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

Brian C. Miller\textsuperscript{a} Marcela V. Maus\textsuperscript{b}

\textsuperscript{a}Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA; \textsuperscript{b}Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA

Keywords
CAR T cell · CD19 · T cell therapies · Adoptive transfer · B cell · Chimeric antigen receptor · Immunotherapy

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.

© 2015 S. Karger GmbH, Freiburg

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 tolerance 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
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 CD8α or CD28 [7]. To transmit signals into the T cell, CARs were first designed with a single intracellular signaling domain (typically the CD3ζ 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 CD3ζ, 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].

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-
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 CD3ζ and CD28 intracellular signaling domains that is retrovirally transduced into T cells [29, 30], UPenn has selected a second-generation CAR with CD3ζ 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 evidence 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 CD3ζ 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.

CD19-Targeted CAR T Cells for B Cell Malignancies

Oncol Res Treat 2015;38:683–690 685
in 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 (CD3ζ 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 (lymphodepleting 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 Ila 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°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)-γ, 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-α, 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.

Acknowledgement

Dr. Miller is supported by T32CA009172, and Dr. Maus is supported by K08CA166039.

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


