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Paving the Road Ahead for CD19 CAR T Cell Therapy

Anandani Nellan¹ and Daniel W. Lee¹

¹Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD

Abstract

Purpose of Review—Modern immunotherapies, most notably in the form of anti-CD19 chimeric antigen receptor (CAR) T cells, have produced significant clinical responses in otherwise refractory pre-B cell acute lymphoblastic leukemia (ALL) patients. Several groups have simultaneously reported robust response rates in children and adults alike. These early studies indicate an impending shift in paradigm for the treatment of ALL. Incorporating CD19 CAR T cell therapy into upfront or salvage regimens has its challenges and opportunities.

Recent findings—Most CD19 CAR T cell products in trial today are excellent at inducing minimal disease (MRD) negative remissions, and most responding patients experience cytokine release syndrome (CRS) and/or neurotoxicity. The challenges facing the CAR community involve how best to minimize the severity of CRS and neurotoxicity while maximizing anti-tumor efficacy, determining what role this therapy will play for the prophylaxis and treatment of CNS leukemia, and its implications on subsequent hematopoietic stem cell transplant (HSCT) given the emergence of CD19 negative relapses.

Summary—CD19 CAR T cell therapy is a powerful new tool in the oncologist's arsenal. How it is incorporated into standard practice and how it will shift survival curves are the exciting questions that are waiting to be answered.

Keywords

Chimeric antigen receptor; Cytokine release syndrome; Acute lymphoblastic leukemia; CNS leukemia; CD19

Introduction

Pre-B acute lymphoblastic leukemia (ALL), the most common childhood malignancy, has a cure rate that exceeds 85% but remains one of the leading causes of cancer death in children [1]. Blinatumomab is the first new agent for ALL to be FDA approved in the last decade, but despite its novel mechanism of action, patients relapsing after hematopoietic stem cell transplant (HSCT), the 1% of patients primarily refractory to chemotherapy, and those with central nervous system (CNS) leukemia have limited effective therapeutic options. Early experience with CD19 chimeric antigen receptor (CAR) T cells has demonstrated complete

Corresponding Author: Daniel W. Lee, MD, Pediatric Oncology Branch, National Cancer Institute, 10 Center Dr. Bldg 10, Room 1-3750, MSC 1104, Bethesda, MD 20892. leed3@mail.nih.gov. ph: 301-435-6236.

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molecular remissions in these patient groups, but in some cases with significant, life-threatening cytokine release syndrome (CRS), and neurologic toxicity [2–6]. Several questions remain unanswered: 1) How can we minimize the severity of CRS while maximizing the anti-tumor effect, 2) What is the role of CD19 CAR T cells in treating CNS leukemia, and 3) Will CD19 CAR T cell therapy replace HSCT?

Minimize risk, maximize returns

We previously reported on 20 children and young adults treated with anti-CD19 CAR T cells for relapsed and refractory pre-B ALL at the National Cancer Institute. In an intent-to-treat analysis, 70% achieved complete remission, including patients with CNS2 leukemia and, remarkably, 6 of 6 patients with primary, refractory disease [2]. No patients with a history of allogeneic HSCT had any evidence of graft versus host disease with CAR T cell infusion. Other groups have reported similar response rates, though preparative regimens differed even within a trial and patient inclusion was not intent-to-treat [3–6].

CRS is the primary toxicity of CAR T cell therapy and has resulted in the deaths of some adult but not pediatric patients to date [7, 8]. Characterized by fever, hypotension, coagulopathy, respiratory distress and end organ dysfunction, CRS appears to be driven by supra-physiologic secretion of pro-inflammatory cytokines including IL-6 and $\text{INF}\gamma$. C-reactive protein (CRP) can serve as a biomarker for IL-6 production and CRS, though its measured level generally reflects the clinical picture occurring 12–24 hours prior [6]. Regardless, CRP remains useful for tracking the onset of CRS and documenting its resolution, but additional biomarkers would be helpful to predict the severity of CRS in a particular patient so that anti-cytokine therapy can be instituted early in an effort to avoid severe toxicity.

Currently, the anti-IL-6 receptor monoclonal antibody, tocilizumab, with or without corticosteroids is the treatment of choice for severe CRS in most centers if aggressive supportive care fails [2–4, 6]. Whether this negatively impacts the cytotoxic potential of the activated CAR T cells is not known. For this reason, our group has instituted a novel CRS grading and treatment algorithm with the aim of limiting grade 4/5 CRS to <10% and maximizing leukemia remission by tolerating a moderate degree of CRS symptoms but instituting early intervention with anti-cytokine therapy in the event of high-grade CRS [2, 6].

One consistent finding across all published CD19 CAR T cell clinical trials is that higher tumor burden poses an increased risk for the development of severe CRS [2–4]. Until and if we determine that prophylactic anti-cytokine therapy in some form does not limit the anti-leukemia capacity of CAR T cells, we postulate that lowering disease burden in patients with M3 bone marrow involvement by intensifying the preparative chemotherapy regimen prior to CD19 CAR T cell infusion will lower the risk of severe CRS. Our first cohort of patients treated on our CD19 CAR T cell trial received a light preparative regimen consisting of fludarabine and cyclophosphamide in an attempt to lymphodeplete rather than eradicate significant tumor outright [2]. Lymphodepletion is thought to increase homeostatic cytokine levels such as IL-7 and IL-15, which may result in improved CD19 CAR T cell

expansion and remains a necessary component [9]. In an effort to reduce the risk of severe CRS, we have adopted a more intensive chemotherapy regimen for patients with high-burden disease that is lymphodepleting while also likely to reduce tumor burden prior to CAR cell infusion.

There may be some settings where a lymphodepleting regimen is not necessary. Donor-derived CD19 CAR T cells are administered to patients who relapsed after allogeneic HSCT without lymphodepleting chemotherapy in one clinical trial [5]. While the response rate was less than other reports in the few patients treated, those that do respond have tolerable CRS [5].

In our trial, 5/5 patients with MRD-level disease at the time of enrollment achieved a MRD-negative remission and had low-grade CRS. Another trial treating adults with relapsed pre-B ALL allowed a “physician’s choice” reinduction regimen, followed by lymphodepleting cyclophosphamide for all patients [3]. Those who achieved a MRD-only status prior to receiving CAR T cells had less severe CRS and were more likely to achieve a molecular remission. Unfortunately, as this was a highly chemotherapy-refractory population, only a fraction of enrolled patients achieved MRD-only status. This approach might be more successful in the recently diagnosed patients where the rate of initial response to chemotherapy is very high. In both trials, low-dose chemotherapy was utilized. One might postulate that similar responses may be achieved without a preparative regimen in low-burden leukemia patients, although this has not been tested.

More intense chemotherapy regimens are not without risk. Acute toxicities including more profound myelosuppression and increased incidence of infectious complications may predispose patients to more morbidity or higher mortality if a subsequent HSCT is performed.

Despite these added risks and since the severity of CRS has been shown to directly correlate with disease burden, a tailored reinduction regimen to reduce but not necessarily eliminate leukemia burden, while still being lymphodepleting prior to CAR T cell therapy may decrease the risk of severe CRS. It remains to be determined if the additional risks of a more intensive chemotherapy preparative regimen outweigh the potential reduction in the incidence of severe CRS. This may be an effective approach for children with leukemia as they can generally tolerate higher doses of chemotherapy compared to adults. Our current trial (www.clinicaltrials.gov NCT01593696) aims to answer this question.

In a recent review on CAR T cells, Maude et al. suggested treatment with prophylactic tocilizumab in patients with high burden disease to reduce toxicities of CRS [10]. While short term data suggests that tocilizumab does not impact the chance of achieving a complete remission from CD19 CAR T cell therapy, long term follow up from a larger number of patients is necessary to determine whether prophylactic or early tocilizumab has any impact on CAR T cell persistence and risk of relapse [2,4]. In fact, the Seattle group has begun to treat patients with tocilizumab at the first sign of fever, the harbinger of CRS (Jensen M, personal communication).

CD19 CAR T cells to replace intrathecal chemotherapy and cranial irradiation

Several groups have published that intravenously (IV) delivered CD19 CAR T cells are found in the cerebrospinal fluid (CSF) of patients [2–4]. Some had active CNS leukemia at the time of therapy, and CAR T cells clearly eradicated disease. Most patients, though, did not even have a history of CNS leukemia yet CD19 CAR T cells were still present in the CSF of 80% of these patients at the time of Day 28 restaging [2].

ALL, the most common childhood malignancy, is most commonly diagnosed in children less than 8 years of age, a critical time in brain development [11]. Multiple doses of prophylactic, intrathecal chemotherapy in children without CNS disease is necessary to avoid the significant risk of CNS relapse. In the case of CNS disease, cranial or craniospinal irradiation up to 1800 cGy is commonly employed in addition to more intensive intrathecal chemotherapies [11]. Even in children without CNS disease, leukoencephalopathy along with measurable and significant cognitive declines have been documented [12]. For those requiring irradiation, the effects are even more pronounced, and patients are predisposed to additional secondary malignancies. It is therefore highly attractive to consider replacing intrathecal chemotherapy and radiation with CAR T cells for the prevention of CNS relapse and the treatment of CNS disease.

Only one grade 3 neurologic toxicity (dysphasia) was encountered in the initial cohort on our clinical trial, and no grade 4 or 5 neurotoxicities occurred. Five other patients experienced grade 1 visual hallucinations [2]. Other groups have reported significant neurotoxicities including seizures, aphasia, obtundation and confusion lasting days and prompting intubation for airway protection [3,4]. These events appear to be more severe in older adults than in children. The pathophysiology of neurotoxicity is not known, but it can be seen entirely outside the context of the classic symptoms of CRS. Some hypothesize it is due to a cytokine effect in the brain as suggested by the finding of high IL-6 and other cytokines in the CSF and the onset of neurotoxicity after administration of tocilizumab, which decreases the clearance of IL-6 from the blood by blocking endocytosis resulting in dramatically increased plasma IL-6 [6]. Others are considering whether there is some degree of CD19 expression on cells within the CNS that is not presently recognized.

Clearly, more study is needed to determine how IV administered CD19 CAR T cells mediate neurotoxicity. In these early days, some adult patients have experienced life-threatening neurologic events that in most cases were entirely reversible [3]. But, the vast majority of adults and children treated do not have any neurologic effects. In fact, neither of the two adolescents with CNS disease treated on our trial experienced significant neurotoxicity though one had transient, focal inflammation identified by MRI in the splenium of his corpus callosum coinciding with grade 1 visual hallucinations [2].

A more complete understanding regarding the pathophysiology and subsequently the best approach to treat or prevent severe neurotoxicity is of utmost importance. Davila et al. found no CAR T cells in the CSF of patients who exhibited neurotoxicity [3]. However, we found that higher absolute numbers of CD19 CAR T cells in the CSF correlated with any grade of

neurotoxicity [2]. Since tocilizumab as it is currently administered may temporarily worsen neurotoxicity, we rely on high-dose steroids to mitigate severe neurologic symptoms and in the process potentially handicap CAR T cells from realizing their full anti-leukemia potential. This is likely not the best approach, and more study is needed. For example, if elevated IL-6 in the CSF indeed contributes to neurotoxicity, CSF concentrations (as a surrogate for parenchymal concentrations) of tocilizumab delivered IV may not be sufficient to competitively inhibit IL-6 receptors. It is not known how effectively, if at all, tocilizumab can penetrate the blood brain barrier, so consideration could be given to studies of alternate routes of administration.

CD19 expression in the CNS remains a controversial subject. One of 6 donor human brains analyzed for CD19 protein expression as part of the Allen Brain Atlas did indeed stain for CD19 in the left inferior frontal gyrus [13]. Regional or temporal expression of CD19 on neuronal or glial cells may be present in some patients. Alternatively, CD19 CAR T cell killing of infiltrating leukemia or B lymphocytes in key regions may induce surrounding inflammation or bystander killing that is reversible or reparable with time. We aim to learn more by opening enrollment in our protocol to patients with isolated CNS disease and those with CNS3 disease provided no focal neurologic symptoms are present.

CD19 CAR T cells have not replaced hematopoietic stem cell transplant

It is tempting to declare HSCT unnecessary in patients who have achieved MRD-negative remissions after CD19 CAR T cell therapy. All of our patients have standard indications for HSCT, spanning early first relapse, multiple relapses or primary refractory disease [14]. Starting with the first patient we treated, our approach has been to recommend HSCT to patients achieving MRD-negative remission with CAR T cells who have not previously had a HSCT, who are medically fit, and who have an appropriate donor. Twelve of 20 ALL patients we treated in the initial cohort achieved an MRD-negative remission. Of these, 2 had contraindications to a second HSCT and relapsed with CD19 negative ALL at 3 and 5 months. Only one of 10 who had HSCT immediately following CAR therapy has relapsed with CD19+ ALL at 2 years post-HSCT. No CD19 negative relapses occurred in this cohort [2].

Other groups have taken the opposite approach where the vast majority of patients achieving an MRD-negative response do *not* proceed to HSCT [4]. For example, Maude and colleagues treated 30 children and young adults with a CD19 CAR that differs from our construct in that 4-1BB replaces our CD28 co-stimulatory domain [4]. Of note, these constructs shows differential persistence with 4-1BB expressing CARs typically persisting more than 6 months, in contrast to CD28 expressing CARs which disappear within the first 60 days [15]. Twenty-two patients achieved MRD-negative remission, but only 3 went on to a subsequent HSCT. Of the 19 that did not receive any further therapy, 7 patients (37%) relapsed between 6 weeks and 8 months, with 3 of these relapsing with CD19 negative ALL.

Recognizing that the sample size is small for both studies, given that our relapse rate in patients proceeding to HSCT of 10% is low, and the standard of care for relapsed, refractory pre-B ALL patients who achieve an MRD-negative remission by any means is HSCT

provided a donor is available [14] we continue to refer patients to HSCT if possible. As we continue to treat more children for whom HSCT is not an option (ie: second HSCT where the risk/benefit ratio is not clear, no donor availability, or comorbidities resulting in excessive risk with HSCT) increasing insights will be gained regarding the risk for relapse in patients rendered MRD negative by CAR therapy who do not proceed to transplant. Ultimately, a long-term goal is to spare as many patients as possible the acute and chronic effects of myeloablative HSCT [11].

Next to managing the toxicities associated with CAR therapy, CD19 negative relapse is the most significant threat to successful CAR regimens. Also seen with blinatumomab, the incidence of CD19 negative relapse may be as frequent as 30% [2–4,16]. It remains to be determined whether these relapses represent an outgrowth of pre-existing CD19 negative cells or whether leukemia can actively downregulate CD19 expression. Before the era of CD19-directed immunotherapies, most flow cytometry laboratories gated on CD19+ events in the blast window before analyzing other markers, and 100% of pre-B ALL was generally thought to be CD19+. However, CD19 negative populations even in patients who have not had CD19-targeted therapies may have been missed. The emergence of CD19 negative ALL post immune pressure requires an entirely different approach to flow cytometric analysis. A consortium of experts in the field are working towards a solution to this problem (Stetler-Stevenson M, personal communication).

The emergence of CD19 negative ALL also requires a new therapeutic approach. To this end, we have recently opened a Phase I clinical trial of CD22-directed CAR T cell therapy in the POB (NCT02315612). Though enrollment is open to patients with CD19-positive ALL, the majority so far are indeed CD19 negative. It is likely that combinatorial therapies or those targeting multiple tumor antigens simultaneously will be required to prevent the emergence of antigen escape.

Conclusion

CD19 CAR T cell therapy is poised to shift the treatment paradigm for ALL, but several challenges remain. Strategies for reducing toxicity, capitalizing on the capacity to mediate clearance of CNS leukemia, incorporating into standard treatment regimens, and reducing the need for HSCT in some patients are only a few of the areas yet to be explored.

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Key Points

- CD19 CAR T cell therapy can induce remissions in otherwise refractory children and young adults with ALL.
- Aggressive supportive care and early anti-cytokine therapy is absolutely required to mitigate the life-threatening consequences of severe cytokine release syndrome.
- IV administered CD19 CAR T cells mediate leukemia clearance from the CSF and may be a new alternative therapy for the treatment or prophylaxis of CNS leukemia.
- Until adequate long-term follow up and more experience is available, eligible responders should proceed with standard of care first HSCT.
- Antigen escape from CD19-directed therapies necessitates continued development of immunotherapeutic approaches to other leukemia antigens.