Tisagenlecleucel for treatment of children and young adults with relapsed/refractory B cell acute lymphoblastic leukemia

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Abstract

The treatment landscape for cancer therapy has changed drastically over the past decade. Tisagenlecleucel, the first genetically engineered adoptive cellular therapy approved by the United States Food and Drug Administration, has revolutionized this field by demonstrating impressive clinical success in children and young adults with relapsed/refractory B cell acute lymphoblastic leukemia (r/r B-ALL). Now three years since its approval, we have gained a deeper understanding on the basic immunobiology and clinical efficacy of this drug. This review will provide an updated summary of the clinical efficacy of tisagenlecleucel in childhood and young adults with r/r B-ALL, common side effects and their associated management strategies, as well as barriers that remain to be addressed in order to realize the maximum potential of this drug.

Introduction

Rapid, iterative improvements in treating B-ALL, especially in younger patients, has been one of the great success stories of cancer therapy. Initial observations that naval personnel exposed to mustard gas during World War II experienced toxic changes in the hematopoietic cells in the bone marrow, creating the fundamental basis of chemotherapy, have given way to the current ability for scientists to identify specific genetic lesions in leukemic blasts for precise targeting of driver kinases. The pace of discovery coupled with clinical relevance has been remarkable[1]. However, with the exception of tyrosine kinase inhibitors in Philadelphia-chromosome positive B-ALL, other targeted therapies are still in development for B-ALL. The large majority of patients who respond to conventional therapies do well, but the prognosis for patients with relapsed/refractory (r/r) B-ALL remains dismal. Thus, development of new therapies remains vital.

In 2017, the autologous chimeric antigen receptor (CAR) T cell therapy tisagenlecleucel became the first gene therapy and the first genetically engineered adoptive cell therapy to be approved by the Food and Drug Administration (FDA), with an indication for patients up to 25 years old with B-ALL that is refractory or in second or greater relapse[2]. Shortly after, in May 2018, a second indication for treatment was added, including adult patients with relapsed or refractory large B cell lymphoma after two lines of therapy[3]. This review will discuss the use of tisagenlecleucel in children and young adults with r/r B-ALL, including its clinical efficacy, common side effects, and current challenges.

B-ALL and Therapies Available

Approximately 3,500 cases of childhood leukemia are diagnosed each year, making it the most common cancer among children. However, despite excellent therapies, it is still the second most frequent cause of death from cancer before 20 years of age [4-6]. Pediatric B-ALL has provided a model for improvement of survival among patients with cancer by progressive improvements in the efficacy of multiagent chemotherapy in large, randomized clinical trials. Such advances have led to an increase in survival rate from less than 10% in the 1960s to greater than 90% today [5,7]. Where we are today has been a triumph of clinical trial

development and multicenter patient enrollment by cooperative groups, both in the US and abroad, but, despite these improvements, relapse occurs in 15-20% of patients[8].

By contrast to the steadily improved outcome of patients with newly diagnosed B-ALL, less progress has been made in the treatment of r/r B-ALL. Several factors contribute to the prognosis after relapse, including time to relapse, immunophenotype, and site of relapse[5]. Medullary relapse within 36 months of initial diagnosis portends the worst prognosis with a 5-year overall survival rate of only 10-20% [9-11]. General treatment algorithms for relapsed B-ALL include multi-agent chemotherapy followed by haemopoietic stem cell transplantation (HSCT) for patients stratified as high-risk, and approximately 2 years of chemotherapy for those with lower or standard risk features. Radiation is often incorporated into regimens for patients who relapse with leukemia in the central nervous system (CNS). Toxicities from such treatment regimens are significant, including, but not limited to, metabolic syndrome and obesity, increased risk for secondary malignancy, and long-term impairment of cardiovascular, cerebrovascular, and peripheral nervous systems[5]. In addition, limited information on the long-term cognitive effects of intrathecal chemotherapy, used universally as CNS prophylaxis, exists. Few new agents have been FDA-approved for relapsed B-ALL, with clofarabine and vincristine sulfate liposomal injection approved by the FDA in 2004 and 2012, respectively[12,13], based on complete remission rates of 20 to 30%[14-16].

Since the 1950s, there have been three established pillars of cancer therapy: surgery, radiation therapy, and chemotherapy. To this list, a fourth pillar can now be added: immunotherapy. Immunotherapies provide an alternative mechanism of action and, in the case of CAR T, selective targeting of antigens on cancer cells, often limiting unwanted "off target" side effects. From 2014 to 2017, three novel and distinct immunotherapy drugs were approved by the FDA for the treatment of r/r B-ALL, a feat that was unprecedented in the prior 25 years[17]. (1) Blinatumomab, a bi-specific T cell engager (BiTE) designed to link CD19+ B cells with CD3+ T cells, (2) Inotuzumab ozogamicin, an anti-CD22 antibody conjugated to a calicheamicin-class cytotoxic drug (3), and most recently, in August 2017, the FDA granted full approval to tisagenlecleucel, a CD19-directed CAR T cell product, which will be the focus of this review.

Clinical efficacy of tisagenlecleucel in B-ALL

Phase 1

The first trials to determine the safety and feasibility of tisagenlecleucel (produced at that time at the University of Pennsylvania as CTL019) in B-ALL were phase 1/2a single arm, single center, open label studies [clinicaltrials.gov NCT01626495 and NCT01029366] conducted at the Children's Hospital of Philadelphia (CHOP) [18] and the University of Pennsylvania (U Penn). In 2014, 30 patients (25 children, 5 adults) with relapsed or refractory CD19+ B-ALL were reported from these two trials [19]; 18 patients had previously undergone allogeneic HSCT. The overall response rate, defined as either CR or CRi, was 90% one-month post-infusion. Nineteen patients (63%) demonstrated continued remission at time of publication with a median follow up time of 7 months (range 1-24 months).

Updated results focused on the pediatric cohort (n=59) presented at the American Society of Clinical Oncology (ASCO) annual meeting in 2016 demonstrated 55 patients (93%) were in complete remission at one-month post-infusion with negative MRD in 52 patients (88%). Relapse-free survival (RFS) was 76% at 6 months and 55% at 12 months, and overall survival was 79% at 12 months. Twenty patients subsequently relapsed with the majority (13) demonstrating antigen escape with a CD19-negative phenotype. CTL019 persistence was accompanied by B cell aplasia, which continued up to last assessment (1-39 months) in 24 of 34 patients with ongoing CR [20], showing that B cell aplasia could be used as a widely available pharmacodynamic marker for functional CAR T persistence. The first patient treated on that trial remains in continuous CR without further therapy at 8 years.

Phase 2

The first multicenter trial of a CAR T product was the Novartis-sponsored ENSIGN trial, which was conducted in the US [NCT02228096]. In the ENSIGN study, CTL019 was produced at the U Penn GMP facility,

and GMP lentiviral vector was made at the CHOP vector core. This study developed the infrastructure to do multicenter CAR T cell therapy, including creating a logistics "cold chain" for shipping cryopreserved cells from and to the treating center. Subsequently, the FDA approval of tisagenlecleucel was based on results of the ELIANA study [NCT02435849], which was a single cohort, multicenter study to test the safety and efficacy of tisagenlecleucel for children and young adults with relapsed or refractory B-ALL. ELIANA was the first global CAR T cell study, enrolling patients at 25 centers in 11 countries, and was conducted in its entirety using cells manufactured in Novartis's GMP facility, which is currently used for commercial manufacturing. In the primary analysis, 97 patients were enrolled, 79 were infused, and 18 were excluded due to tisagenlecleucel product-related issues, death, or other adverse events that precluded tisagenlecleucel infusion. The 79 patients who received tisagenlecleucel had undergone a median of 3 previous therapies and had a median bone marrow blast percentage of 74% at enrollment. A majority of them (61%) had previously undergone allogeneic HSCT. Lymphodepleting chemotherapy (moderate dose fludarabine and cyclophosphamide) was given prior to tisagenlecleucel infusion in 96% of patients; 3 patients did not receive lymphodepleting chemotherapy due to leukopenia. The overall response rate for patients who received tisagenlecleucel was 82% (95% CI 712-90) at 3 months, with the vast majority of the responders (98%) achieving a MRD negative state by multiparameter flow. For responders, RFS was 66% (95% CI 52-77) at 12 months and 62% (95% CI 47-75) at 24 months. In those patients who experienced a relapse, it was largely driven by CD19-negative escape variants. Eight patients underwent allogeneic HSCT while in tisagenlecleucel-induced remission, including 2 patients who were MRD+ and 2 patients with evidence of early B cell reconstitution[21]. Updated trial data presented at ASH in 2018 showed an OS among all infused patients of 76% (95% CI 63-86) at 12 months and of 66% (95% CI, 54-76) at 24 months [22].

Common Side Effects

Cytokine release syndrome (CRS)

CD19-directed CAR T cell products, including tisagenlecleucel, report similar treatment-related toxicities with the most common being CRS. CRS describes a constellation of inflammatory symptoms resulting from cytokine elevations associated with T cell expansion, proliferation, immune system activation, and tumor cell elimination and is not restricted to anti-CD19 therapies [23,24]. CRS is initially characterized by fevers and myalgias and can progress to hypotension, hypoxia, and/or multiorgan toxicity[25,26]. The overall goal of management of CRS is to minimize symptoms and avoid life-threatening organ toxicity without compromising the CAR T cell function. For pediatric patients with mild to moderate CRS symptoms, management includes supportive care and close monitoring for hypotension, tachypnea, and hypoxia. Severe CRS (grade 3-4) is characterized by unstable hypotension or significant respiratory insufficiency. The management of severe CRS was revolutionized by the observation in our initial patients that interleukin 6 (IL-6) is a key driver of the CRS reaction [18,27]. Severe CRS is treated with the IL-6 receptor inhibitor tocilizumab, which is currently the only FDA-approved therapy for CRS, as targeted anti-cytokine therapy[23]. Tocilizumab has allowed corticosteroids, which are lympholytic and at high and prolonged doses can jeopardize the function and persistence of CAR T cells, to be avoided as first-line management. Severe CRS from tisagenlecleucel is managed in a stepwise fashion, starting with tocilizumab in one or two doses, then adding corticosteroids, followed by another tocilizumab dose. After these interventions, most severe CRS is controlled, as indicated by resolution of fever and minimal to no need for pressor support. In cases where CRS continues, which are generally the most challenging to manage, other interventions such as high-dose steroids and siltuximab, a direct IL-6 antagonist [25], may be considered. Although siltuximab has been proposed as a first-line agent in one publication[25], no clinical experience or published data exists to support its use as first-line management [28]. The utility of siltuximab is an especially important question during the COVID-19 pandemic, where tocilizumab is being used off-label to treat COVID-19 CRS [29], and there may be concerns about drug supply. Having tocilizumab available in the pharmacy for patients undergoing cell therapy is required by the risk evaluation and mitigation strategy (REMS) of the product, and remains standard of care. For now, the place for siltuximab in the management of CRS remains an important area of active study [30,31]. In addition, recent pre-clinical studies have also demonstrated potential activity of dasatinib, a multityrosine kinase inhibitor, for patients refractory to standard CRS treatment by potently and reversibly inhibiting

CAR T cell function [32,33]. Investigators from multiple centers recently convened to unify CRS grading across trials and commercial CAR T products, producing the ASTCT grading scale for CRS (Table 1)[34].

CRS in phase 2 trial

In the global trial ELIANA, CRS occurring in 50 of 65 patients (77%). Fifty-three percent of patients experienced hypotension that required intervention, and 24% required high-dose vasopressors. Fifteen percent of patient were intubated, and 10% of patients required dialysis. Thirty-nine percent of patients received tocilizumab. and 20% received corticosteroids. All cases of CRS were reversible[22]. Baseline disease burden, as defined by the percentage of blast cells in bone marrow before infusion, correlated with the severity of CRS. Patients with severe CRS also had higher levels of CAR positive CD8 and CD3 positive cells.

Neurotoxicity

CAR T-related neurotoxicity, also known as immune effector cell (IEC) therapy-associated neurotoxicity syndrome (ICANS), is pathophysiologically distinct from CRS [34]. Symptoms observed are global encephalopathy, which can include aphasia, confusion, hallucination, tremor, and agitation; focal deficits; and seizures [19,21,35]. While the pathophysiology of neurotoxicity remains to be fully elucidated, it is hypothesized to be an off-target toxicity with some evidence to suggest the diffusion of inflammatory cytokines through the blood-brain barrier and/or direct CNS toxicity by the engineered T cells may play a role [36]. In limited available data, ICANS does not appear to be readily reversed or ameliorated by IL-6 receptor blockade, possibly due to upregulation of IL-6, inefficient distribution into the CNS (as most monoclonal antibodies do not cross blood brain barrier), or the involvement of other cytokines. Furthermore, although it is known that tisagenlecleucel crosses the blood brain barrier and can persist for months, no clear correlation exists between the presence of tisagenlecleucel in the CNS and severity of symptoms [37,38]. Treatment for ICANS is mainly focused on supportive care after ruling out other potential causes of symptoms [31].

Neurotoxicity in phase 2 trial

The incidence of neurotoxicity in the ELIANA trial was 30 of 75 patients (40%) within 8 weeks after infusion. Ten patients had grade 3 neurologic events, but no grade 4 events or cerebral edema were reported. The majority of neurologic events occurred during CRS or shortly after its resolution. Severe neurologic events occurred more frequently in patients with higher-grade CRS (grade 3 neurologic events occurred more frequently in patients with grade 4 CRS than among those with grade 0 through 3 (32% vs. 7%; 95% CI for the difference, -1 to 50 percentage points)). Among grade 3 neurologic episodes that resolved, 50% resolved within 10 days, and 75% resolved within 18 days[21]. Interestingly, neurotoxicity does not appear to be correlated with CNS involvement, as 3 of 17 patients (18%) with CNS disease compared to 12 of 43 patients (28%) without CNS disease developed encephalopathy in CTL019 trial [39]. The grading for ICANS has now also been presented in the ASTCT consensus paper [34]. This scale now provides consistent grading for encephalopathy as well as other major events such as seizures or cerebral edema, while not including headache (very common in patients with even mild CRS), or CNS hemorrhage, which is graded separately as a bleeding event.

Current challenges

Although impressive progress has been made in revolutionizing the landscape of anti-cancer treatment through the development of cellular therapy, many challenges remain, such as ensuring successful leukapheresis and CAR T cell manufacturing, non-response and disease relapse, and managing its unique toxicity profile. Continued insights into these barriers will allow us to maximize the potential benefit of this powerful therapy with the goal of extending the application of CAR T cell therapy beyond B cell malignancies.

Successful leukapheresis and CAR T cell manufacturing

The current recommended dose of tisagenlecleucel contains 0.2 to 5.0 x10^6 CAR-positive viable T cells per kg of body weight for patients 50kg or less, or 0.1 to 2.5 x10^8CAR-positive viable T cells for patients more than 50kg[40]. In order to achieve this dose, an adequate quantity of T cells must first be collected from the

patient; therefore, a minimum absolute lymphocyte count (ALC) of ˜500 cells/uL and a CD3+ cell count of ˜150 cells/uL is recommended prior to starting apheresis [41]. Factors that would affect both ALC and CD3+ cell count include timing of proximal cytotoxic therapy or progressive leukemic disease leading to bone marrow replacement of cancer cells. Some patients are never able to achieve these minimal peripheral blood parameters due to the nature of their highly refractory B-ALL. For these patients, the prospect of an allogeneic CAR T cell product, which remain in early clinical investigation, is attractive [42].

Once an adequate quantity of T cells is collected, characteristics of the leukapheresis product may directly affect the quality and/or performance of the CAR T cell product. Predicting the performance of CAR T cell products is quite difficult using in vitro testing, so at this time, performance is best assessed after infusion into the patient using the metrics of disease response, in vivo proliferation and CAR T cell persistence. Expansion is a vital element to disease response. In the ELIANA trial, expansion (measured as the geometric mean of the area under the concentration-time curve in peripheral blood from time 0 to day 28 as expressed in copies per microgram of DNA times days) was 315,000 in patients with a response and 301,000 in patients without a response[21]. In addition, responders to tisagenlecleucel have a shorter median time to maximum expansion of 11 days compared to 13 days in non-responding patients[21,37]. Much research has been dedicated to understanding the mechanisms behind poor expansion and persistence of the T cell product in order to maximize the anti-leukemic property of this drug.

First, recent studies have demonstrated that T cell phenotype plays an important role in predicting a CAR T cell product's subsequent clinical activity. The presence of naïve and early memory T cells with significant proliferative potenital in the pre-manufactured product was found to correlate with a biomarker of successful CAR performance in pediatric B-ALL [43]. Peripheral blood samples that contained a higher percentage of naïve and stem central memory cells directly correlates with T cell expansion potential in vivo[44], and CAR T cell products that contain more central memory T cells persist longer, which can mediate a more successful clinical response [45,46]. Interestingly, it has also been recently shown that the distribution of T cell subsets in peripheral blood samples varied across different pediatric cancers, thus indicating that disease biology may further play a role in altering the patients' T cell developmental phenotype at collection[44], which can inform collection practices as CAR T cells are applied to other diseases.

Another factor that can contribute to differences in T cell fitness lies in the previously exposed chemotherapy regimen. For example, chemotherapy regimens containing clofarabine or doxorubicin has been implicated in both quantitatively insufficient and poor-quality CAR T cell products [44,47] (we strongly discourage use of clofarabine prior to collection). Additionally, clinical data suggest that prior treatment with cyclophosphamide and cytarabine selectively reduces early lineage T cells that are associated with productive CAR T cell expansion [43]. Therefore, it is important to understand how different chemotherapies affect T cells as it can have a direct impact on the quality of T cells collected. Early collection of T cells prior to intensive regimens of cytotoxic chemotherapy should be considered in patients identified as having a high risk of relapse or those with relapsed disease, which may improve the quality of the apheresis product and, thus, the resultant manufactured CAR T cell product.

Finally, differences in the CAR design and manufacturing processes may also play an important role in predicting the clinical performance of the final CAR T product. CAR T cell products that have shown efficacy in clinical trials to this point, including tisagenlecleucel, are second generation products [48-50]. Tisagenlecleucel utilizes a 4-1BB based co-stimulatory domain and has been shown to persist in the blood for a median duration of 168 days (range 20-167 days) compared to CAR constructs using CD28 co-stimulatory domains, whose persistence is approximately 1 to 2 months [21,47,51]. There are patients from the first CHOP studies with persistent CAR T cells for 5-10 years. This longer persistence is likely due to the reduced propensity for T cell exhaustion induced by tonic CAR signaling when co-stimulation is mediated by a 4-1BB domain [52]. Data thus far suggests that CAR persistence is an important factor in achieving a durable remission in ALL without further anti-leukemia therapy. This association is harder to discern in lymphoma patients treated to date [53].

Non-response and disease relapse

When a patient is able to be treated at an institution that offers tisagenlecleucel, another challenge lies in the failure for them to either achieve remission or to maintain a sustained remission. Although tisagenlecleucel has the potential to be definitive therapy for patients with r/r B-ALL who achieve long-term CAR T cell persistence, approximately 10-20% of patients fail to enter remission after receiving anti-CD19 CAR T cell therapy, and 30-50% of patients who achieve remission will have either antigen-positive or -negative disease relapse, the majority within 1 year of infusion[21,51,54]. Specifically, in the ELIANA trial, 38% of patients who initially achieved a CR after CAR T cell infusion when on to relapse within 24 months [22] [21]. Of those 19 patients who relapsed in that timeframe, 14 (74%) showed evidence of CD19 escape. Other institutions across the country have also reported similar antigen negative relapse rates with other CD19 CAR T cell constructs [47,51,55,56].

CD19 antigen negative relapse

Patients treated CD19 CAR T cells may experience CD19-negative relapse. Three mechanistic hypotheses have been suggested in: antigen loss or modulation, inherent tumor heterogeneity with pre-existing CD19 negative subclones, and lineage switching. In antigen loss and modulation, pathways leading to the loss of CD19 includes alternate splicing (exon 2 skipping) [57], interruption in the transport of CD19 to the cell surface due to mutations [58], or mutations in the CD19 chaperone protein CD81 [59]. Secondly, it is also possible that pre-existing CD19 negative subclones are present at diagnosis, which allows for emergence of CD19 negative leukemic blasts[60]. Specifically, patients with BCR-ABL1 positive B-ALL have been shown to harbor CD19 negative malignant precursor cells[61]; although, further studies are needed to determine whether certain cytogenetics are associated with higher occurrences of inherent tumor heterogeneity with more CD19 negative subclones. Thirdly, lineage switch is another mechanism for CD19 loss. Although it is traditionally associated with infant KMT2A-rearranged leukemic subtypes[62], CAR T cell clinical trials have also reported lineage switch from lymphoid to myeloid leukemic subtypes regardless of KMT2A rearrangement [56,63]. This phenomenon has also been seen with other CD19 directed therapy, such as blinatumomab[64], which highlights the concept that leukemic blasts with certain cytogenetics may show exceptional plasticity in response to their microenvironment; therefore, careful monitoring for escape variants cannot be overemphasized. Though the use of blinatumomab is effective in disease control in with relapsed/refractory B-ALL[65], especially at MRD levels of disease. its prior use may contribute to the increased risk of immune evasion after CAR therapy due to selection pressure for CD19-negative malignant cells. Due to these concerns, treatment with prior anti-CD19 therapy such as blinatumomab was an exclusion criterion in the ELIANA trial[21]. Subsequent analysis of 166 patients who received tisagenlecleucel confirmed this suspicion by demonstrating that prior therapy with blinatumomab was associated with a significantly higher rate of failure to achieve MRD negative remission or subsequent loss of remission with antigen escape that was not associated with the presence of dim CD19 or rare CD19 negative events by flow cytometry prior to tisagenlecleucel infusion [66]. However, we do not regard prior CD19-directed therapy as. A contraindication for tisagenlecleucel infusion. Lastly, a unique case was recently reported showed introduction of the CAR gene into a single leukemic B cell during T cell manufacturing. An ALL patient treated on our studies relapsed with CD19 negative leukemia 9 months after he received tisagenlecleucel. It was unclear whether the CAR gene resulted in the relapse, but one hypothesis was that the CD19 molecule was masked but the CRA protein. In this single case, the relapse resulted from a single ALL cell as shown by site integration analysis [67].

For patients with antigen negative relapse, targeting of other antigens such as CD22 using inotuzumab ozogamicin or a CD22-directed CAR T product are viable options, but no data on the curative potential of either exist and further prospective studies are needed[68,69]. Therefore, at this time, allogeneic HSCT remains as the only option for definitive therapy after achievement of second remission in this population.

Early B cell recovery and CD19 antigen positive relapse

Our group has defined early B cell recovery as B cell recovery within six months of infusion, which indicates. Loss of CAR T function. Risk of relapse is considered higher with early B cell recovery due to failure of disease surveillance by circulating CAR T cells. Current therapies offered after early B cell recovery include retreatment with CAR T cells, with or without additional therapy to augment CAR T cell activity,

and/or hematopoietic stem cell transplant. Limited published data exists on re-infusion for early B cell recovery with the same CAR product. Gardner et al. administered a second infusion of anti-CD19 CAR T cells to 8 patients with B-ALL who had evidence of engraftment loss. Of the eight patients, only two had CAR T cell expansion after re-infusion; however, the six patients who did not respond to the re-infusion of CAR T cells did not receive what most consider now to be the standard lymphodepleting preparatory chemotherapy regimen prior to their retreatment[54]. In the CTL019 trial, 17 of 55 patients were received repeat infusion of murine CTL019 for poor persistence at 3 and/or 6 months after initial infusion. Reinfusion induced B cell aplasia for a second time in 1 of 7 children treated for B cell recovery, while 6 of 7 patients reinfused for CD19 positive hematogones demonstrated continued B cell aplasia six to 21 months after repeat infusion. Of this group, 6 remained in remission 9 to 24 months after initial infusion, and one experienced CD19 negative relapse[70]. Methods being tested to improve success rates of CAR persistence after re-infusion include concurrent treatment with PD-1 checkpoint inhibitor and infusion of different CAR constructs such as humanized CD19 CARs to overcome immune-mediation rejection of murine-derived anti-CD19 CARs. At our center, concurrent treatment with programmed death-1 (PD-1) checkpoint inhibitor in those with early CAR T cell loss/no response to CAR T cell therapy has shown encouraging results. Fourteen patients received pembrolizumab or nivolumab, and three of six patients who received pembrolizumab for early B cell recovery re-established B cell aplasia. Two of these patients had persistent B cell aplasia with ongoing pembrolizumab therapy[71].

Treatment for antigen positive relapse with further CAR T is possible, since the CD19 antigen is still expressed. Lee et al. described 3 patients who received re-infusion of CAR product with a CD28 endodomain for recurrent CD19+ disease, but none had an objective response[47]. In the phase 1 CTL119 (humanized CD19 scFv) trial, 6/9 patients treated with CTL119 CAR for relapse after prior CD19 CAR T therapy achieved MRD negative CR[72]. Work is ongoing to assess whether immunogenicity of the murine CAR plays a role in such events, which might be alleviated by a humanized CAR. Despite the different CAR constructs, lack of CAR-specific T cell responses in such patients suggest a possible mechanism of immune-mediated rejection upon repeat dosing. In very early data testing the addition of a checkpoint inhibitor to CAR T therapy resulted in 2 partial and 2 complete responses [71].

Advances in toxicity management

Despite advances in knowledge and management of CRS, it continues to be a significant cause of morbidity in patients treated with tisagenlecleucel. The challenge in toxicity management lies in controlling CRS symptoms without compromising clinical efficacy. Early recognition of CRS symptoms may allow for better control of symptoms through implementation of treatments before patients become critically ill. Our group at CHOP/UPenn demonstrated that IL6, CRP and ferritin all are strongly associated with development of severe CRS, but are not useful as predictive biomarkers early after infusion[73]. Other analytes collected in the first 72 hours, such as a model incorporating sgp130 + IFN-g + IL1RA, strongly predicted later CRS. Use of prophylactic treatment strategies is also being explored. Gardner et al. reported on use of early administration of tocilizumab with and without dexamethasone after treatment with a 4-1BB second generation CAR T cell product with encouraging data of a modest decrease in severe CRS without an effect on engraftment and persistence of CAR T cells [74][clinicaltrials.org NCT02028455]. A similar clinical trial at CHOP [clinicaltrials.gov, NCT02906371] was conducted to evaluate the efficacy of early administration of tocilizumab after treatment with tisagenlecleucel in pediatric patients with high disease burden in an attempt to mitigate severe CRS. Patients with high disease burden (>40% BM blasts) on day-1 were allocated to receive a single dose of tocilizumab at the time of fever, after which they received standard CRS management. This trial testing pre-emptive tocilizumab met the endpoint for grade 4 CRS reduction (by 1/3) in these high-risk patients [74,75]. Finally, our understanding of the mechanisms underlying neurotoxicity remains poor. There are no predictive models for the development or severity of neurotoxicity, although there is a strong correlation with severe CRS. Treatment with tocilizumab has not been effective, and clinicians often revert to corticosteroids for severe neurologic toxicities to avoid feared cerebral edema; although, this complication has not been reported after treatment with tisagenlecleucel [76].

The unification into a single grading system will allow for an objective assessment and reporting of clinical severity between different clinical trials and CAR T cell constructs going forward in order to further advance our current understanding and management of CRS[17,24,25,77-82].

Future directions and Conclusion

The treatment landscape for cancer therapy has evolved significantly over the past few decades. Cellular therapies, such as tisagenlecleucel, offer tremendous promise in patients with treatment refractory B-ALL.

However, much work remains to be done to optimize the utility of cellular therapies in order to harness their full potential, including increasing accessibility to these agents, managing potentially life-threatening side effects, preventing disease relapse, and translating these successes in B ALL to solid tumors and other diseases. In addition, studies are underway to expand the success of tisagenlecleucel beyond r/r B-ALL to patients earlier in therapy. The Children's Oncology Group phase II trial (AALLL1721) is evaluating the 5-year disease free survival rate in patients with high-risk B-ALL who are MRD positive at the end of consolidation to then receive tisagenlecleucel to determine if earlier administration of CAR T cells would have an impact on EFS and the ability to avoid HSCT. Continued understanding into basic immunology, genetic engineering, and ALL biology will surely result in more advances in cellular therapy that continue to improve outcomes in r/r childhood B-ALL and offer the opportunity for curative therapy in our sickest and most refractory patients.

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TABLE 1 ASTCT Consensus Grading of CRS

Hosted file

`image1.emf` available at https://authorea.com/users/361230/articles/482710-tisagenlecleucel-for-treatment-of-children-and-young-adults-with-relapsed-refractory-b-cell-acute-lymphoblastic-leukemia

FIGURE 1 **Schematic of CAR structure in relation to CD19 expressing tumor cells** . CARs are composed of single-chain variable fragment (shown here as FMC63) joined to an intracellular CD3 zeta signaling domain. Tisagenlecleucel is a second-generation CAR construct that incorporates a second additional co-stimulatory endodomain (4-1BB).

FIGURE 2 **Diagram of CAR T cell manufacturing and treatment process** . The treatment process starts with leukapheresis of the patient's peripheral blood mononuclear cells, which is then frozen and shipped to the appropriate manufacturing facility for ex-vivo modifications. T cells are activated and expanded using

anti-CD3/anti-CD28 coated beads and expression of CAR is transduced using lentiviral vector. Successfully manufactured products are shipped back to the treating facility, where it is given intravenously back to the patient.

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temp ≥ 38°C	Temp ≥ 38°C	Temp ≥ 38°C	Temp ≥ 38°C
Hypotension	None	No vasopressors	One vasopressor with or without vasopressin	Multiple vasopressors
Hypoxia	None	Low flow nasal cannula or blow-by	High flow nasal cannula, facemask, nonrebreather mask, or Venturi mask	Requires positive pressure