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Using gene therapy to manipulate the immune system in the fight against B cell leukemias

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Abstract

Introduction—Over 20 years ago, chimeric antigen receptors (CARs) were created to endow T-cells with new antigen-specificity and create a therapy that could eradicate cancer and provide life-long protection against recurrence. Steady progress has led to significant improvements with CAR design and CAR T-cell production, allowing evaluation of CAR T-cells in patients. The initial trials have targeted CD19, which is expressed on normal and malignant B-cells.

Areas covered—We review data from trials for patients with chronic lymphocytic leukemia (CLL) and B-cell acute lymphoblastic leukemia (B-ALL). In addition, we discuss the on-target toxicities, B-cell aplasia and cytokine release syndrome (CRS), which is uniquely associated with T-cell immunotherapies.

Expert Opinion—We compare the results when targeting the same antigen in CLL or B-ALL and speculate on reasons for outcome differences and future directions to enhance outcomes. Furthermore, the dramatic results targeting B-ALL require further analysis in Phase II trials, and we discuss important components of these future trials. We also suggest a management scheme for CRS. The next several years will be critical and may lead to the first clinical indication of a gene-engineered cell therapy for cancer.

Keywords

Adoptive T cell therapy; B cell acute lymphoblastic leukemia; Chimeric Antigen Receptors; Chronic Lymphocytic Leukemia; Gene Therapy; Immunotherapy

1. Introduction

Since the original chimeric antigen receptor (CAR) was described over two decades ago, the construct has seen several incarnations. In its most basic form, a CAR consists of an antigen-specific single chain variable fragment (scFv) linked to transmembrane and T cell

Declaration of Interest

The authors have no other relevant disclosures.

stimulatory domains [1, 2]. By freeing the antigen receptor from dependence on the major histocompatibility complex, the CAR allows for a universally applicable but patient-specific immunotherapy. Furthermore, in creating a link between the immune system and cancer, CAR T cell therapy harnesses existing cellular resources and maximizes their efficiency.

Early experiments with first generation CARs, while promising, demonstrated limited expansion and efficacy in vivo [3-5]. The addition of a co-stimulatory domain (Figure 1) led to long-term eradication of cancer and increased the persistence of CAR T cells in vivo [6-8]. While CD28 was the initial focus of co-stimulatory signals for second generation CARs, other co-stimulatory domains have also proven successful at enhancing in vivo CAR T cell function such as CD27, OX40, 4-1BB, and ICOS [9-12].

The advance brought by second generation CARs allowed for their evaluation in clinical trials. The most extensively studied CARs in clinical trials have been targeted to CD19, a protein that stands out in B cell malignancies as an attractive target because of its specificity to malignant and normal B cells from the early pro to mature stage of development [13]. It was therefore reasoned that other than B cell aplasia, which can be medically managed with antibiotics and/or gamma globulin, there would be low potential for other off-target toxicities. Our discussion will focus mostly on second-generation CD19-targeted CARs, which include 1) 19-28z [14, 15], which is constructed from an anti-CD19 antibody, the CD28 co-stimulatory domain, and the CD3 ζ signaling domain and 2) the 19BBz CAR, which includes the CD137 (4-1BB) intracellular signaling domain in lieu of CD28 [16]. With the extensive clinical experience targeting CD19 in patients with chronic lymphocytic leukemia (CLL) and B cell acute lymphoblastic leukemia (B-ALL), we can now make important insights about their safety, efficacy, and future directions.

2. Targeting CLL with gene-engineered T cells

Multiple groups have targeted CD19 in patients with CLL or other non-Hodgkin lymphoma (NHL) (Table 1). Each trial has differed with regards to patient selection, conditioning chemotherapy, and CAR T cell design. Researchers at Baylor [3] performed an elegant trial comparing CAR T cell constructs designed to express the CD3 ζ intracellular domain alone (CAR.CD19 ζ) or in tandem with the CD28 co-stimulatory domain (CAR.CD19-28 ζ). Six patients with NHL were infused simultaneously with equal amounts of CAR.CD19 ζ and CAR.CD19-28 ζ T cells at 3 different total cell doses ($2 \times 10^7/\text{m}^2$, $1 \times 10^8/\text{m}^2$, $2 \times 10^8/\text{m}^2$). CAR.CD19-28 ζ cells displayed a 6.82-fold change over immediate post-infusion values of CAR T cells by qPCR, while CAR.CD19 ζ T cells failed to expand in vivo with construct copy numbers diminishing at weeks one and two [3]. CAR.CD19-28 ζ T cells remained detectable at four to six weeks (42.6 $\pm$ 19.5 copies/ μg of DNA), while CAR.CD19 ζ cells were nearly absent (4.3 $\pm$ 2.2 copies/ μg of DNA) at six weeks [3]. CAR T cells with two intracellular signaling domains also expanded more readily when stimulated ex vivo. This study confirms that co-stimulation augments both persistence and CAR T cell proliferation in vivo and that second generation CAR T cells should prove more beneficial clinically than first generation CAR T cells.

At the National Cancer Institute (NCI), Kochenderfer et al [15, 17] reported on eight patients treated with gammaretrovirus transduced CAR T cells containing an FMC63-derived anti-CD19 scFv along with human CD28 and CD3 ζ intracellular domains. This cohort was comprised of three patients with follicular lymphoma (FL), one with splenic marginal zone lymphoma, and four with CLL [15, 17]. Patients were apheresed and then pre-treated with cyclophosphamide and fludarabine. Following infusion of CAR T cells, IL-2 was administered every eight hours until toxicity limited further dosing. Of note, the patients with FL tolerated five to ten doses of IL-2, while patients with CLL received only one or two total doses of IL-2, suggesting that T cell activation (and associated toxicity) was greater with circulating tumor burden compared to lymphomatous disease. Of the four CLL patients in this trial, one achieved a 15-month complete remission (CR), two were in partial remission (PR), and one had stable disease (SD). These patients were treated with 0.3×10^7 - 2.8×10^7 CAR T cells/kg, although CAR T cell number did not correlate with response. This group reported the predicted on-target toxicity of B cell aplasia, which was evident as prolonged B cell depletion (<61 B cells/ μ L) in two CLL and two FL patients for at least four months after treatment [15, 17]. Toxicities during the trial included hypotension, fevers, fatigue, renal failure, and obtundation. Cytokine elevations were seen coincident with the toxicities, so they are believed to be secondary to a cytokine release syndrome (CRS). The NCI group recently reported on 15 additional patients who received cyclophosphamide and fludarabine plus CD19-targeted CAR T cells without exogenous IL-2 [18]. Of the four CLL patients in this report, three achieved CRs lasting 14 months, 15 months, and 23 months while one patient had PR at four months. The CAR T cells were produced more rapidly (10 days) for this cohort compared to the earlier cohort, and this may contribute to differences in anti-CLL efficacy [18]. In both reports from the NCI, a total of seven patients with CLL received CAR T cell therapy and 43% achieved a CR [15, 17, 18].

At the University of Pennsylvania (UPenn), June and colleagues have utilized a lentiviral CAR construct targeting CD19, which has a 4-1BB signaling domain (CD137) in tandem with CD3 ζ [16]. This group previously reported that inclusion of a 4-1BB signaling domain enhanced in vivo persistence of CAR T cells in mice [16]. All three patients reported by the group at UPenn were heavily treated with various chemotherapeutic regimens prior to the trial [19, 20]. Before CAR T cell infusion, UPN01 received one cycle of bendamustine, UPN02 received rituximab and bendamustine for two cycles and then bendamustine alone, while UPN03 received pentostatin and cyclophosphamide. Importantly, the trial design at the time required the patient's disease be sensitive to chemotherapy [19, 20]. CAR T cells were infused over the course of three days in a split-dose fashion. UPN01, UPN02, and UPN03 received 1.6×10^7 , 1.0×10^7 , and 1.46×10^5 CAR T cells/kg or 1.1×10^9 , 5.8×10^8 , and 1.4×10^7 total CAR T cells respectively. Both UPN01 and UPN03 achieved a CR lasting >10 months [19, 20]. UPN01 and UPN03 had very low levels of tumor burden in blood when CAR T cells were infused, so Kalos et al [19] calculated systemic tumor burden by measuring CLL tumor load in blood, bone marrow (BM), lymph node, and spleen. An extensive review of this data and other groups' data in relation to the impact of tumor burden on CAR T cell efficacy has been reported [21].

Infused CAR T cells were shown to persist in all three patients for at least six months, as measured by qPCR [19, 20]. Ex vivo cytotoxicity assays performed with peripheral blood

extracts and CD19+ NALM6 cell lines showed effector function at 56 and 169 days after CAR T cell infusion. This finding demonstrates that persistent CAR T cells may account for durable remissions and prolonged B cell aplasias. Therefore, Kalos et al [19] measured plasma cell levels of bone marrow before and after CAR T cell infusion, and all patients had a reduction in the percentage of plasma cells (UPN01 1% to 0% at Day +174, UPN02 2-3% to 1% at Day +188, and UPN03 3-5% to 1-2% at Day +174), but only UPN01 had a long-term B cell aplasia. B cell aplasia suggests persistent CAR T cell activity and more data is needed to assess whether persistent B cell aplasia will be associated with long-term morbidity and/or mortality. The UPenn group also noted toxicities following CAR T cell infusion [19, 20]. UPN01 had fevers, rigors, and hypotension 10 days after infusion, while UPN02 was hospitalized with fevers to 40°C, rigors, and dyspnea 11 days after infusion. UPN03 experienced tumor lysis syndrome and acute kidney injury 22 days after infusion of CAR T cells with elevated uric acid, lactate dehydrogenase, and a rising creatinine. Cytokine abnormalities were also noted in these patients coincident with the toxicities. The results from the NCI and UPenn groups suggest that the toxicities associated with CRS are a byproduct of CAR T cell activation by CLL.

Follow-up studies from the UPenn group were reported at the 2013 American Society of Hematology meeting [15, 17, 18, 22]. In total, 14 patients with relapsed/refractory CLL were treated as above. Target ranges for total infused CAR T cell dose was $5 \times 10^8 - 2 \times 10^9$ with an average of dose of 1.4×10^8 CAR T cells (range 0.14×10^8 to 5.9×10^8). Three patients received fludarabine and cyclophosphamide, five patients received pentostatin and cyclophosphamide, and six patients received bendamustine. Six of the patients did not receive all three required doses due to fevers within the first 24 hours of CAR T cell infusion. At the time of abstract submission, a median of 9.4 months had elapsed [22]. Three patients achieved CR (21%) with reported follow up times of 11, 34, and 35 months, while five patients achieved a PR (36%). Two of the five PR patients progressed after four months. Six patients (43%) had no response. All responding patients (CR and PR) developed a delayed CRS [22].

Brentjens et al [23] reported for the MSKCC group the results of a Phase I trial that treated eight patients with CLL with 19-28z CAR T cells. The CLL protocol consisted of three CLL patients treated with $1.2-3 \times 10^7$ 19-28z CAR T cells/kg without cyclophosphamide pre-conditioning and five patients treated with 1.5 or 3.0 g/m² cyclophosphamide followed by $0.4-1.0 \times 10^7$ 19-28z CAR T cells/kg. In the three patients without cyclophosphamide pre-conditioning, no objective disease response was observed [23]. CLL-4 was pre-treated with cyclophosphamide and infused with a total of 3.2×10^9 19-28z CAR T cells. This patient developed hypotension, fevers, and renal failure and died within 48 hours of T cell infusion. An extensive workup, including autopsy, suggested he died because of an infection and not CRS [24]. The other four CLL patients (CLL-5 – CLL-8) were pre-conditioned with cyclophosphamide and received lower total T cell doses (4×10^8 , 4×10^8 , 7.6×10^8 , and 1.4×10^9 19-28z CAR T cells, respectively) split over two days. Other than the death from a probable infection, the 19-28z CAR T cells were well tolerated and not associated with CRS toxicities [23]. Brentjens et al [23] observed an inverse correlation between tumor burden and 19-28z CAR T cell persistence suggesting that overwhelming tumor burden may limit the efficacy of CAR T cells. The T cells that persist after infusion retained cytotoxic

capability for at least 42 days [23]. In addition, this study highlights the importance of pre-conditioning therapy as three out of four patients with cyclophosphamide pre-treatment exhibited stable disease or reduction of peripheral lymphadenopathy. While the early results from the UPenn group were striking and confirmed the proof of principle of CD19-targeted CAR T cell therapy, it is important to note that as the number of patients treated by the NCI, UPenn, and MSKCC groups has increased, anti-CLL efficacy appears to be limited. Targeting CLL and other NHL was the initial focus of CD19-targeted CAR T cell therapy (Table 1). These studies revealed the importance of conditioning therapy and inclusion of the co-stimulatory domain within second generation CARs. In addition, studies demonstrated the proof-in-principle of this therapy through anecdotal reports of rapid CR inductions, B cell aplasia, and long-term persistence of CAR T cells [15, 20]. Unfortunately, as more patients have been treated [17, 18, 22] the overall clinical efficacy appears limited, especially in comparison to the considerable efficacy of recently approved therapies for patients with relapsed/refractory CLL [25, 26]. However, applying what we learned from these early clinical trials to new trials against relapsed/refractory B-ALL set the stage to witness the potential of CD19-targeted CAR T cells against a rapidly fatal disease.

3. Targeting B-ALL with Gene-engineered T cells

Multiple clinical trials are ongoing to evaluate CAR T cells as a therapy for acute leukemias (Table 2). The MSKCC and UPenn groups [27-29] have illustrated the profound anti-tumor effect that CAR T cell therapy has in adults and children with relapsed/refractory B-ALL. Patients with relapsed B-ALL have bleak remission rates of approximately 30% following salvage chemotherapy [30, 31]. In a Phase 1 trial of 16 patients, Davila et al [28] reported an 88% overall complete response rate to CD19-targeted CAR T cell immunotherapy. Most of the patients (88%) in the cohort were refractory to their most recent previous therapy and 56% of the patients had morphologic residual disease at the time of CAR T cell infusion [27, 28]. Of the nine patients with morphological residual disease, 78% had a CR after treatment with CD19-targeted CAR T cells. Furthermore, 75% of all patients achieved a molecular remission (CRm) as assessed by qPCR and/or deep sequencing for the clonal IgH rearrangements associated with the malignant B-ALL cells, which suggests these high-quality remissions may be durable [28].

The patients were enrolled, apheresed, and treated with physician's choice salvage chemotherapy while their autologous T cells were transduced with the 19-28z CAR. Regardless of the salvage chemotherapy that was given, patients received 1.5-3.0 g/m² cyclophosphamide prior to infusion with 3.0×10⁶ CAR T cells/kg (in 15/16 patients) [28]. After infusion, patients were monitored in the hospital; the persistence of the CAR T cells and the fate of the B-ALL were monitored via qPCR and/or deep sequencing of peripheral blood and/or BM. In this way it was possible to watch the expansion (50 to 1000 fold in the blood) and persistence (up to 2 months in the BM) of CAR T cells and the reduction of tumor cells after CAR T cell infusion [28].

Grupp et al [29] reported the results of two children with relapsed/refractory B-ALL treated with CD19-targeted CAR T cells containing the CD137 (4-1BB) signaling domain. Patient 1 was a young girl with a second recurrence of B-ALL that did not respond to intensive

chemotherapy given six weeks prior to CAR T cell infusion. She received 1.2×10^7 CAR T cells/kg and one month later a CRm was confirmed by deep sequencing. At the time of publication this patient had maintained a CRm for nine months [29]. Patient 2 was in a second relapse after an unrelated umbilical cord transplant complicated by graft vs. host disease (GVHD). She was treated with blinatumomab, did not respond, and subsequently received 1.4×10^6 CAR T cells/kg in a single dose following etoposide and cyclophosphamide. At one-month post infusion Patient 2 achieved a CR but relapsed two months later with CD19-negative tumor cells [29]. The refractory cells were derived from the initial dominant CD19+ B-ALL tumor cells by virtue of their sharing identical clonal IgH rearrangements [29]. Retrospective analysis showed the presence of a CD19-negative clone in the blood as early as 23 days after CAR T cell infusion, suggesting that all the targeted CD19+ disease was eradicated leaving the CD19-negative cells free to expand. It is possible that the CD19-negative B-ALL tumor cells were selected by treatment with blinatumomab, which has been shown to result in CD19-negative B-ALL tumor cells [32]. Recently, the UPenn group has published a larger cohort (n=30) of their experience treating B-ALL patients (pediatric and adult) with CD19-targeted CAR T cells [33]. In addition, the NCI group also recently reported results from their Phase I intention to treat study of CD19-targeted CAR T cells for pediatric patients (n=21) with relapsed B-ALL [34]. These larger reports have confirmed the clinical outcomes we first described with CR rates up to 90% in B-ALL patients with chemotherapy-refractory disease.

While all these trials [27-29, 33, 34] had remarkable successes in patients with dismal prognoses and few treatment options, they also contended with the major associated toxicity of CAR T cell therapy, CRS (Figure 2). Cases spanned from mild with fevers, chills, fatigue, and headaches, all the way to life threatening with hypotension, tachycardia, pulmonary edema, altered mental status, and seizures [27-29]. The most severe cases required pressor support and mechanical ventilation. Generally these symptoms begin to manifest as early as two days after CAR T cell infusion and are associated with a concomitant rise in cytokines and CAR T cells [27-29]. Given the range and potential severity of the CRS observed following CAR T cell infusion, it is essential to accurately describe and define the disorder.

The diagnosis of CRS is complicated by other possible explanations for clinical deterioration post immunotherapy such as tumor lysis syndrome, sepsis, or hemophagocytic lymphohistiocytosis/macrophage-activation syndrome (HLH/MAS) [35]. The distinction between CRS, HLH/MAS and tumor lysis syndrome appears to hold more weight in certain patients than others. Patient 1 described in the UPenn trial displayed symptoms of CRS starting on day four, with high fevers followed by hypotension and rapid respiratory and cardiovascular deterioration requiring mechanical ventilation and pressors [29]. Cytokine analysis showed elevations 75 fold for IFN γ , IL-6, IL-2, and IL-1B, and smaller increases of IL-1 α , IL-2 γ , and TNF α . This patient had hypofibrinogenemia, hepatosplenomegaly, elevated ferritin and aminotransferases, coagulopathy and elevated d-dimers, all suggestive of HLH/MAS [29]. Furthermore, abnormalities in uric acid values, such as those seen in tumor lysis syndrome, were absent. In contrast, the second patient reported by the group at UPenn developed a reaction characterized by fever, elevated d-dimers, aminotransferase, ferritin, and confusion but no other major clinical complications requiring glucocorticoids or anti-cytokine therapy; she also did not show any evidence of GVHD even though the cells

collected at apheresis were of donor origin [29]. However, she did show abnormal uric acid values on days eight through ten, supporting a diagnosis of tumor lysis syndrome, as well as significant (above 75-fold) elevations in IFN γ , IL-6, and IL-10.

The MSKCC cohort shows no cytokine patterns consistent with HLH/MAS [28]. These patients had many varied elevations in the 39 cytokines examined, particularly in 7 cytokines highlighted in their report. Some of the cytokines would be expected to increase in large scale T cell activation, such as IL-2, IFN γ , and IL-10, whereas others stand out as less expected; for example, IL-1b is produced by macrophages, dendritic cells, endothelial cells, and hepatocytes and IL-12 by macrophages and dendritic cells [28, 36]. Fibrinogen, d-dimer, ferritin and aminotransferase levels were not reported for the MSKCC patients.

The data from UPenn and MSKCC illustrate that the toxicities observed with CAR T cell immunotherapy does not fall easily into any one category of an existing syndrome. Ultimately, this may be reflected by the heterogeneity of this highly personalized form of therapy. Therefore, Davila et al [28] performed a retrospective analysis of their clinical and laboratory data and provided a three-prong definition of clinically significant, severe CRS associated with CAR T cell activation (sCRS). This definition consists of 1) fevers for at least three consecutive days, 2) two cytokine maximum fold changes of at least 75-fold from baseline or one cytokine max fold change of at least 250-fold from baseline (out of a pre-selected group of 7 cytokines found to be commonly elevated in patients with CRS), and 3) at least one clinical sign of toxicity such as hypotension (requiring at least one intravenous vasoactive pressor), hypoxia (PO₂ < 90%), or neurologic disorders (including mental status changes, obtundation, and seizures) [28]. In addition, others have proposed a similar CRS classification and management scheme [37].

Patients fulfilling these criteria required intensive medical interventions and remained in the hospital an average of 41.6 days longer than patients who did not have CRS or had only a mild variation of CRS [28]. The severity of CRS and cytokine elevation correlates significantly with tumor burden at the time of CAR T cell infusion. In the cohort described from MSKCC, patients without morphologic residual disease showed minor or undetectable cytokine elevations, whereas all the patients who developed sCRS had morphological residual leukemia [27, 28]. Distinguishing the severity of CRS and determining when to treat the toxicities is essential to avoid premature, unnecessary intervention that could limit the efficacy of CAR T cells. The first three patients who displayed the symptoms of sCRS at MSKCC were treated with high dose steroids to reduce the toxicities [27, 28]. While this goal was achieved, a fivefold decrease in CAR T cells could be seen in the BM of these patients compared with later patients who did not receive steroids [27, 28]. Furthermore, all three of the patients treated with steroids relapsed. In contrast, in patients treated with the IL-6 receptor (IL-6R) blocking antibody tocilizumab, the sCRS was ameliorated but T cell expansion was not affected. In fact, Davila et al [28] reported expansion and persistence of T cells (up to 20-fold expansion in one patient) after tocilizumab.

The use of tocilizumab is intriguing given that not all patients suffering from sCRS demonstrate notably high levels of IL-6, but they still respond to this intervention [27, 28]. The IL-6R is expressed on the cell surface of a few cell types, mainly hepatocytes and

subsets of leukocytes [38]. IL-6 binding to the IL-6R leads to anti-inflammatory signals and is referred to as classical signaling [38]. However, the IL-6R can also be secreted as a soluble factor (sIL-6R) [39]. When the sIL-6R soluble receptor binds to IL-6 it forms an agonist (sIL-6R/IL-6) that can bind gp130, a cell surface receptor present on nearly all cells, and mediate pro-inflammatory signals [39]. Therefore, processes that initiate IL-6R secretion and/or IL-6 production may mediate widespread pathogenic inflammation. This process has been identified at the center of various rheumatologic diseases and can be successfully treated with tocilizumab [38]. Interestingly, C-reactive protein (CRP) can cause an increase in the release of membrane-bound IL-6R from neutrophils at high concentrations [40]. The correlation between CRP, IL-6R, and inflammation suggests a common pathogenic inflammatory mechanism behind CRS. This may support monitoring of common inflammatory markers as an indicator of the CRS, which is what the MSKCC group demonstrated.

An important clinical obstacle is determining when patients need intensive monitoring because of impending clinical toxicities. Patients with morphologic residual leukemia are at the greatest risk of clinical toxicities from sCRS, but their respective clinical courses are heterogeneous enough to preclude identifying when patients will begin developing their toxicities. Monitoring cytokine levels would allow a definitive determination of the initiation and maximal periods of sCRS, but the MSKCC group sought to identify a substitute laboratory test that can be followed rapidly and daily. Davila et al [28] illustrated a correlation between CRP and severity of CRS, with the sickest patients reaching CRP elevations of 40-50 µg/mL. There was a significant difference in average CRP in patients with or without sCRS, and the ROC curve analysis supports the assertion that maximum CRP is a significant indicator for sCRS [28]. Therefore, the MSKCC group determined that once CRP levels reach 20 µg/mL, patients should begin an intensive monitoring program because they are at risk for impending clinical toxicities.

At this time there is insufficient data to conclusively attribute differences in CRS to differences in the CAR used. Murine studies suggest differences in the kinetics of the 1928z and 4-1BB CARs, with 1928z CAR T cells expanding more rapidly to antigen but persisting for less time after the stimulus has disappeared [14, 16]. The 4-1BB CARs are associated with long-term persistence, a more antigen-independent signaling mechanism, and extended B cell aplasias, potentially because of a higher accumulation over time despite the slower kinetics [19, 20, 29]. The CAR T cells described by Grupp et al [29] were observed in the blood, BM, and CSF of Patient 1 up to 180 days post infusion. Both of these conditions have benefits, with the 1928z CAR enabling normal B cell recovery as well as preservation and restoration of normal hematopoiesis, while the 4-1BB CAR provides potentially longer lasting anti-tumor action. It is unclear at this point whether the limited persistence seen with the 19-28z CAR is sufficient for durable remissions, but considering the rapidity of CRm achieved, the limited half-life might not limit long-term beneficial clinical outcomes [27, 28]. Persistence of the 4-1BB containing CAR T cells should provide durable remissions, but it is unknown if the resultant long-term B cell aplasias could lead to morbidities and/or mortalities from infectious complications.

The use of CAR T cell therapy as a bridge to allogeneic stem cell transplant (allo-SCT) represents a major clinical advance for this patient population. Using current standard treatments, only 5% of adults with relapsed or refractory B-ALL transition to allo-SCT after salvage chemotherapy [41]. Furthermore, patients who transition to allo-SCT with minimal residual disease have a significantly worse prognosis than those who proceed without any detectable disease [42, 43]. Therefore, CAR T cell therapy can have an immediate impact on the clinical outcomes of this very poor-risk adult patient population by increasing the number of patients who can receive an allo-SCT and by increasing the duration of remissions after allo-SCT due to the high-quality remissions at the time of transplant. The conditioning chemotherapy and/or radiotherapy associated with an allo-SCT will eradicate residual CAR T cells. However, considering that the lifespan of infused CAR T cells in many patients is less than 3 months, then withholding allo-SCT until after B cell recovery, an indirect measure of loss of CAR T cell function, will ensure patients get the full benefit of CAR T cell-mediated killing of malignant B cells [27, 28, 33, 34]. Of the patients treated at MSKCC, 70% of eligible patients received an allo-SCT after CAR T cell therapy, and there have been no relapses reported to date (follow up ranging from 2 – 24 months), which is supportive of the potential of adoptive CAR T cell therapy as a bridge to allo-SCT, improving the clinical outcomes of this disease for patients with few to no treatment options [27, 28].

4. Conclusions

CD19-targeted CAR T cells were initially evaluated in patients with indolent B cell leukemia's due to their large incidence and incurability, as well as the slow nature of the disease, which would not complicate the collection, genetic-modification, and infusion of autologous T cells back into patients (Table 1). Early results from two groups were striking with strong evidence of CAR T cell eradication of the B cell malignancy and the predicted on-target toxicity of B cell aplasia [15, 17-20]. However, as more patients have been treated, it is clear that the overall response rate and CR rates for CLL are not as high as once hoped and are not comparable to recently FDA approved therapies [22, 25, 26]. These early studies, however, showed the safety and promise of CD19-targeted CAR T cell therapy and paved the way for their evaluation in other B cell malignancies. Targeting patients with relapsed/refractory B-ALL was a much more dubious prospect considering that these patients have a very short predicted median overall survival (OS), are treated with multi-agent, high-doses of chemotherapy, and require such frequent therapy that collecting autologous T cells, genetically-modifying and infusing them back into patients leads to a small therapeutic window [30]. However, B-ALL is exquisitely sensitive to CD19-targeted CAR T cells with CR rates reaching 90% in clinical trials from three independent groups, where a 30% CR rate would be expected with chemotherapy alone [27-29, 33, 34]. Furthermore, sensitive analyses have revealed that most of these remissions are high-quality CRm, which suggests that these patients will have more durable remissions than expected with salvage chemotherapy. The results, however, do suggest some obstacles for the application of CD19-targeted CAR T cells for patients with B-ALL. The CRS with its associated clinical toxicities requires intensive medical management and is likely the most significant clinical obstacle. While longer follow up will allow us to make more concrete

conclusions, the impressive results to date strongly suggest that CD19-specific CARs should be further evaluated for clinical efficacy.

5. Expert Opinion

5.1 B-ALL vs. CLL

One of the important conclusions from the last five years of clinical trials using CAR T cells to target CD19 in B cell malignancies is that B-ALL is much more responsive than CLL. One possibility could be related to the autologous T cells. Patients with CLL are often treated with T cell suppressive therapies such as fludarabine and alemtuzumab, so it may be that the autologous T cells, while functional in vitro and in immunodeficient animal models, are not functional, or are dysfunctional, in patients [45]. Alternatively, it may be the nature of the disease itself that renders the T cells dysfunctional. Evidence supporting that T cell function may be responsible for decreased efficacy comes from comparisons of CLL and B-ALL patients treated on clinical trials; the CAR T cells produced from CLL patients are mostly CD4+ (helper), while the CAR T cells produced from B-ALL patients are mostly CD8+ (cytotoxic) [17, 18, 20, 23, 27-29, 33, 34]. Another T cell property that may explain the different outcomes is that CAR T cells may not traffic to lymph nodes, although this is not suggested by lymph node biopsies from an anecdotal case report [24]; CAR T cells readily traffic to the BM, usually the sole location of disease for B-ALL [46]. In the future, trials targeting CLL should include more lymph node biopsies to evaluate this possibility. Another possibility could be that the pure bulk of disease in the blood, BM, and lymph nodes may be suppressive to CAR T cell adoptive therapy. This possibility has some pre-clinical [47-49] and clinical [23] support from studies demonstrating the importance of lymphodepletion or tumor debulking before CAR T cell infusion. However, it may be more definitively answered by a clinical trial from the MSKCC group, which is evaluating CD19-targeted CAR T cells in CLL patients with minimal residual disease after chemotherapy ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01416974) identifier NCT01416974). In addition, T cells from patients with CLL may be more prone to “exhaustion” thereby limiting their anti-leukemia efficacy, which can potentially be reversed by lenalidomide [50]. At the least, it appears that CLL would be an ideal target for the evaluation of CD19-targeted third generation or armored CARs to determine if additional CAR functions may enhance anti-CLL efficacy [51].

5.2 Toxicities

The success of this therapy has led to the identification of a new set of toxicities to be managed by the medical oncologist. First of all, B cell aplasias were a predicted complication of CD19-targeted CAR T cell therapies and were considered to be an acceptable trade-off for patients with life-threatening B cell malignancies. B cell aplasias have been prolonged in patients treated by the UPenn group [19, 20, 29], which suggests that the CD137 (4-1BB) co-stimulatory domain may enhance persistence of CD19-targeted CARs compared to the CD28 co-stimulatory domain. However, the expansion and contraction of 19-28z CAR T cells in response to abundant and lack of target antigen, respectively, suggests it may represent a physiologic immune response [27, 28]. The number of patients with prolonged B cell aplasias is still small, and their cumulative duration of

aplasia is small, but treatment with infusional gamma globulin and antibiotics appears to be sufficient for managing this complication, at least in the near-term.

A more worrisome toxicity is CRS, which can result in fevers, hypotension, and neurologic changes and has occurred in trials at all major CAR T cell centers [28, 33, 34]. These toxicities can be severe and warrant intensive medical management and interventions including intubation for airway protection due to decreased mental status, infusional pressor support, aggressive fluid support, and anti-epileptics. While these toxicities have been severe they have been self-limiting and patients recover without persistent neurologic deficits. Neuroimaging studies by all groups could not document any anatomical cause for the neurologic abnormalities. However, all groups have documented presence of CAR T cells in the cerebrospinal fluid of patients with CRS [28, 33, 34]. Therefore, this suggests that CAR T cells may be mediating, directly or indirectly, the neurologic toxicities. What is unknown and needs to be evaluated further is if CAR T cells are migrating to the central nervous system in response to antigen, which could be in the form of residual B-ALL or possibly aberrant CD19 expression in normal neural tissue [52].

It is likely that the T cells are at the center of CRS considering similar toxicities are seen in patients treated with an anti-CD28 antibody or blinatumomab, a CD19/CD3 bi-specific T cell engaging antibody [53, 54]. Initial attempts at managing the CRS have included steroids and tocilizumab, an IL-6R blocking antibody [38]. Steroids have been very effective at ameliorating the clinical toxicities of the CRS, but at the cost of anti-leukemia efficacy, since all the patients treated with steroids have relapsed [19, 28]. In contrast, patients treated with tocilizumab recovered from their CRS but with more durable remissions [28]. While we must wait for longer follow up to determine if there are any relapses after tocilizumab, it is advisable to use tocilizumab as a front-line agent for CRS management and reserve steroids for second-line. Uniformity of CRS management will be critical when adopting CAR T cell therapy to other centers to ensure optimal supportive care is achieved; toward this end CRS management algorithms have been proposed [28, 37].

5.3 A new standard of care for relapsed/refractory B-ALL

It is too soon to claim clinical efficacy, but considering the poor outcomes of patients with relapsed/refractory B-ALL and the clinical indications for the most recently approved therapies for relapsed/refractory B-ALL [55-57], there is a strong possibility that the first clinical indication of a gene-modified cell therapy will be CD19-targeted CAR T cells. Therefore, the most critical issue facing the further development of CD19 CAR T cells is the design of the Phase II trials evaluating their clinical efficacy. The first issue within clinical trial design is determination of a proper endpoint. The three most recently approved clinical therapies for relapsed/refractory B-ALL were evaluated in clinical trials with CR as the primary endpoint [55-57]. CR is a suitable endpoint since re-induction of a CR is the main obstacle to providing patients with the only potential curative treatment, an allo-SCT [30]. It is likely that by improving the CR rate there will also be an improvement in the OS of these patients. OS is another potential suitable endpoint, since it would account for mortality in patients waiting for T cells, or from the CRS or prolonged B cell aplasia, or from complications related to allo-SCT.

These trials must be multi-center to ensure that collection of autologous T cells, production of CAR T cells, and management of CRS can be adapted across multiple sites [58]. However, the small number of relapsed B-ALL patients makes randomized Phase II trials or large Phase III trials unrealistic. In fact, the three most recently FDA approved therapies for relapsed B-ALL were single-arm Phase II trials [55-57]. Furthermore, the FDA granted blinatumomab breakthrough therapy status and orphan product designation because B-ALL is such a rare disease. Therefore, comparing the CR rates of a single-arm Phase II trial of B-ALL patients receiving the same CAR T cell dose to historical, observed CR rates in patients treated with standard salvage therapies is sufficient for evaluation of clinical efficacy. In addition, it is a valid concern that CR rates and/or mortality could be affected by the unique nature of this personalized therapy, so all trials for clinical efficacy should include intent to treat analyses to ensure that patients are not dying because they do not have an adequate number of T cells collected or the required dose is not achieved, or while waiting for their T cells to be produced.

The trials should also include post-CAR T cell therapy directions. B-ALL patients are often treated intermittently for years with maintenance therapy, intrathecal prophylaxis, and/or tyrosine kinase inhibitors [59]. At the least, considering the high historical relapse rate in patients with relapsed/refractory B-ALL and the short follow-up available for patients already treated with CD19-targeted CAR T cells, all patients with a suitable stem cell donor and reasonable mortality risks should be offered an allo-SCT as a curative treatment option. It is true that relapses still occur after allo-SCT, but the high CRm rate associated with CD19-targeted CAR T cells may decrease this relapse rate.

5.4 Future Directions

Despite the exciting results from CD19-targeted CAR T cells as a therapy for B-ALL, there is much work that remains to advance CAR technology. The excitement from the dramatic results with CD19 CAR T cells is tempered by the CRS toxicities. There is a need for animal models [60] and more correlative data from clinical trials to understand the mechanism behind this syndrome and define the immune cells and cytokines that contribute to its pathology. This knowledge will allow the development of targeted, supportive therapies that will decrease CRS toxicities without impacting anti-leukemia efficacy.

The most critical advance in CAR design the past two decades was the inclusion of a co-stimulatory domain in addition to the CD3 ζ domain, which led to CAR T cells that were effective and persistent in vivo. Subsequent pre-clinical studies have resulted in various modifications in CAR design with putative advances, but most have not been validated in humans [47, 61-63]. The most extensive results we have in comparing CAR designs come from the three different B-ALL trials [28, 29, 44], which have similar outstanding outcomes despite using two different anti-CD19 scFv's (FMC63 vs SJ25C1) and two different co-stimulatory domains (CD28 vs 4-1BB). These results lead to the opinion that, at least for B-ALL, the various second-generation CD19-targeted CARs have equivalent anti-leukemia efficacy.

As we treat other cancers we will determine if CAR T cells represent a bona fide cancer therapy or if it is a therapy for a singular disease. Targeting solid tumors with their highly

immunosuppressive tumor microenvironment will represent a significant challenge. However, the advent of third-generation and/or armored CARs may allow the adoptively transferred T cells to overcome tumor-mediated immunosuppression so certain co-stimulatory domains and/or other CAR design elements may be necessary to treat other cancers. Targeting cancers other than B cell malignancies also has safety issues that need to be addressed early in CAR development. CD19, which is expressed only on B cells, was deemed safe without significant concerns for off-target toxicities. However, other antigens, especially those where we have limited knowledge of their tissue expression, can be dangerous, such as when ERBB2-specific CAR T cells were infused into a patient with colorectal cancer [64]. Engagement of CAR T cells with respiratory epithelium, which expresses low levels of ERBB2, likely led to respiratory failure shortly after infusion. There is a dire need to develop new CARs that are both effective and safe, which will require some manner of extensive screening before these CARs are brought to clinical trials. If this should be done with screenings in silico, in vivo, in vitro, or some combination thereof remains to be seen. These future directions will lead to important advances in the use of CAR T cells as a cancer therapy and build on the promise first noted when targeting B-ALL with CD19-targeted CAR T cells.

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Article Highlights

- Dramatic results have been reported with targeting B-ALL with CD19-targeted CAR T cells. The CR and CRm rates (reaching 90%) are much higher than expected with salvage chemotherapy.
- The cytokine release syndrome (CRS) is a set of clinical toxicities including fevers, hypotension, and neurologic changes associated with en masse activation of CAR T cells by their target antigen. The severe illnesses associated with this syndrome represent the main clinical limitation to adapting CAR T cells to a larger population of patients.
- Toxicity management of the CRS should involve tocilizumab as a first-line agent and steroids as a second-line agent. Both of these therapies are effective at ameliorating the CRS, but steroids are also lymphotoxic and lead to relapses.
- Phase II trials to determine the clinical efficacies of CD19-targeted CAR T cells are being designed. These trials should be multi-center, include intention to treat analyses, and endpoints of CR, CRm, or OS.
- CLL has not been nearly as susceptible to CD19-targeted CAR T cells as B-ALL. It is likely that third-generation or armored CAR T cells will be required to enhance efficacy against CLL.

Figure 1. Evolution of CAR T Cell design

The immunoglobulin heavy and light chains from a monoclonal antibody (Mab) are fused to a glycine-serine linker to create a scFv, which is the antigen-binding component of the CAR. First generation CARs are fused only to CD3 ζ signal domains, which are normally associated with the T cell receptor. Further addition of CD28, 4-1BB, or OX40 cytosolic domains provide co-stimulation (signal 2) making 2nd or 3rd generation CARs.

Figure 2. Administration and toxicities associated with CAR T cell therapy

Patients with leukemia are infused with T cells that have been engineered to express MHC-independent CARs specific for CD19. Engagement of CAR T cells with circulating leukemia cells leads to the production of cytokines such as IL-2, IL-6, IL-10, and IFN γ and recruitment of other immune cells such as macrophages and NK cells. The elevation of inflammatory markers and cytokines can lead to clinical toxicities such as hypotension, hypoxia, fevers and neurologic disorders such as seizures. Steroids ameliorate the clinical toxicities but also kill CAR T cells so they limit clinical efficacy. In contrast, Tocilizumab, an IL-6R blocking antibody, can successfully manage CRS without compromising clinical efficacy. Etanercept, a TNF inhibitor, may also prove beneficial in the treatment of CRS.

Table 1

Active and inactive clinical trials using CAR T cells to target CLL/NHL

Clinical trial identifier	Phase	Active	CAR: Target/Signal domains	Gene transfer	Disease	Conditioning therapy	Trial Site
NCT00881920	1	Y	Ig kappa/CD28	γ-retrovirus	Relapsed/refractory NHL, MM, CLL	Cyclophosphamide	BCM
NCT00586391	1	Y	19/28z	γ-retrovirus	Relapsed/refractory NHL, ALL, CLL	Ipilimumab post T cells	BCM
NCT00709033	1	Y (not recruiting)	19/28z (virus-specific T cells)	γ-retrovirus	Relapsed/refractory NHL, CLL	Cyclophosphamide	BCM
NCT01853631	1	Y	19/28z and/or 19/CD28BBz	γ-retrovirus	B cell NHL	Cyclophosphamide	BCM
NCT01653717	1	Y	19/28z	Electroporation with Sleeping Beauty	B-CLL (8 weeks from last chemo)	Cyclophosphamide + fludarabine	MDACC
NCT01416974	1	Y	19/28z	γ-retrovirus	B-CLL	Cyclophosphamide	MSKCC
NCT00840853	1/2	Y	19/28z (virus-specific T cells)	γ-retrovirus	Relapsed/refractory NHL, ALL, and CLL	Post allo-SCT	BCM
NCT01865617	1/2	Y	19/BBz	lentivirus	Relapsed/refractory CLL, NHL, ALL		FHCRC
NCT01475058	1/2	Y (not recruiting)	19/ (virus-specific T cells)		In CR (high-risk) or relapse post allo-SCT Ph- ALL, CLL, DLBCL, mantle cell		FHCRC
NCT00466531	1/2	Y	19/28z	γ-retrovirus	Relapsed/refractory CLL or NHL	Cyclophosphamide	MSKCC
NCT00924326	1/2	Y	19/28z	γ-retrovirus	Progressive or stable CD19+ B Cell malignancies	Cyclophosphamide + fludarabine	NCI
NCT01864889		Y	19/BBz	γ-retrovirus	Relapsed/refractory CD19+ B Cell malignancies		CPLAGH
NCT01735604		Y	20/BBz	γ-retrovirus	CD20+ B cell malignancies		CPLAGH

BCM: Baylor College of Medicine

MDACC: MD Anderson Cancer Center

MSKCC: Memorial Sloan Kettering Cancer Center

FHCRC: Fred Hutchinson Cancer Research Center

NCI: National Cancer Institute

CPLAGH: Chinese PLA General Hospital

Table 2

Current Active and inactive clinical trials using CD19 antigen for B-ALL

Clinical trial identifier	Phase	Active?	CAR: Target/Signal domains	Gene transfer	Disease	Conditioning therapy	Trial Site
NCT02050347	1	Y	19/28z	γ -retrovirus	Relapsed/refractory NHL, ALL, CLL		BCM
NCT00586391	1	Y	19/28z	γ -retrovirus	Relapsed/refractory NHL, ALL, CLL	Ipilimumab post T Cells	BCM
NCT01626495	1	Y	19/BBz	lentivirus	Relapsed/refractory Pediatric B-ALL		UPenn
NCT01430390	1	Y	19/28z (virus-specific T cells)	γ -retrovirus	Refractory post-Allo-SCT Pediatric B-ALL	Physician's choice	MSKCC
NCT01860937	1	Y	19/28z	γ -retrovirus	Relapsed/refractory Pediatric/Young adult B-ALL	Cyclophosphamide	MSKCC
NCT01044069	1	Y	19/28z or 19/BBz	γ -retrovirus	Relapsed/refractory B-ALL	Cyclophosphamide	MSKCC
NCT01087294	1	Y	19/	γ -retrovirus	Relapsed/refractory post allo-SCT CD19+ B Cell malignancies	Pentostatin + Cyclophosphamide	NCI
NCT01593696	1	Y	19/	γ -retrovirus	Relapsed/refractory Pediatric/young adult B-ALL or lymphoma	Physician's choice	NCI
NCT01683279	1	Y	19/	lentivirus	Relapsed/refractory Pediatric B-ALL	Cyclophosphamide	FHCRC
NCT00840853	1/2	Y	19/ (virus-specific T cells)	γ -retrovirus	Relapsed/refractory NHL, ALL, CLL	Post allo-SCT	BCM
NCT01865617	1/2	Y	19/BBz	lentivirus	Relapsed/refractory CLL, NHL, ALL		FHCRC
NCT01475058	1/2	Y (not recruiting)	19/ (virus-specific T cells)		In CR but high-risk or Relapsed post allo-SCT: Ph-ALL, CLL, DLBCL, mantle cell		FHCRC
NCT00924326	1/2	Y	19/28z	γ -retrovirus	Relapsed/refractory CD19+ B Cell malignancies	Fludarabine + Cyclophosphamide	NCI
NCT02028455	1/2	Y	19/BBz	lentivirus	Relapsed/refractory after allo-SCT Pediatric/young adult CD19+ B-ALL	Physician's choice	FHCRC
NCT02030847	2	Y	19/BBz	lentivirus	Relapsed/refractory B-ALL		UPenn
NCT01864889		Y	19/BBz	γ -retrovirus	Relapsed/refractory All CD19+ malignancies		CPLAGH
NCT01029366		N	19/BBz	lentivirus	Relapsed/refractory CD19+ malignancies	Physician's choice	UPenn