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ACUTE LYMPHOCYTIC LEUKEMIAS (K BALLEN, SECTION EDITOR)

Chimeric Antigen Receptor Therapy in Acute Lymphoblastic Leukemia Clinical Practice

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Abstract Over half of patients diagnosed with B-cell acute lymphoblastic leukemia (ALL) develop relapsed or refractory disease. Traditional chemotherapy salvage is inadequate, and new therapies are needed. Chimeric antigen receptor (CAR) T cell therapy is a novel, immunologic approach where T cells are genetically engineered to express a CAR conferring specificity against a target cell surface antigen, most commonly the pan-B-cell marker CD19. After infusion, CAR T cells expand and persist, allowing ongoing tumor surveillance. Several anti-CD19 CAR T cell constructs have induced high response rates in heavily pre-treated populations, although durability of response varied. Severe toxicity (cytokine release syndrome and neurotoxicity) is the primary constraint to broad implementation of CAR T cell therapy. Here, we review the experience of CAR T cell therapy for ALL and ongoing efforts to modify existing technology to improve efficacy and decrease toxicity. As an anti-CD19 CAR T cell construct may be FDA approved soon, we focus on issues relevant to practicing clinicians.

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Introduction

Incremental improvements to standard therapy for acute lymphoblastic leukemia (ALL) have increased the likelihood of successful cure in adults diagnosed with the disease. Advancements such as the addition of tyrosine kinase inhibitors to the treatment of Philadelphia chromosome-positive ALL [1–3], increased use of intensive asparaginase-based pediatric-style chemotherapy regimens in younger adults [4–6], and better allocation to and conduct of allogeneic hematopoietic stem cell transplant (HSCT) have all helped bring more patients from first complete remission (CR) to cure [7–9]. Despite progress, approximately 20% of the adult patients will have primary refractory disease and most who do achieve CR will eventually relapse. Overall survival (OS) in newly diagnosed adult patients is approximately 40% [10, 11]. Although the outlook is improved in pediatric ALL where 80 to 90% of the children are cured, relapsed ALL still represents a significant unmet for pediatric oncology [12].

Standard salvage treatment is typically based on administration of chemotherapy agents different than those given during induction such as high-dose cytarabine [13], clofarabine [14], liposomal vincristine [15], and/or nelarabine [16], with the latter agent approved specifically for T cell ALL. Chemotherapy is seldom curative in the relapsed/refractory setting, and the goal of salvage therapy is to induce CR so that a patient may become eligible for curative potential allogeneic HSCT consolidation. Unfortunately, most patients are not cured via this approach with durable remissions reported to be at most 20% in adults [10, 17–19]. More effective salvage

therapy for children and adults with resistant disease is a major unmet clinical need.

Development of monoclonal antibody-based therapy has shown promise for treatment of relapsed and refractory ALL, as recently reviewed by DeAngelo et al. [20]. Blinatumomab, a bi-specific T cell engager (BiTE) monoclonal antibody that simultaneously targets CD19 (present on most precursor B-cell ALL tumor cells) and CD3 (present on cytotoxic T cells), is the first novel antibody to have received FDA approval for relapsed/refractory ALL and ALL in remission but with persistent minimal residual disease (MRD). Inotuzumab ozogamicin, an anti-CD22 antibody conjugated to calicheamicin, is another antibody-based therapy in late stages of clinical development. Randomized phase III trials have shown that both antibodies can induce superior response rates and longer survival compared to standard chemotherapy, although duration of remission is short [21–23].

The development of cellular therapy for B-cell ALL, specifically CD19-directed chimeric antigen receptor (CAR) T cell therapy, represents another promising immunologic approach for treatment of relapsed/refractory disease. Initial trials conducted at three centers—Children's Hospital of Philadelphia/University of Pennsylvania (CHOP/UPENN), Memorial Sloan Kettering (MSK), and the National Cancer Institute (NCI)—have shown remarkable efficacy, although with significant toxicity. Multi-center studies are underway, and leukemia clinicians may soon have the opportunity—and the challenge—of offering this therapy to patients. Here, we review outcomes reported thus far for patients with relapsed and refractory B-cell ALL treated with CAR T cells, and highlight clinical challenges and novel approaches to these challenges. There has been little success thus far applying CAR T cell therapy to T-cell ALL and is not included in the scope of this review.

Introduction to Anti-CD19 CAR T Cell Therapy

A CAR T cell is a T cell that has been genetically engineered to express a recombinant CAR receptor which confers major histocompatibility complex (MHC)-independent specificity against a designated target antigen. CAR T cells with specificity against CD19, an antigen expressed exclusively on B cells, have been most extensively developed and are the subject of this review. CARs in clinical trials today express "second-generation" receptors which are comprised of three components: an extracellular antigen-recognition domain derived from the single-chain variable fragment of a monoclonal antibody (scFv), an intracellular signaling domain (the CD3z chain from the T cell receptor), and a co-stimulatory domain (most commonly, 4-1BB or CD28, Table 1) [24]. CARs are transduced into T cells harvested from the patient via leukapheresis using either a lentiviral or a retroviral gene transfer technique resulting in CAR protein expression on the T cell surface (Fig. 1). This process effectively redirects an entire population of T cells against one target antigen, independent of MHC presentation. When the scFv encounters its target antigen, the CAR T cell is activated via the intracellular CD3z chain. Absent in first-generation iterations of CAR technology, the co-stimulatory domain (4-1BB or CD28) provides the important "signal 2" that allows activated CAR T cells to expand and persist, resulting in bulk (and ongoing) killing of malignant cells expressing the target [24–29]. Without a second signal, activated CAR T cells become anergic and clinically ineffective. The addition of the co-stimulatory domain was therefore the critical feature that effectively brought CAR T cells from bench to bedside [30, 31]. Early studies of second-generation CAR T cells have demonstrated deep and durable disease responses in CD19-positive B-cell hematologic malignancies, including for relapsed and refractory B-cell ALL [32, 33••, 34••, 35••].

Clinical Results Associated with Early Trials

Initial clinical successes of anti-CD19 CAR T cell therapy for B-cell ALL was published by three groups: UPENN/CHOP, MSK, and the NCI. The CAR constructs manufacturing techniques, and patient populations varied between studies, but all reported high CR rates paving the way for further clinical development of CAR T cell technology for the clinic (Table 1).

UPENN/CHOP reported a 90% CR rate in a report of the first 30 patients (25 pediatric, 5 adult) with relapsed and refractory B-cell ALL treated on a phase I/II study of their anti-CD19 CAR T cell construct, now termed CTL019 [35••]. This extraordinary CR rate was observed in a very high-risk population that included 3 patients with primary refractory disease, 18 patients relapsed after allogeneic HSCT, and 3 patients refractory to blinatumomab. MRD assessments were conducted in most patients who achieved CR with 22 of 25 (88%) confirmed to be MRD negative. Responses were remarkably durable with 7 relapses and 19 ongoing remissions (2 to 24 months) at the time of the publication. It was notable that most of the ongoing remissions were in patients who received no further therapy (15 of 19 patients who achieved CR received no further therapy). The CAR T cell product (CTL019) developed by UPENN/CHOP (and subsequently in collaboration with Novartis) incorporates the 4-1BB co-stimulatory domain which is believed to support prolonged CAR T cell persistence. Indeed, high rates of CAR T cell persistence, with associated B-cell aplasia, was described in this study with a 68% probability of CTL019 persistence at 6 months, and documented cases of persistence (along with B-cell aplasia) of up to 2 years. Event-free survival (EFS) and OS at 6 months were 67 and 78%, respectively. Updated results at the American Society of Hematology (ASH) meeting

Table 1 CD19 CAR T cell constructs for B-ALL

	MSKCC [33••, 37]	NCI [34••]	CHOP/UPENN [35••, 36]	FHCRC [51•]
Vector	Retroviral	Retroviral	Lentiviral	Lentiviral
Transmembrane	CD28	CD28	4-1BB	4-1BB
Signaling domain	CD3z	CD3z	CD3z	CD3z
Persistence	Short (~30 days)	Short (~30 days)	Long (~4 years)	Long
CR rate in ALL	~90%	~80%	>90%	~90%
Defined CD4/CD8 ratio	No	No	No	Yes

CAR chimeric antigen receptor, *ALL* acute lymphoblastic leukemia, *MSKCC* Memorial Sloan Kettering Cancer Center, *NCI* National Cancer Institute, *CHOP* Children's Hospital of Philadelphia, *UPENN* University of Pennsylvania, *FHCRC* Fred Hutchinson Cancer Research Center, *CR* complete remission

in 2015 after treatment of 53 children and young adults confirmed initial results with 50 (94%) patients achieving CR—the majority (48) MRD negative. At a median follow-up of 10.6 months, 29 were reported to have ongoing CR. Twelve-month EFS and OS were 45 and 78%, respectively [36].

In contrast to the UPENN/CHOP study which included mostly pediatric patients, MSK reported a similarly high CR rate (88%) in their initial report of 16 adult patients (median age 50 years) with relapsed and refractory B-ALL treated with anti-CD19 CAR T cell therapy [33••]. As in the UPENN/CHOP experience, most CRs were MRD negative but CAR T cell persistence was shorter (1–3 months). Of note, the CAR T cell constructs developed by MSK and NCI both contain a CD28 co-stimulatory domain in contrast to the 4-1BB domain used by UPENN/CHOP. The MSK results were also updated at the ASH meeting in 2015 where it was reported that 37 of 45 (82%) evaluable adult patients treated with CAR T cell therapy achieved a CR with MRD-negative remissions documented in 30 of 36 (83%) patients [37]. Like the UPENN/CHOP cohort, the MSK cohort represented a very high-risk population with 26 patients having received 3 or more lines of therapy and 18 patients relapsed after allogeneic HSCT. Of those achieving CR, 13 of 37 transitioned to allogeneic HSCT, although interestingly, OS at 6 months was not different between those who were and were not consolidated with allogeneic HSCT (79 and 80%, respectively).

Finally, the NCI reported early results of their anti-CD19 CAR T cell construct (with a CD28 co-stimulatory domain) among 20 pediatric and young adult patients with relapsed and refractory B-cell ALL [34••]. The response rate reported in this trial was slightly lower than reported in the initial UPENN/CHOP and MSK studies, with a CR rate of 70% (MRD negative in 12 of 14 patients). Persistence was difficult to determine as 10 of the 14 responding patients were bridged to allogeneic HSCT, but the longest documented persistence of CAR T cells was reported to be 68 days, comparable to the MSK experience.

Overall, despite differences in CAR T cell constructs and manufacturing methods used in these initial studies, as well as differences in the populations treated, the uniformly high CR rates among very high-risk ALL populations were notable. Taken together, these initial studies confirmed the impressive efficacy of this modality for treating very high-risk relapsed and refractory B-cell ALL patients of all ages, as well highlighting differences in persistence and durability between products. The different attributes of the co-stimulatory domain have been most discussed, with both pre-clinical and clinical data suggesting that the 4-1BB domain may be associated

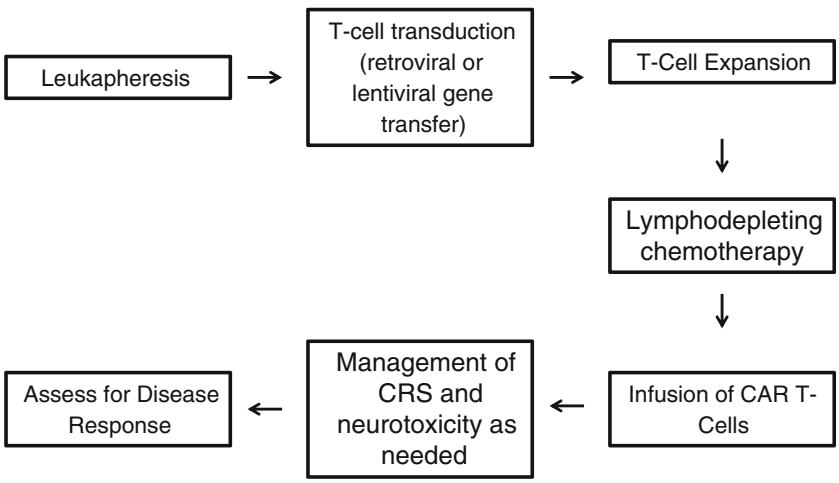

Fig. 1 Major steps of CAR T cell therapy

with enhanced persistence and more durable remissions [25, 34••, 35••, 38•]. However, the relative merits of CD28 and 4-1BB-based products remain to be defined, ideally through direct comparison in a randomized trial.

Bringing CAR T Cells to the Clinic: Challenges

Getting the Product to the Patient Promptly

Manufacturing of CAR T cells for each patient is a complex process involving multiple steps including apheresis collection of T cells from the patient, modification of the patient's T cells to express the CAR via a lentiviral or retroviral transduction technique, and then expansion of the product [24]. After completion of CAR T cell manufacturing, the modified T cells are re-infused into the patient after administration of lymphodepleting chemotherapy (Fig. 1). As ALL is often a rapidly progressive disease requiring immediate treatment, maintaining clinical stability while the steps required for CAR T cell infusion are completed can be difficult. Some investigators are exploring ways to shorten manufacturing times [39] and even considering the concept of "off the shelf" CAR T cells [40–43]. Efforts to improve ability to get CAR T cells to patients expeditiously continue, although initial results from a Novartis-run, global phase II trial (ELIANA) for pediatric and young adult ALL have shown that CAR T cell products can be successfully distributed across a global network [44•].

Toxicity: Cytokine Release Syndrome and Neurologic Toxicity

Concurrent with the realization that anti-CD19 CAR T cells can generate rapid and impressive disease responses was the recognition that this therapy is associated with a unique and severe side effect profile. The two major toxicities associated with CAR T cell therapy are cytokine release syndrome (CRS) and neurotoxicity.

CRS is a systemic inflammatory response that results from CAR T cell activation and proliferation. As recently reviewed by Frey et al. [45], CRS is associated with high fevers, hypoxemia, hypotension, capillary leak, and multi-organ dysfunction. CRS generally occurs during the first 2 weeks after CAR T cell infusion with fever almost always the first sign. CRS is sometimes mild and easily managed with fluids and antipyretics. In other cases, the initial fever, which can exceed 104–105 °F, is followed quickly by distributive shock, respiratory distress, and organ failure. The clinical syndrome of CRS is associated with laboratory evidence of inflammation including elevation of acute phase reactants (ferritin, C-reactive protein [CRP]), effector cytokines (interferon-γ, soluble IL-2 receptor-α), and cytokines associated with macrophage activation (IL-6 and IL-10).

In the initial CHOP/UPENN report of anti-CD19 CAR T cell therapy for ALL, all patients experienced signs and symptoms of CRS with 8 of 30 patients requiring care in the intensive care unit (ICU) [35••]. Rates of CRS were similar in the early reports from the MSK and NCI groups with severe CRS reported in 28 (NCI) and 43% (MSK) of the patients [33••, 34••]. Indications for ICU transfer were need for vasopressors or high levels of supplemental oxygen. The major predictor for CRS is disease burden but does not seem to be as closely related to the infused dose of CAR T cells presumably because the relationship between the infused and the final biological dose of CAR T cells after in vivo T cell expansion is affected by many factors.

The NCI Common Terminology Criteria for Adverse Events (CTCAE) grading scale for CRS was developed for CRS syndromes associated with antibody-based therapy, such as blinatumomab. Given that CAR T cells cannot be turned on and off like infusion therapies, a need to create grading systems that better reflect the toxicity of CAR T cell-related CRS was quickly recognized. The two systems now used most commonly are the 2014 NCI consensus grading system and a grading system developed by the UPENN/CHOP group for their clinical trials [38•, 46]. Unfortunately, the two scales grade toxicity differently and therefore careful attention to which grading scale is being used to report adverse events is important when reviewing the results of clinical studies. Ideally, grading of CRS will become more consistent across trials of CAR T cell therapy to permit more facile comparison of results.

In addition to supportive care, specific interventions to halt the underlying pathophysiology and ameliorate the symptoms of CRS are needed. Steroids are the mainstay of management for CRS secondary to blinatumomab [21]. In the case of CAR T cells, however, it is believed that steroids may negatively impact the effectiveness of treatment by impairing T cell function. Fortunately, early in clinical development of CAR T cells at CHOP, tocilizumab, an anti-IL6 receptor antibody, was found to be effective and has become the mainstay of management for severe CRS secondary to CAR T cells across all centers. Tocilizumab is well tolerated and rapidly effective in most cases. Whether tocilizumab should be given for mild CRS (or prophylactically to prevent CRS) is uncertain. Averting severe life-threatening CRS is obviously desirable, but whether early intervention will impact the effectiveness of CAR T cells by altering the cytokine milieu during early phases of T cell expansion has not been established. A recent report by Gardner et al. has suggested that administration of tocilizumab and dexamethasone for early signs of CRS (rather than waiting for dose limiting toxicity) decreased rates of severe CRS (30 versus 15%) without affecting efficacy (MRD-negative CR rate) or T cell engraftment and persistence [47]. To direct prophylactic therapies most effectively, predictive biomarkers for severe CRS are needed. Teachey et al. recently

reported a cytokine signature that could accurate predict CRS; unfortunately, the more readily available clinical tests (ferritin, CRP) were not reliable predictors for severe CRS [48•].

Neurologic toxicity is another unique side effect associated with CAR T cell therapy. Neurologic symptoms range from mild confusion to obtundation. Interestingly, central nervous system (CNS) toxicity does not always track with presence of systemic CRS and the mechanism is not known. In most circumstances, CNS toxicity resolves completely with supportive care; however, recently, there have been several fatalities due to cerebral edema and herniation in phase II trials of anti-CD19 CAR T cells for ALL run by Juno Therapeutics [49]. Why one CAR T cell construct has been associated with fatal cerebral edema while others have been associated with significant, but reversible, neurotoxicity is an unanswered question.

It deserves mention that the severe and life-threatening nature of the toxicities associated with CAR T cell therapies places extraordinary burden, not only on patients and treating oncologists but also on non-oncology clinicians (including "frontline" clinicians that are often trainees or physician extenders) and hospital infrastructure. A high-volume CAR T cell program may frequently require the use of multiple ICU beds as well as assistance of the critical care, neurology, and other specialty services.

In summary, the severity of CRS and neurologic toxicity are a major limitation to extending this therapy to older patients and patients with co-morbidities. Developing better approaches to pre-empt and manage CRS will decrease resource burden on healthcare systems and increase the number of patients with ALL who can benefit from CAR T cell therapy.

The Way Ahead: Preventing Relapse and Reducing Toxicity

Not all patients respond to the second-generation anti-CD19 CAR T cell therapies currently in clinical trials, many relapse, and the majority experience severe toxicity. Multiple approaches to enhance the effectiveness and reduce toxicity of CAR T cell therapy are being investigated, recently expertly reviewed by others [50]. Select approaches particularly relevant for treatment of B-cell ALL are described as follows (Tables 2 and 3).

Improving Efficacy

Optimizing CAR T Cell Product Composition Investigators working at the Fred Hutchinson Cancer Research Center (FHCRC) have focused on designing T cell products composed of defined proportions of CD4 and CD8 T cell subsets [51•]. The hope is that more precise management of the T cell product will enhance ability of investigators to define the total dose and T cell subset composition that optimizes efficacy and toxicity [52]. Others are focused on enriching T cell products with T cell subsets likely to have enhanced persistence and efficacy, specifically less differentiated T cells, such as central memory T cells [53, 54].

Humanized CARs Most current CAR T cells in clinical trials have murine derived scFvs. It is hoped that humanized CARs, where the scFv is of human origin, will reduce antimurine immune-mediated rejection and thereby increase persistence and thereby efficacy. Maude et al. from the CHOP/UPENN group presented early results of a phase I trial of a humanized anti-CD19 CAR T cell (CTL119) for pediatric B-ALL and reported a CR rate of 57% (8/14) in patients previously treated with CTL019 and 100% (22/22) in those who were CAR T cell naive (Table 2) [55]. The safety profile appears similar to CTL019 though longer follow-up is needed to determine how long-term outcomes compare between the humanized and the murine products.

Third-Generation CARs The addition of a co-stimulatory domain was the critical improvement that led to the clinical success of second-generation CARs. So-called third-generation CARs try to double down on the benefit of co-stimulation and include two co-stimulatory domains. Pre-clinical studies have suggested superior efficacy [56, 57], and clinical trials are underway (NCT01853631).

Armored CARs This term refers to CAR T cells that are engineered to secrete pro-inflammatory cytokines in order to shield CAR T cells from inhibitory microenvironment, induce resistance to regulatory T cells, and improve CAR T cell cytotoxic function [50, 58]. A pre-clinical mouse model of anti-CD19 CAR T cells constitutively expressing IL-12 showed efficacy, including ability to eradicate disease in the absence of a conditioning regimen [59•]. Constitutive expression of CD40L on T cells is also being explored as a method for enhancing CD8 T cell cytotoxicity. When CD40L binds CD40 on dendritic cells, the release of inflammatory cytokines IL-12 and interferon-γ is triggered which in turn improves T cell proliferation and CD8 T cell efficacy [58]. Anti-CD19 CAR T cells with constitutive CD40L expression performed better than second-generation CAR T cells in in vitro experiments [60]. Finally, constitutive expression of 4-1BBL is being investigated as a method for improving CAR T cell function via autostimulation and co-stimulation of endogenous T cells and enhancing dendritic cell IL-12 secretion [58, 61].

Combination with Checkpoint Inhibitors Another approach to counteract the immunosuppressive tumor microenvironment and improve efficacy is through the co-administration of checkpoint inhibitors—monoclonal antibodies against PD-1 axis and CTLA4—with CAR T cells. Pre-clinical data supports this approach [62•], and ongoing

Table 2 Novel anti-CD19 and anti-CD22 CAR T cells in B-cell ALL: American Society of Hematology Meeting 2016

Study	Phase	Population	Construct	Efficacy	Toxicity
Shah et al., NCI [68•]	Phase I	Pediatric *N* = 17	Anti-CD22 scFv with NCI construct	CR: 8/10 (80%)	CRS in >50%. No severe or irreversible neurotoxicity
Maude et al., CHOP/UPE-NN [55]	Pilot/phase I	Pediatric/young adult *N* = 36	Humanized anti-CD19 scFv domain with CHOP/UPENN construct.	CR: 8/14 (57%) in re-treatment cohort (patients treated previously with murine CAR T cell); 22/22 (100%) in CAR T cell naive. Six-month RFS: 75% in re-treatment cohort, 83% in CAR T cell naive cohort	Safety profile similar to murine CAR T cell counterpart. CRS mild in most. Neurologic toxicity fully reversible.

CAR chimeric antigen receptor, *ALL* acute lymphoblastic leukemia, *NCI* National Cancer Institute, *CR* complete remission, *CRS* cytokine release syndrome, *CHOP* Children's Hospital of Philadelphia, *UPENN* University of Pennsylvania, *RFS* relapse-free survival

studies are evaluating the combination of an anti-CD19 CAR in combination with anti-PD-L1 antibody in relapsed and refractory B-cell lymphomas (NCT02926833, NCT02706405). A similar approach combining ipilimumab (anti-CTLA4) with an anti-CD19 CAR is underway at Baylor for treatment of relapsed B-cell lymphomas and B-cell ALL (NCT00586391).

Minimizing Toxicity: Controlling the CAR

As severe toxicity is a major limitation for clinical implementation of CAR T cell technology, the ability to effectively eliminate CAR T cells on demand in the setting of severe CRS or neurotoxicity would significantly increase the number of treatment-eligible patients. Suicide gene technology has been pursued for this purpose with approaches including integration of an inducible caspase-9 (iCasp9) and EGFR elimination genes (non-functional, truncated version of EGFR receptor) [50]. The FHCRC is currently evaluating the use of an EGFR elimination gene (where administration of cetuximab leads to CAR T cell elimination via activation of antibody-dependent cytotoxicity) in an anti-CD19 CAR T cell trial (NCT01865617). Another approach is an inducible CAR system whereby CAR T cells are functional only in the presence of a pharmacologic agent allowing CAR T cells to be turned on and off. Sakemura et al. described an "inducible" CAR system using a tetracycline regulation system activated by doxycycline. In the presence of doxycycline, Tet-CD19 CAR T cells showed antitumor activity in a xenograft model; without doxycycline, CAR expression and CAR T cell function were lost [63, 64•].

Table 3 Approaches to improving anti-CD19 CAR T cell therapy

Efficacy
Engineering of CAR T cell product composition
Humanized CAR T cells
Third-generation CAR T cells (two co-stimulatory domains)
Armored CAR T cells (secrete pro-inflammatory cytokines)
Combine with checkpoint inhibitors
Minimizing toxicity
Suicide gene technology
Inducible CAR T cells
Approaching CD19-negative relapse
Anti-CD22 CAR T cells and bi-specific CAR T cells (CD22 and CD19)

CAR chimeric antigen receptor

Addressing CD19-Negative Relapses: Alternative Target

CD22 is another antigen expressed almost universally on B-cells, including on B-cells of patients who experience CD19-negative relapses after anti-CD19 CAR T cell therapy [65]. Therefore, anti-CD22 CAR T cells are being developed [66, 67] with ongoing trials that are being conducted by NCI and UPENN (NCT02650414, NCT02588456, NCT02315612). Shah et al. recently presented phase I data from 17 pediatric patients treated at NCI with anti-CD22 CAR T cell 69% of whom had received prior CD19 CAR T cell therapy and all whom were relapsed post allogeneic HSCT (Table 2). In total, 56% had CD19-negative relapsed disease. Of 10 patients treated, 8 of 10 patients achieved MRD-negative CRs highlighting the efficacy of this approach [68•]. The optimal use of anti-CD22 CAR T cell therapy will need to be defined with possibilities including treatment of CD19-negative relapses or upfront combination treatment with anti-CD19 CAR T cells to prevent such relapses (with either infusion of two CARs or development of bi-specific CARs as recently described by Qin et al. [69]). Finally, it is important to remember that not all relapses are driven by loss of CD19 and further investigation into the mechanisms of relapse is needed.

In summary, modifications to second-generation CAR T cell technology are being pursued to improve efficacy and persistence of CAR T cells, minimize toxicity, and address a common mechanism of failure (loss of CD19 antigen expression) (Table 3). Each of these interventions will need to be

carefully evaluated. More broadly, investigators need to be vigilant about defining all the components that contribute to functional characteristics of the infused product (CAR design, CAR delivery vector, expansion method, manufacturing conditions, dose and type of cells infused, and associated preconditioning regimen) as small alterations in the production of CAR T cells may impact the behavior of a CAR T cell product in unanticipated ways [24].

Financial

The cost of manufacturing and delivering CAR T cells is high with expenditures associated with maintaining manufacturing facilities capable of reliably producing CAR T cells, as well as the associated health care personnel and infrastructure costs associated with managing patients coping with significant complications from the disease or CAR T cell treatment. Although the costs are steep—estimated to be several hundred thousand dollars per patient—they are likely comparable to the costs associated with allogeneic HSCT, and justified if efficacy is high [70]. Additionally, as investigational approaches to increase efficacy and decrease toxicity mature, the cost benefit ratio (value) is likely to improve. Finally, as CAR T cells move from academia to the pharmaceutical industry for commercial development, it is anticipated that competition will encourage pharmaceutical companies to present the best value product (in terms of efficacy, toxicity, and cost) to clinicians and insurers.

Registration Trials

While efforts to develop the next generation of CAR T cells continue, registration trials for second-generation projects are underway (Table 4). Grupp et al. recently reported early results from a global registration trial (ELIANA) led by Novartis that is underway for the CHOP/UPENN construct (CTL019) in pediatric and young adults with relapsed, refractory B-cell ALL [44•]. Initial reports demonstrate the feasibility of global implementation of CAR T cell therapy as CTL019 cell processing occurs at a US-located Novartis facility and then distributed globally to 25 sites in North America, Europe, and Asia. So far, among 81 patients enrolled, 62 patients were successfully infused (with 6 dying before treatment and 5 experiencing manufacturing failures). Among 50 patients analyzed for efficacy, 82% achieved MRD-negative remissions, comparable to earlier phase trials of CTL019. Six-month relapse free and OS was 60 and 89%, respectively. In terms of toxicity, 48% experienced grade 3 or 4 CRS and 15% experienced transient neurologic events though there were no deaths from either CRS or cerebral edema.

Kite Pharma is conducting several phase 1 and 2 trials in B-cell malignancies including for pediatric (ZUMA-4) and adult (ZUMA-3) ALL using the NCI construct [71]. The number of patients treated so far is small, but CR/CRi rate appears to be is the same as that reported for CTL019 (82%, 9 of 11) with all CRs being MRD negative. Five of 13 patients each experienced grade 3 or higher CRS or neurologic events. No cerebral edema or deaths from CRS were reported.

Juno Therapeutics was also developing CAR T cells for B-cell ALL based on constructs from MSK and FHCRC. Results from their pediatric study reported at the ASH meeting in 2016 showed that 93% of the 43 treated patients achieved an MRD-negative CR with 12 months EFS and OS reported to be 51 and 70%, respectively [72]. Severe CRS and neurotoxicity reported in 23 and 49%. However, Juno's CAR T cell trials for ALL have been suspended due several cases of fatal cerebral edema [49]. The reasons for the association of an increased rate of cerebral edema with Juno's product are not certain and are being investigated.

Table 4 Pharmaceutical company trials of anti-CD19 CAR T cells for relapsed/refractory B-Cell ALL

Study	Phase	Population	Construct	Efficacy	Toxicity
Novartis (Grupp et al. [44•])	Phase II (ELIANA)	Pediatric/young adult Relapsed/refractory $N = 50$	CHOP/UPENN	CR/CRi 82%—all MRD negative 6 month OS—89% 6 month RFS—60%	48% grade 3+ CRS 15% neurotoxicity (no cerebral edema)
Kite Pharma (Shah et al. [70])	Phase I/II (ZUMA-3 and ZUMA-4)	Pediatric/adult Relapsed/refractory $N = 11$	NCI	CR/CRi 82%—all MRD negative	5/13 grade 3+ CRS 5/13 grade 3+ neurotoxicity (no cerebral edema)
Juno Therapeutics (Gardner et al. [71])	Phase I (PLAT-02)	Pediatric/young adult Relapsed/refractory $N = 43$	FHCRC	CR 93%—all MRD negative 12 month OS—70% 12 month EFS—51%	23% severe CRS 49% neurotoxicity (several cases of cerebral edema)

CAR chimeric antigen receptor, *ALL* acute lymphoblastic leukemia, *CHOP* Children's Hospital of Philadelphia, *UPENN* University of Pennsylvania, *CR* complete remission, *CRi* complete remission with incomplete count recovery, *MRD* minimal residual disease, *OS* overall survival, *RFS* relapse-free survival, *CRS* cytokine release syndrome, *NCI* National Cancer Institute, *FHCRC* Fred Hutchinson Cancer Research Center

Conclusion: Where Will CAR T Cell Therapy Find Its Space in ALL Treatment Paradigm?

The challenge for investigators and practicing clinicians is to optimally develop and deploy CAR T cell therapy in the care of patients with B-cell ALL. Current efforts are focused on optimizing CAR T cell technology to improve efficacy and reduce toxicity. Ongoing trials, including a phase III registration trial, are focused on patients with relapsed or refractory disease, or persistent MRD after initial therapy. Given the toxicity of ALL chemotherapy, an obvious future question is whether CAR T cell therapy can be developed for use earlier in the course of disease, as consolidation therapy (in place of allogeneic HSCT), or even as up front therapy for patients ineligible for standard induction therapy. The efficacy of CAR T cell therapy will need to be confirmed in comparative trials, and toxicity reduced, in order to bring it into the front line (and first salvage) setting, but this exciting possibility is on the horizon. Other critical questions include whether allogeneic HSCT consolidation is necessary after CAR T cell therapy as well as the optimal product for different clinical scenarios. These questions will ultimately have to be answered carefully and incrementally via well-designed, collaborative multi-center trials. Despite the challenges ahead, CAR T cell already represents an effective salvage therapy for patients B-cell ALL who otherwise have few therapeutic options and we look forward to broader availability of this therapy for our patients.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by any of the authors.

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