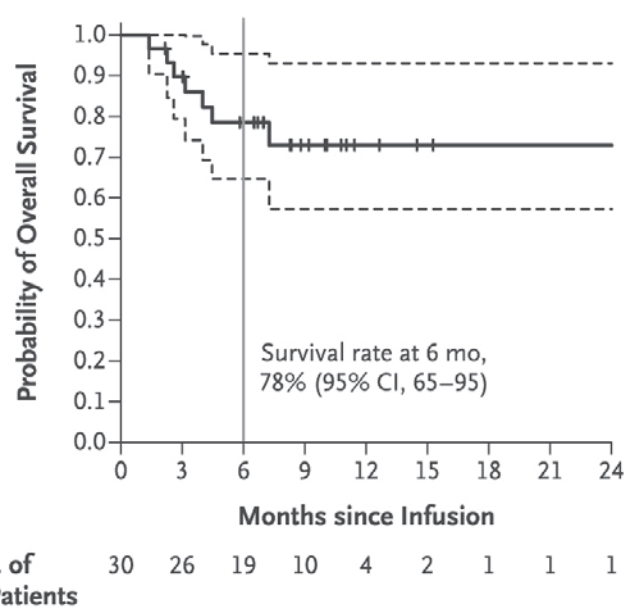
In summary, multiple groups have now reported promising results for the use of CD19-directed CAR T cells to treat refractory/relapsed ALL. Response rates in the more advanced trials range from 70 to 91% of patients achieving a CR. Follow-up is problematic, as many patients go on to receive allogeneic SCT once in remission. In those patients not receiving SCT, relapses are seen, sometimes with CD19-negative leukemic cells, suggesting this as one mechanism for escape. In addition, relapses often correlate with the absence of detectable CAR T cells, suggesting temporary control but incomplete eradication of the leukemic clone by the modified T cells. The use of CAR T cells as a bridge to transplant versus a means of obtaining long-lasting disease control is still unclear, and may depend on the properties of the CAR and persistence of the modified cells.

## NHL

NHL are a diverse group of lymphoid malignancies, with nearly 70,000 new cases per year in the United States, many of which express CD19. Aggressive forms such as diffuse large B cell lymphoma (DLBCL) can sometimes be cured with chemotherapy but are difficult to treat when recurrent or refractory, while indolent forms such as follicular lymphoma (FL) have a slower clinical course but cannot be cured without SCT [47].

In an early publication from the City of Hope, a first generation anti-CD19 CAR (CD3 $\zeta$  intracellular signaling domain only) was used to treat 2 patients with FL who failed prior rituximab therapy [48]. Neither patient had detectable CAR T cells in the peripheral blood more than a few days after infusion, despite multiple infusions and increasing doses of cells. Both patients progressed rapidly after treatment. The authors postulated that cell-mediated immunoreactivity against the CAR T cells may have contributed to the poor persistence.

The NCI has conducted the majority of clinical trials for treatment of CD19-positive lymphomas with anti-CD19 CAR T cells. They first published a case report in 2010 of a patient with advanced FL treated with CAR T cells after lymphodepletion, followed by intravenous IL-2 [49]. He had a PR that lasted 8 months, with CAR T cells that were detectable for up to 27 weeks after transfusion. This patient was later retreated with the same CAR T cells, and had another PR ongoing at 26 months after his original treatment [50]. In their 2012 paper, Kochenderfer et al. [36] reported on 3 additional patients with NHL (2 FL, 1 splenic marginal zone lymphoma (SMZL)) treated on the same protocol (lymphode-



**Fig. 2.** Overall survival after treatment with CD19-directed chimeric antigen receptor (CAR) T cells. 30 patients with relapsed/refractory acute lymphoblastic leukemia were treated with CD19-directed CAR T cells (CTL019). This difficult-to-treat cohort of patients had an excellent 6-month survival, demonstrating the potential benefit of CD19 CAR T cell therapy. Dotted lines represent 95% confidence interval [27] (© (2014) Massachusetts Medical Society; reprinted with permission from Massachusetts Medical Society).

pleting pretreatment, infusion with T cells, then IL-2 until dose-limiting toxicity). 1 patient passed from influenza, but the other 2 patients both had a PR lasting 8–12 months after infusion.

Under a different treatment protocol, Kochenderfer et al. [37] treated 6 patients with NHL (2 DLBCL, 4 mantle cell lymphoma) who had progressed after allogeneic SCT and donor-lymphocyte infusion. Of the 6 patients, 5 had SD following infusion and 1 had a PR. The authors have also reported on 1 patient with DLBCL who had PD despite treatment [46]. In their largest series to date, Kochenderfer et al. [38] treated 11 patients with NHL (5 with DLBCL, 4 with primary mediastinal B cell lymphoma, 1 with SMZL, and 1 with an indolent NHL). Of these 11 patients, 1 was lost to follow-up and 1 passed away 16 days after infusion (attributed to cardiac arrhythmia of unknown etiology). Of the remaining 9 patients, 5 had a CR (some ongoing as long as 23 months, 1 relapse after 6 months), 3 had a PR, and 1 had SD lasting 1 month.

A group from UPenn has recently reported preliminary outcomes from a phase IIa clinical trial of CTL019 in patients with NHL. Of 18 patients evaluable for response (12 with DLBCL, 6 with FL), the overall response rate was 67% (with 50% of DLBCL patients and 100% of FL patients responding) [51].

Although numerous clinical trials have included patients with NHL, the numbers of patients are small and response rates are variable. Although much more work needs to be completed in this area, recent results are encouraging. For patients with relapsed/refractory NHL with few treatments options, CD19 CAR T cells hold promise.

## Toxicities

Although CAR T cells are proving to be a powerful new therapeutic against cancer, they also have the potential for significant toxicity. Fatalities have been caused by CARs directed against a tumor antigen (HER2/neu) also found on normal pulmonary epithelium, for example [52]. On-target toxicity of CD19-directed CAR T cells results in B cell aplasia and hypogammaglobulinemia, which can be managed with immunoglobulin infusions [32, 34]. Depending on the CAR construct used, different groups have reported aplasia lasting from 1 month to over 4 years [34, 46, 53]. Many groups are using B cell aplasia as a marker of CAR T cell persistence and activity, with some reports of a tight correlation between CLL disease response and B cell aplasia [27].

The most life-threatening complication of CD19 CAR T cell treatment is the development of CRS, a macrophage hyperactivation-like condition attributed to robust T cell proliferation and activation. Davila et al. [44] defined a set of diagnostic criteria for severe CRS: i) fevers  $> 38.0^{\circ}\text{C}$  for at least 3 consecutive days; ii) 2 cytokine max fold changes of at least 75 or 1 max fold change of at least 250 over baseline; iii) at least 1 clinical sign of toxicity such as hypotension, hypoxia, or neurologic dysfunction. Commonly elevated cytokines include interferon (IFN)- $\gamma$ , IL-6, and IL-10, although the clinical severity of CRS may or may not correlate with the degree of cytokine elevation [36, 54]. The onset of CRS ranges from a few days to 3 weeks post infusion. For patients with ALL, the development of CRS correlates with pre-treatment tumor burden [43, 44, 46].

The treatment of severe CRS often requires intensive care unit (ICU)-level supportive care while treating the underlying cause with steroids and tocilizumab, an antibody that inhibits the IL-6 receptor [54]. The majority of patients have rapid reversal of CRS with tocilizumab, without any clear decrease in efficacy of the CAR T cells [53, 54]. Although there is concern that steroids may blunt CAR T cell activity [43], in some cases a short course of steroids has been administered to mitigate CRS without clear evidence of diminished CAR T cell efficacy. Other cytokine-directed approaches have been tried, such as etanercept to target TNF- $\alpha$ , without benefit [54]. The optimal treatment algorithm for CRS is under development by each sponsor, with cooperation among various investigators [55].

Tumor lysis syndrome, a condition of electrolyte imbalances caused by the rapid lysis of tumor cells which can result in kidney failure, cardiac arrhythmias, seizures, and death, has also been observed after treatment with CD19 CAR T cells, particularly in patients with CLL [32, 33, 37, 56]. Of note, no cases of graft-versus-host disease have been reported due to donor-derived CAR T cells

in patients treated after allogeneic SCT [37, 40]. Despite the theoretical concern, there have also been no reports of insertional mutagenesis resulting in new T cell malignancies.

## Conclusion and Future Directions

CD19-directed CAR T cells have shown remarkable promise for relapsed or refractory cases of CLL, ALL, and NHL. Studies are ongoing to expand the field of potentially treatable malignancies, including multiple myeloma. Although multiple myeloma cells do not typically express high levels of CD19, a group at UPenn has reported clinical responses with CTL019 [57].

To safely expand the access to CAR T cell therapies from a few expert centers to more institutions, it will be important to develop standardized approaches to grading and treatment of CRS and better understand who is likely to develop this condition. In addition, much is still unknown about how to optimize CAR efficacy while minimizing toxicity. Variables such as the design of the CAR construct, ex vivo cell growth conditions, transduction/transfection methods, and patient preparation prior to infusion all need to be studied. The interplay of CAR T cells with the local immunosuppressive microenvironment of the tumor is also an area of active research [58].

Finally, relapses after CD19 CAR T cells are well documented, and can occur with both CD19-expressing and CD19-negative tumor cells [26, 27]. In the case of retained CD19 expression, reinfusion of CD19-directed CAR T cells may be sufficient in some cases to reinduce remission, although this has not always been seen [36, 46]. For CD19-negative relapses, alternative approaches will need to be used, such as utilizing CARs directed against other B cell surface molecules (i.e. CD22) [59]. CD19 CAR T cells will undoubtedly have a significant role in the future treatment of B cell malignancies.

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## Disclosure Statement

Dr. Miller does not declare any conflicts of interest. Dr. Maus is listed as an inventor in patents related to T cell therapies held by the University of Pennsylvania and Novartis.

## References

- Coley WB: The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the streptococcus erysipelas and the bacillus prodigiosus). *Proc R Soc Med* 1910;3:1–48.
- Gross G, Waks T, Eshhar Z: Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proceedings of the National Academy of Sciences* 1989;86:10024–10028.
- Deeks SG, Wagner B, Anton PA, Mitsuyasu RT, Scadden DT, Huang C, Macken C, Richman DD, Christopher C, June CH, Lazar R, Broad DF, Jalali S, Hege KM: A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. *Mol Ther* 2002;5:788–797.
- Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, Vogel AN, Kalos M, Riley JL, Deeks SG, Mitsuyasu RT, Bernstein WB, Aronson NE, Levine BL, Bushman FD, June CH: Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Sci Transl Med* 2012;4:132ra153.
- Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P: A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006;12:6106–6115.
- Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, Gratama JW, Stoter G, Oosterwijk E: Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* 2006;24:e20–22.
- Maus MV, Grupp SA, Porter DL, June CH: Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood* 2014;123:2625–2635.
- Sadelain M, Brentjens R, Riviere I: The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013;3:388–398.
- Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, Huls MH, Liu E, Gee AP, Mei Z, Yvon E, Weiss HL, Liu H, Rooney CM, Heslop HE, Brenner MK: Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008;14:1264–1270.
- Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Griesman HA, Qian X, James SE, Raubitschek A, Forman SJ, Gopal AK, Pagel JM, Lindgren CG, Greenberg PD, Riddell SR, Press OW: Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 2008;112:2261–2271.
- Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, Samanta M, Lakhal M, Gloss B, Danet-Desnoyers G, Campana D, Riley JL, Grupp SA, June CH: Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol Ther* 2009;17:1453–1464.
- Singh H, Moyes JS, Huls MH, Cooper LJ: Manufacture of T cells using the sleeping beauty system to enforce expression of a CD19-specific chimeric antigen receptor. *Cancer Gene Ther* 2015;22:95–100.
- Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat R, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M: Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008;118:3132–3142.
- Flynn JK, Gorry PR: Stem memory T cells (TSCM) – their role in cancer and HIV immunotherapies. *Clin Transl Immunology* 2014;3:e20.
- Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, Ramsay AG, Gribben JG: T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood* 2013;121:1612–1621.
- Themeli M, Riviere I, Sadelain M: New cell sources for T cell engineering and adoptive immunotherapy. *Cell Stem Cell* 2015;16:357–366.
- Kim JV, Latouche JB, Riviere I, Sadelain M: The ABCs of artificial antigen presentation. *Nat Biotechnol* 2004;22:403–410.
- Maus MV, Thomas AK, Leonard DG, Allman D, Addya K, Schlienger K, Riley JL, June CH: Ex vivo expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. *Nat Biotechnol* 2002;20:143–148.
- Wrzesinski C, Paulos CM, Kaiser A, Muranski P, Palmer DC, Gattinoni L, Yu Z, Rosenberg SA, Restifo NP: Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. *J Immunother* 2010;33:1–7.
- North RJ: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med* 1982;155:1063–1074.
- Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, Taylor C, Yeh R, Bortido S, Borquez-Ojeda O, Olszewska M, Bernal Y, Pegram H, Przybylowski M, Hollyman D, Usachenko Y, Pirraglia D, Hosen J, Santos E, Halton E, Maslak P, Scheinberg D, Jurcic J, Heaney M, Heller G, Frattini M, Sadelain M: Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* 2011;118:4817–4828.
- Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002;298:850–854.
- Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA: Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005;23:2346–2357.
- Depoil D, Fleire S, Treanor BL, Weber M, Harwood NE, Marchbank KL, Tybulewicz VL, Batista FD: CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nat Immunol* 2008;9:63–72.
- Scheuermann RH, Racila E: CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy. *Leuk Lymphoma* 1995;18:385–397.
- Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, Milone MC, Levine BL, June CH: Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013;368:1509–1518.
- Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL, Grupp SA: Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014;371:1507–1517.
- Evans AG, Rothberg PG, Burack WR, Huntington SF, Porter DL, Friedberg JW, Liesveld JL: Evolution to plasmablastic lymphoma evades cd19-directed chimeric antigen receptor T cells. *Br J Haematol* 2015; Epub ahead of print.
- Hollyman D, Stefanski J, Przybylowski M, Bortido S, Borquez-Ojeda O, Taylor C, Yeh R, Capacio V, Olszewska M, Hosen J, Sadelain M, Brentjens RJ, Riviere I: Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy. *J Immunother* 2009;32:169–180.
- Kochenderfer JN, Feldman SA, Zhao Y, Xu H, Black MA, Morgan RA, Sadelain M, Rosenberg SA: Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. *J Immunother* 2009;32:689–702.
- Nabhan C, Rosen ST: Chronic lymphocytic leukemia: a clinical review. *JAMA* 2014;312:2265–2276.
- Porter DL, Levine BL, Kalos M, Bagg A, June CH: Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011;365:725–733.
- Kalos M, Morgan RA, Porter DL, Katz S, Grupp SA, Bagg A, June CH: T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011;3:95ra73.
- Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, Bagg A, Marcucci KT, Shen A, Gonzalez V, Ambrose D, Grupp SA, Chew A, Zheng Z, Milone MC, Levine BL, Melenhorst JJ, June CH: Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med* 2015;7:303ra139.
- Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M: 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* 2010;18:666–668.
- Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, Stetler-Stevenson M, Phan GQ, Hughes MS, Sherry RM, Yang JC, Kammula US, Devillier L, Carpenter R, Nathan DA, Morgan RA, Laurencot C, Rosenberg SA: 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* 2012;119:2709–2720.

- 37 Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, Hakim FT, Halverson DC, Fowler DH, Hardy NM, Mato AR, Hickstein DD, Gea-Banacloche JC, Pavletic SZ, Sportes C, Maric I, Feldman SA, Hansen BG, Wilder JS, Blacklock-Schuber B, Jena B, Bishop MR, Gress RE, Rosenberg SA: Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* 2013;122:4129–4139.
- 38 Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, Yang JC, Phan GQ, Hughes MS, Sherry RM, Raffeld M, Feldman S, Lu L, Li YF, Ngo LT, Goy A, Feldman T, Spaner DE, Wang ML, Chen CC, Kranick SM, Nath A, Nathan DA, Morton KE, Toomey MA, Rosenberg SA: 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* 2015; 33:540–549.
- 39 Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, Kamble RT, Bollard CM, Gee AP, Mei Z, Liu H, Grilley B, Rooney CM, Heslop HE, Brenner MK, Dotti G: CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J Clin Invest* 2011;121: 1822–1826.
- 40 Cruz CR, Micklethwaite KP, Savoldo B, Ramos CA, Lam S, Ku S, Diouf O, Liu E, Barrett AJ, Ito S, Shpall EJ, Krance RA, Kamble RT, Carrum G, Hosing CM, Gee AP, Mei Z, Grilley BJ, Heslop HE, Rooney CM, Brenner MK, Bollard CM, Dotti G: Infusion of donor-derived CD19-redirection virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase I study. *Blood* 2013;122:2965–2973.
- 41 Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, Reaman GH, Carroll WL: Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol* 2012;30: 1663–1669.
- 42 Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G, Durrant IJ, Luger SM, Marks DI, Franklin IM, McMillan AK, Tallman MS, Rowe JM, Goldstone AH; Medical Research Council of the United Kingdom Adult ALLWP, Eastern Cooperative Oncology G: Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL): an MRC UKALL12/ECOG 2993 study. *Blood* 2007;109:944–950.
- 43 Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, Bartido S, Stefanski J, Taylor C, Olszewska M, Borquez-Ojeda O, Qu J, Wasielewska T, He Q, Bernal Y, Rijo IV, Hedvat C, Kobos R, Curran K, Steinherz P, Jurcic J, Rosenblatt T, Maslak P, Frattini M, Sadelain M: CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* 2013;5: 177ra138.
- 44 Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, 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 DC, Arcila ME, Gonen M, Roboz GJ, Maslak P, Douer D, Frattini MG, Giral S, Sadelain M, Brentjens R: Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 2014;6: 224ra225.
- 45 Sadelain M, Brentjens R, Riviere I, Park J: CD19 car therapy for acute lymphoblastic leukemia. *Am Soc Clin Oncol Educ Book* 2015;35:e360–363.
- 46 Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroneck D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS, Mackall CL: T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015;385:517–528.
- 47 Guerard EJ, Bishop MR: Overview of non-Hodgkin's lymphoma. *Dis Mon* 2012;58:208–218.
- 48 Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, Forman SJ: 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* 2010;16:1245–1256.
- 49 Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, Maric I, Raffeld M, Nathan DA, Lanier BJ, Morgan RA, Rosenberg SA: Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* 2010; 116:4099–4102.
- 50 Kochenderfer JN, Rosenberg SA: Chimeric antigen receptor-modified T cells in CLL. *N Engl J Med* 2011; 365:1937–1938;author reply 1938.
- 51 Stephen JS, Jakub S, Sunita N, David LP, Elise AC, Yolanda M, Simon FL, Joseph JM, Anne C, Gaurav S: Phase IIa trial of chimeric antigen receptor modified T cells directed against CD19 (CTL019) in patients with relapsed or refractory CD19+ lymphomas. *Blood* 2014; 124:3087–3087.
- 52 Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA: Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010;18:843–851.
- 53 Maude SL, Teachey DT, Porter DL, Grupp SA: CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood* 2015;125:4017–4023.
- 54 Maude SL, Barrett D, Teachey DT, Grupp SA: Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J* 2014;20:119–122.
- 55 Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL: current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 2014;124:188–195.
- 56 Howard SC, Jones DP, Pui CH: The tumor lysis syndrome. *N Engl J Med* 2011;364:1844–1854.
- 57 Garfall AL, Maus MV, Hwang WT, Lacey SF, Mahnke YD, Melenhorst JJ, Zheng Z, Vogl DT, Cohen AD, Weiss BM, Dengel K, Kerr ND, Bagg A, Levine BL, June CH, Stadtmauer EA: Chimeric antigen receptor T cells against CD19 for multiple myeloma. *N Engl J Med* 2015;373:1040–1047.
- 58 Lee JC, Hayman E, Pegram HJ, Santos E, Heller G, Sadelain M, Brentjens R: In vivo inhibition of human CD19-targeted effector T cells by natural T regulatory cells in a xenotransplant murine model of B cell malignancy. *Cancer Res* 2011;71:2871–2881.
- 59 Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, Dimitrov DS, Morgan RA, FitzGerald DJ, Barrett DM, Wayne AS, Mackall CL, Orentas RJ: Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. *Blood* 2013;121:1165–1174.