

## ACUTE LYMPHOBLASTIC LEUKEMIA

# CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia

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**Relapsed and refractory acute lymphoblastic leukemia (ALL) remains difficult to treat, with minimal improvement in outcomes seen in more than 2 decades despite advances in upfront therapy and improved survival for de novo ALL. Adoptive transfer of T cells engineered to express a chimeric antigen receptor (CAR) has emerged as a powerful targeted immunotherapy, showing striking responses in highly refractory populations. Complete remission (CR) rates as high as 90% have been reported in**

**children and adults with relapsed and refractory ALL treated with CAR-modified T cells targeting the B-cell-specific antigen CD19. Distinct CAR designs across several studies have produced similar promising CR rates, an encouraging finding. Even more encouraging are durable remissions observed in some patients without additional therapy. Duration of remission and CAR-modified T-cell persistence require further study and more mature follow-up, but emerging data suggest these factors**

**may distinguish CAR designs. Supraphysiologic T-cell proliferation, a hallmark of this therapy, contributes to both efficacy and the most notable toxicity, cytokine release syndrome (CRS), posing a unique challenge for toxicity management. This review will discuss the current landscape of CD19 CAR clinical trials, CRS pathophysiology and management, and remaining challenges. (*Blood*. 2015;125(26): 4017-4023)**

### Introduction

Overall survival for acute lymphoblastic leukemia (ALL) in children exceeds 85%.<sup>1</sup> Improved survival primarily stems from decreased incidence of relapse, with very little improvement for more than 20 years in survival rates for children who relapse.<sup>2-4</sup> In contrast, overall survival for adults with ALL is quite poor (30% to 40%),<sup>5,6</sup> and relapsed ALL remains particularly challenging for all age groups, making it a leading cause of cancer deaths in children and carrying a dismal prognosis in adults.<sup>2,4,7</sup> Most children in first relapse will achieve a second complete remission (CR2), in contrast to the adult population, in which CR2 rates are 50% at best.<sup>2,4,7</sup> Even for patients who achieve CR2, those remissions are frequently not sustained.<sup>2,4</sup> With each subsequent relapse, achieving remission is harder and long-term survival is extremely poor.<sup>4</sup> Refractory ALL is also challenging, with long-term survival close to 30%.<sup>8</sup> For those who do not achieve a remission, options are limited.

Several ALL subtypes with high-risk genotypes have been characterized beyond *BCR-ABL1*-driven Philadelphia chromosome-positive ALL. In the future, genetic lesions may be targeted by inhibitors aimed at intracellular or extracellular molecules expressed by leukemic blasts, as suggested by the use of imatinib.<sup>9,10</sup> Although promising examples exist,<sup>11-13</sup> the majority of ALL cases do not have a known driver lesion. Thus, the current state of ALL therapy is that these genomic discoveries will first be used to detect patients at higher risk of recurrence, a group of patients for whom novel therapies are needed.

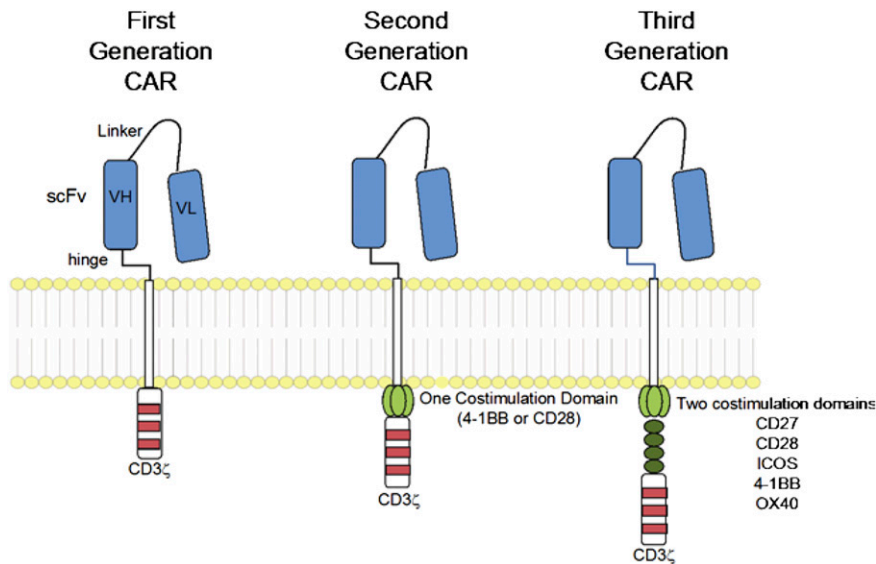
Targeted immunotherapy is another attractive alternative that has emerged as a potent therapy and will be the subject of this review. Chimeric antigen receptor (CAR)-modified T cells targeting CD19, the best-studied CAR T-cell therapy to date, will be discussed, with a focus

on clinical trials for ALL demonstrating efficacy as well as toxicity and toxicity management.

### Immunotherapy rationale

Potentially malignant cells are continuously eliminated by apoptosis and the immune system, but cancers have escaped these mechanisms. For immune-mediated clearance of leukemia to be possible, tolerance has to be overcome. This is the basis for the graft-versus-leukemia (GVL) effect, which contributes in part to the efficacy of allogeneic stem cell transplantation (SCT) and is the rationale for donor lymphocyte infusion in leukemia.<sup>14</sup> Although donor lymphocyte infusion has been less efficacious in ALL, GVL efficacy in ALL is recognized. A recent Children's Oncology Group trial demonstrated that relapse risk was significantly lower in patients who developed acute graft-versus-host disease (GVHD) after allogeneic SCT for ALL compared with those who did not.<sup>15</sup> However, the GVL effect carries the significant risk of GVHD.

Use of autologous T cells would remove the risk of GVHD but requires a means of breaking tolerance to self. One method involves reprogramming T cells to identify and eliminate malignant cells through tumor-specific antigen recognition. CARs link an extracellular antigen-recognition domain, derived from a monoclonal antibody fragment (scFv), to intracellular signaling domains of the T-cell receptor complex. T cells engineered to express such CARs engage an antigen on a tumor cell through the extracellular antibody domain, thereby activating the



**Figure 1. CAR structure, according to signaling domains.** CAR molecules link an extracellular single-chain variable fragment (scFv) to intracellular signaling domains. The intracellular component of the T-cell receptor either alone (first generation) or in combination with 1 (second generation) or 2 (third generation) costimulatory domains. Reprinted with permission from Maus et al.<sup>19</sup>

T cells in a major histocompatibility complex–independent manner and stimulating a potent cytotoxic response.

## T-cell engineering and manufacture

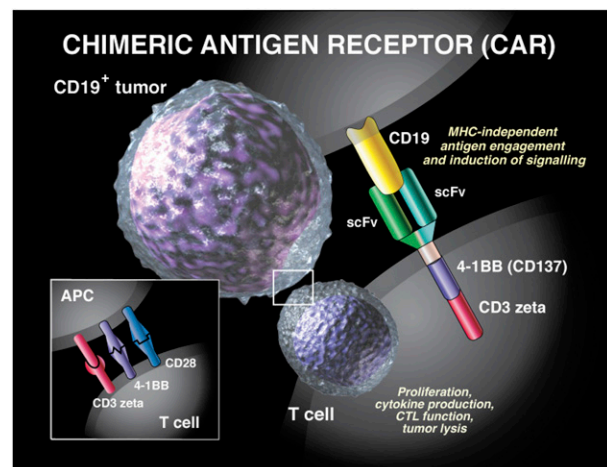
The concept of CARs was first described 25 years ago as a means of introducing tumor specificity into adoptive cell therapy.<sup>16</sup> The initial design joined an antibody-derived scFv to the CD3 $\zeta$  intracellular signaling domain of the T-cell receptor through hinge and transmembrane domains. Although the first CARs showed evidence of function preclinically and limited responses in clinical trials (reviewed in Barrett et al,<sup>17</sup> June et al,<sup>18</sup> and Maus et al<sup>19</sup>), fine-tuning was needed to optimize both *in vitro* and *in vivo* T-cell proliferation and persistence. As iterative modifications were made, this fundamental design was dubbed a “first-generation” CAR. “Second-generation” CARs incorporate an additional domain, CD28 or 4-1BB, to supply a costimulatory signal. Two costimulatory domains, a combination of CD27, CD28, 4-1BB, ICOS, or OX40, make up “third-generation” CARs.<sup>20–23</sup> First-, second-, and third-generation CARs are depicted in Figures 1 and 2.

CAR designs differ not only in their components but also in their functional properties. The CD3 $\zeta$  signaling domain of the T-cell receptor, when engaged, will activate and induce proliferation of T cells but can lead to anergy. The addition of a costimulatory domain in second-generation CARs improved replicative capacity and persistence of modified T cells. Which costimulatory domain is optimal is unknown. Similar antitumor effects are seen *in vitro* with CD28 or 4-1BB CARs, but preclinical *in vivo* studies<sup>22</sup> suggest that 4-1BB CARs (Figure 2) may produce superior proliferation and/or persistence.<sup>21,24</sup> Moreover, clinical trials suggest that both of these second-generation CARs are capable of inducing substantial T-cell proliferation *in vivo*, but CARs containing the 4-1BB costimulatory domain appear to persist longer.<sup>25–27</sup>

Another variable that may influence replicative capacity and persistence is the *ex vivo* cell culture system used to manufacture large quantities of engineered T cells. Various systems have been developed, with current systems employing antibodies and/or artificial antigen-presenting cells to engage CD3, with costimulation provided by a second signal or cytokine.<sup>28–30</sup> It appears that the *in vitro* culture system for T-cell expansion plays an important role in influencing the final

composition of effector, naive, and memory T cells in the manufactured product. Although effector T cells may mediate potent cytotoxicity, they are terminally differentiated with minimal replicative capacity compared with naive and memory T cells, which have significant replicative capacity and potential for long-term persistence, respectively. Several groups, including ours, currently use an anti-CD3/CD28 antibody-coated magnetic bead system, developed by Levine et al.<sup>30</sup> T cells manufactured with this system are capable of maintaining the memory phenotype and long-term persistence.<sup>31</sup>

Gene transfer technologies are used to engineer T cells to express CARs, often simultaneously with expansion techniques. Various modes of gene transfer can be employed, from retroviral and lentiviral vector methods resulting in permanent modification of the genome to RNA-based methods leading to transient gene expression. Retro- or lentiviral approaches have the advantage of long-term gene expression and, therefore, the potential for long-term disease control from a single infusion of engineered T cells (if those T cells persist). The disadvantages of permanent modification are persistent on-target toxicity and the theoretical risk of transformation if gene insertion results in dysregulation of an oncogene. This has been



**Figure 2. Second-generation CAR used in current clinical studies at Penn and CHOP.** CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex. Reprinted with permission from Barrett et al.<sup>77</sup> © Sue Seif

reported with retroviral modification of hematopoietic stem cells, but not with lentiviral transduction and not when mature T cells are used as the target.<sup>32-35</sup> Gene transfer using messenger RNA yields transient expression without integration into the genome, obviating any concern of transformation. Short-term expression may also be desirable for CARs directed against antigens expressed on normal cells when sustained on-target toxicity is a concern. T cells engineered by RNA insertion through electroporation<sup>36</sup> have marked replicative capacity and can produce substantial tumor responses.<sup>37,38</sup> However, expression typically lasts 7 days or less, so long-term disease control, although still possible, would require multiple infusions with this approach.

## CD19: an ideal target?

Ideally, an antigen targeted by CAR-modified T cells would be tumor specific. Beyond that, an ideal antigen would be ubiquitously expressed on tumor cells, but not expressed on normal cells. This requires a cell-surface molecule that is unique to tumor cells, perhaps through altered expression, translocation producing a fusion protein, or mutation leading to altered configuration or antibody binding. Such unique antigens are hard to find. But viable alternatives include antigens that are expressed on a single cell lineage whose function is dispensable or replaceable or antigens that are differentially expressed on tumor cells compared with normal cells.

CD19 is a B-cell surface protein expressed throughout B-cell development; therefore, it is expressed on nearly all B-cell malignancies, including chronic lymphocytic leukemia (CLL), ALL, and many non-Hodgkin lymphomas.<sup>39</sup> This near-universal expression and specificity for a single cell lineage has made CD19 an attractive target for CAR-modified T-cell therapies. Additional B-cell-specific cell-surface molecules, such as CD22, may hold similar promise and are under active investigation.<sup>40</sup>

Target identification for T-cell ALL poses a particular challenge as leukemic blasts express the same antigens as normal T cells; therefore, CAR immunotherapy may not be possible in T-cell ALL. This is because B-cell aplasia is treatable and tolerable, whereas T-cell aplasia is not. Although select T-cell ALL subsets may aberrantly express cell-surface proteins that are not normally expressed on T cells or express abnormal fusion proteins, there is no universal target that is specific to T lymphoblasts.

## CD19 CAR clinical trials: striking activity

CD19-directed CAR-modified T-cell therapies for B-cell malignancies are the most advanced engineered T-cell therapies presently being tested. Clinical trials in CLL and ALL have demonstrated robust activity and striking clinical responses.<sup>20,25-27,41-45</sup> Initial reports included small numbers of patients but were notable for remissions induced in patients with refractory, bulky CLL and relapsed, highly refractory ALL who were considered incurable.<sup>20,41-44</sup>

### CLL

We first showed that CD19-directed CAR T cells could induce complete remissions (CR) for some patients with advanced, heavily pretreated, and high-risk CLL; all 3 of the first patients treated had dramatic antitumor responses, including 2 patients with long-term CRs.<sup>31,43</sup> These initial studies showed that CAR-modified T cells

targeting CD19 and containing the CD3 $\zeta$  activation domain and 4-1BB costimulatory domain (CTL019 cells) could dramatically proliferate *in vivo*, eliminate high volume and bulky disease, and, we now know, persist with ongoing functional activity beyond 3 years.<sup>46</sup> Others have also shown significant responses in small numbers of CLL patients to T cells modified with slightly different CAR constructs also targeting CD19 but containing a CD28 costimulatory domain.<sup>45,47</sup> Responses have occurred even in patients who have relapsed after prior allogeneic SCT.<sup>44</sup> We have now treated over 45 patients with advanced relapsed and refractory CLL. Response rates are ~45% with remissions in some patients extending beyond 4 years (D. L. Porter, unpublished data). All patients who respond and have persistent CAR-modified T cells also develop B-cell aplasia and hypogammaglobulinemia, perhaps considered “on-target” toxicity but also a measure of functional persistence. The most significant and unique toxicity is cytokine release syndrome (CRS), similar to patients with ALL; however, for reasons that are not well defined, the incidence and severity of CRS appear to be lower in CLL than ALL despite CLL patients having very high tumor burdens.

### ALL

The first reports of efficacy in ALL, although hopeful, needed to be expanded to determine the CR rate. With larger studies now published by 3 groups, using different CD19 CAR designs, the efficacy is even better than expected, with CR rates of 70% to 90%.<sup>25-27</sup> Our group has reported a 90% CR rate in 30 pediatric and adult patients with relapsed/refractory ALL treated in Children’s Hospital of Philadelphia (CHOP) and University of Pennsylvania (Penn) phase 1 trials.<sup>26</sup> Davila et al reported an 88% CR rate in a cohort of 16 adults with relapsed B-cell ALL treated at Memorial Sloan-Kettering Cancer Center (MSKCC).<sup>25</sup> Finally, Lee et al recently reported a 70% CR rate in a National Cancer Institute (NCI) intent-to-treat analysis of 20 children and young adults with ALL.<sup>27</sup> All 3 studies included patients with a prior history of allogeneic SCT, and no GVHD was seen.

The initial responses are comparable across different studies, institutions, and CAR designs, which is encouraging in terms of the ability to replicate these promising results. However, persistence of CAR-modified T cells and long-term outcomes can vary and may distinguish CAR designs. In general, CAR T-cell persistence appears to be shorter in some patients with ALL compared with CLL patients who respond, even though CR rates are higher in ALL.<sup>48</sup> The kinetics of tumor growth and elimination are markedly different between these 2 diseases. Combined with the ongoing antigen reservoir provided by bulky disease characteristic of CLL, these factors may account for this difference. However, the persistence of T cells engineered to express a CD19-targeted CAR is also discrepant across ALL studies.

In the MSKCC ALL cohort, Davila et al report a 1 to 3 month persistence of 19-28z CAR T cells.<sup>25</sup> Similarly, with the NCI’s CD19-CAR T cells (CD28 costimulatory domain), the longest persistence reported is 68 days with rapid B-cell recovery seen.<sup>27</sup> In our ALL cohort treated with CTL019 T cells (4-1BB costimulatory domain), many patients had longer persistence (up to 2 years), with the probability of CTL019 persistence at 6 months being 68% (95% CI: 50% to 92%).<sup>26</sup> The duration of B-cell aplasia, also longer at up to 2 years, suggests continued effector function of CTL019 cells.

Durable remissions have also been reported across studies but were only described in the ~50% of patients proceeding to allogeneic SCT in the NCI and MSKCC cohorts. In the CHOP/Penn cohort, sustained remissions of 2 to 24 months were seen in 19 out of 27 responding patients, 15 of whom received no further therapy.<sup>26</sup> Event-free survival at 6 months was 67% (95% confidence interval [CI], 51%-88%; Figure 3), and overall survival at 6 months was 78% (95% CI,

65%-95%). Updates from 39 pediatric patients with ALL were presented at the 2014 annual meeting of the American Society of Hematology.<sup>49</sup> This analysis showed a 92% CR rate and a 6-month probability of sustained response of 76% (95% CI, 61%-94%). The CR rate in the group of patients with >50% bone marrow blasts just prior to T-cell infusion was 82%. Three of the patients in this group went on to SCT. These data suggest that T cells engineered to express a CD19-directed CAR have the potential to produce durable remissions, but more mature follow-up is needed across studies, as there are likely to be differences. Because much of our data to date are from pediatric patients, this could be one factor in the longer persistence our group has observed. However, more data from all groups testing highly proliferative gene-modified T cells will be needed before these differences can be better elucidated.

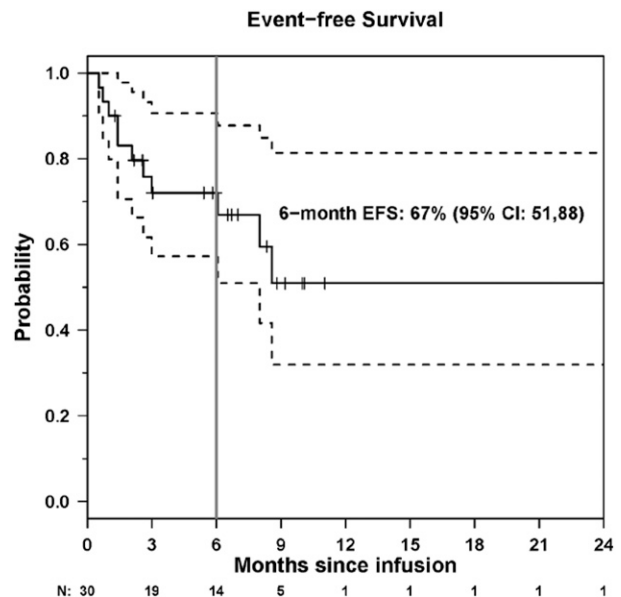
## Toxicity

### CRS

The most common and potentially severe toxicity associated with CAR-modified T-cell therapy is CRS.<sup>25-27,42</sup> Early data from our group and others suggest that there may be a correlation between the development of CRS and the response to therapy; patients who do not develop CRS may be less likely to benefit from CAR-modified T cells, whereas those who develop CRS often respond to the therapy. Although there may be some correlation between *developing* CRS and efficacy, there does not appear to be a strong correlation between the *degree* of CRS and response to therapy. This is because of the confounding and strong impact of disease burden on the risk of severe CRS. Similar to other T-cell engaging therapies, including BiTE (bi-specific T-cell engaging) antibodies,<sup>49,50</sup> we have found that the severity of CRS may correlate with tumor burden at time of infusion of the CAR-modified T cells.<sup>26</sup>

CRS is an inflammatory process related to exponential T-cell proliferation with resultant marked elevations in cytokine levels. Symptoms can range from mild flu-like symptoms to shock and multisystem organ failure. Our group has shown that the cytokine profile after both CAR-modified T-cell therapy or BiTE antibody therapy with blinatumomab mirrors the same profile seen in macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH).<sup>26,50-52</sup> CRS includes marked elevations in soluble interleukin-2 receptor  $\alpha$  (sIL2Ra), interleukin-6 (IL-6), IL-10, and interferon (IFN- $\gamma$ ).<sup>26</sup> Moreover, patients who develop severe CRS after CAR-modified T cells or blinatumomab often develop clinical and laboratory manifestations similar to MAS, including marked hyperferritinemia (>10 000 ng/mL), hepatomegaly/splenomegaly, and hypofibrinogenemia (<150 mg/dL).<sup>26,42</sup> We have performed more extensive cytokine profiling of over 30 different cytokines and chemokines after CAR-modified T cells and consistently observe a pattern that mirrors the profile seen in MAS (D. T. Teachey, unpublished data). Improved understanding of CRS may help determine which cytokines may be required for the therapy to be effective and which cytokines are not required and could be pharmacologically targeted to reduce inflammation and toxicity.

High levels of IFN- $\gamma$  or sIL2Ra would be expected after T-cell engaging therapies. In contrast, high levels of IL-6 or IL-10 would not be anticipated after CAR-modified T cells. IFN- $\gamma$  is a proinflammatory cytokine produced by cytotoxic T cells, natural killer cells, and T-helper cells (T<sub>H</sub>1).<sup>53</sup> IFN- $\gamma$  has a number of important functions including macrophage activation, major histocompatibility complex induction, and T<sub>H</sub>1 differentiation.<sup>53</sup> The high levels of IFN- $\gamma$  released by activated cytotoxic T cells after engagement may be



**Figure 3. Event-free survival in 30 children and adults treated with CTL019 therapy.** Of this group, 5 patients who entered a CR went on to further therapy, 3 of whom received an allogeneic bone marrow transplant. The fourth had refractory T-cell ALL aberrantly expressing CD19, entered remission after CTL019, and subsequently underwent donor lymphocyte infusion. She remains in remission >1 year later. The fifth patient developed myelodysplastic syndrome and received therapy for this condition (this was scored as an event, but she did receive further therapy in ALL remission and was counted among the 5). The rest have not received further therapy to consolidate their remissions. EFS, event-free survival. Figure adapted from Maude et al<sup>26</sup> with permission.

important for the efficacy of CAR-modified T cells. Accordingly, although a number of IFN- $\gamma$  inhibitors are in clinical development, we would be hesitant to use them to ameliorate CRS. In children with genetic (primary) HLH, abnormally activated CD8 T cells secrete high levels of IFN- $\gamma$ , which in turn can stimulate uncontrolled macrophage activation and the CRS seen in primary HLH.<sup>54</sup> We hypothesize the IFN- $\gamma$  produced by the CAR-modified T cells may be driving the secondary MAS/HLH in patients with severe CRS.

sIL2Ra is released by a number of cell types, including cytotoxic T lymphocytes.<sup>55</sup> High levels of sIL2Ra are 1 of 8 criteria used to diagnose primary HLH, but high sIL2Ra levels are also found in patients with a large number of inflammatory disorders, autoimmune diseases, and malignancies.<sup>56-58</sup> A number of sIL2Ra (CD25) inhibitors are in clinical use<sup>59</sup>; however, as CD25 is present on activated T lymphocytes, sIL2Ra inhibitors have a reasonable likelihood of impacting efficacy by eliminating the CAR-modified T cells.

IL-10 is a negative regulator of macrophage function that is markedly elevated in a number of inflammatory conditions, including MAS/HLH.<sup>60</sup> IL-10 is not commonly produced by cytotoxic T cells but is produced by monocytes/macrophages, B cells, mast cells, helper T cells (T<sub>H</sub>2), and regulatory T cells. In HLH, IL-10 is believed to be a negative regulator that may be involved in dampening CRS by inhibiting activated macrophages.<sup>61</sup> Thus, targeting IL-10 could easily enhance CRS. Indeed, preclinical studies targeting IL-10 in mouse models of systemic inflammation have established the protective role of IL-10.<sup>62,63</sup>

IL-6 is a potent inflammatory cytokine that is often elevated after infection or inflammation and in patients with MAS/HLH.<sup>64</sup> It is produced by many different cell types, including monocytes/macrophages, dendritic cells, T cells, fibroblasts, keratinocytes, endothelial cells, myocytes, adipocytes, mesangial cells, and osteoblasts.<sup>65</sup> Our group was the first to target IL-6 signaling in patients who developed severe CRS after CAR-modified T cells and after BiTE antibody therapy.<sup>42,51</sup>

However, although CRS occurs after blinatumomab, in general, it is of lower grade than the CRS that may be seen after second-generation CAR T-cell therapy, with fewer patients requiring intensive care unit-level care for CRS. We chose to target IL-6 as we hypothesized it may be integral to CRS-mediated toxicity; however, preclinical data showing efficacy of CTL019 cells in mouse xenograft models (David Barrett, unpublished data) without production of IL-6 suggested that IL-6 would be unlikely to be necessary for the efficacy of T-cell engaging therapies. We have used the interleukin-6 receptor (IL-6R) inhibitor tocilizumab in over 10 patients and have found it improves CRS without appearing to affect efficacy.<sup>26</sup> Based on our successful use of this drug, other groups have replicated these results.<sup>25,27,66</sup> The majority of patients treated with tocilizumab have rapid and complete resolution of CRS after a single dose, although a minority of the patients we have treated with severe CRS require a second dose within 1-3 days.

We chose tocilizumab because it has been extensively studied in children and adults, and until 2014 it was the only US Food and Drug Administration–approved inhibitor of the IL-6 pathway. Subsequently, other IL-6 inhibitors have been approved by the US Food and Drug Administration for use. Tocilizumab blocks IL-6 binding to membrane and sIL6R.<sup>67</sup> It is well tolerated, with rare toxicities that include liver inflammation and cytopenias.<sup>68</sup> These toxicities have only been reported in patients who receive chronic treatment with the agent. Siltuximab is an IL-6 antagonist that is approved to treat multicentric Castleman disease.<sup>69</sup> It is also well tolerated. Sarilumab (anti-IL-6R) and olokizumab (anti-IL-6) are newer agents that are currently undergoing later stage clinical trials in rheumatologic diseases.<sup>70</sup>

It is unknown whether targeting IL-6 directly would be more effective at improving CRS than IL-6R blockade. Based on the marked efficacy of tocilizumab in CRS post-CAR-modified T cells, it is difficult to switch to an alternative therapy without compelling data. We would, however, consider using siltuximab in a patient who does not respond to tocilizumab.

In addition to using cytokine-blocking drugs, our group and others have used corticosteroids to treat CRS, as corticosteroids are effective in a number of diseases driven by activated T lymphocytes.<sup>25,27,42,43</sup> Corticosteroids are routinely used to treat and prevent CRS with blinatumomab.<sup>71</sup> Concern exists over the potential for corticosteroids to affect the efficacy of CAR T cells. Although corticosteroids have been used successfully to treat CRS, recent data suggest they may hamper the efficacy of the CAR T cells<sup>25</sup> and may eliminate CAR T cells over time, allowing disease to regrow after an initial response.<sup>43</sup> Accordingly, we recommend corticosteroids be used as a second line after cytokine blockade with tocilizumab. We limit the steroid exposure as much as clinically possible, have a high clinical bar for giving steroids, and have observed that 2 mg/kg per day of methylprednisolone will not interfere with clinical response if given for 2 to 4 days at the peak of CRS.<sup>26</sup>

Thus far, the only reproducible predictor of severe CRS across clinical trials is high disease burden at infusion.<sup>26</sup> C-reactive protein has been proposed as an indicator of severe CRS<sup>25</sup>; however, although a high C-reactive protein level correlates with the severity of CRS in several studies, its use as a predictive biomarker is still being assessed.<sup>26,72</sup> We are currently determining if cytokine levels may be better predictors of CRS severity. Our data suggest that peak levels of certain cytokines, including IFN- $\gamma$  and sIL2Ra, are markedly more elevated in severe CRS than mild CRS. We need to identify biomarkers that can be easily measured soon after infusion that may predict which patients will become critically ill before they become sick. The window is short, as patients typically develop CRS within the first 5 days after infusion. We hypothesize that the pattern of a number of cytokines may be able to predict severity of CRS. As a number of clinical labs have

developed clinically-available cytokine array testing, we anticipate in the future cytokine monitoring may be used to predict severity of CRS and to help guide management with cytokine-blocking agents, including tocilizumab.

### Encephalopathy

Neurologic toxicities have been reported after T-cell-engaging therapies, including distinct CAR-modified T-cell therapies as well as blinatumomab.<sup>25,73,74</sup> Global encephalopathy is the most common toxicity, but seizures have also been reported.<sup>25</sup> In the CHOP/Penn experience with CTL019 in ALL patients, in addition to delirium at least partly attributable to high fever, 6 out of 30 patients experienced a distinct encephalopathy syndrome that occurred after resolution of CRS. This encephalopathy is brief and self-limited, resolving over several days without intervention or apparent long-term sequelae. Scans (computed tomography and/or magnetic resonance imaging) and lumbar punctures have revealed no etiology of this syndrome, although CAR T cells are seen in the spinal fluid of most patients, regardless of encephalopathy. As we have seen encephalopathy after administration of tocilizumab, it does not appear to be prevented by IL-6 blockade. Larger studies are needed to further characterize the spectrum of neurotoxicity with CAR therapies as well as the pathophysiology.

### B-cell aplasia

Chronic B-cell aplasia, and resultant hypogammaglobulinemia, is an expected on-target toxicity of successful CD19-directed CAR T-cell therapy. CD19 CAR therapies eliminate normal mature and precursor B cells. As long as CAR-modified T cells persist, B-cell aplasia continues, which provides what appears to be a highly accurate pharmacodynamic marker of CAR function. Although immunoglobulin replacement mitigates most infectious complications, longer follow-up is needed to assess late toxicity of B-cell aplasia.

## Challenges for CD19 CARs

CD19 CAR T cells have shown remarkable promise in ALL. Notwithstanding that success, challenges remain. For CAR T-cell therapies to be available to more patients, the delivery of this therapy needs to be feasible across institutions. Expanding access will require comprehensive training of clinicians and a standardized approach to CRS grading and management.<sup>66</sup> Additional studies aimed at minimizing serious toxicities will be important as this therapy is exported to more sites. Across studies, disease burden appears to be correlated with CRS severity in responding patients. Although disease burden is difficult to modify in a highly refractory population, prophylactic measures, such as early tocilizumab administration, may prove effective in mitigating serious toxicities. The efficacy of this approach in preventing life-threatening CRS without compromising responses has yet to be studied.

Finally, relapse after CAR T-cell therapy remains a challenge. Two modes of disease recurrence have been seen: CD19 positive and CD19 negative. Relapse of ALL that retains surface CD19 expression results from rapid disappearance of CAR-modified T cells or decreased function of those T cells. Optimized CAR designs, manufacturing technologies, or T-cell subset ratios may prevent some of these relapses by prolonging T-cell persistence. There may be differences in persistence between CARs that use CD28 and 4-1BB costimulatory domains or possibly between retroviral and lentiviral vectors

used for cell modification. However, CD19-negative relapses are not prevented by engineered T-cell persistence. Single-target therapy may select for and lead to escape variants. Combination or tandem CARs, which join 2 antigen-recognition moieties, may prevent relapses due to escape variants but need further studies. In this regard, the group at the National Institutes of Health has developed CAR T cells targeting the B-cell antigen CD22,<sup>40,75,76</sup> which can be used for treating CD19-negative relapse and could be combined with a CD19-directed CAR in the future.

## Conclusions

CD19-targeted CARs have paved the way for engineered T-cell therapies with high response rates and durable remissions reported. These results are unprecedented in patients without curative options. As new innovations in CAR design and manufacture develop and toxicity management evolves, the potential uses for this therapy will be expanded, as will access.

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## Authorship

Contribution: S.A.G., S.L.M., D.T.T., and D.L.P. wrote the paper; and S.A.G. and S.L.M. edited the paper.

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