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Chimeric Antigen Receptor Modified T Cells for B Cell Malignancies: An Advance in Cellular Therapy

Jie Wang, Michael Klichinsky and Stephen J Schuster*

Department of General Oncology, University of Pennsylvania, USA

Abstract

CD19 directed Chimeric Antigen Receptor (CAR) modified T cells have recently shown dramatic results in the treatment of relapsed and refractory B cell malignancies. CARs endow autologous T cells with antibody-like specificity and are capable of redirecting them to target tumor antigens and kill tumor cells. Investigational CD19 directed CARs have gained breakthrough therapy designation from the FDA and may represent the beginning of a paradigm shift in the field of cellular therapies. By redirecting the patient's own T cells to tumor cells, CAR modified T cells harness the power of cellular immunity resulting in prolonged remissions in many patients with refractory disease, while avoiding the risks of allogeneic stem cell transplantation. In this review, we address CAR T cell design and function, discuss the clinical experience in CD19 directed CAR T cell therapy, and summarize the efficacy of CD19 CAR T cells for the treatment of different B cell malignancies. CAR T cell therapy addresses several areas of unmet clinical need in the treatment of B cell malignancies in the current era, and represents an important advance in the field of cellular therapies.

Introduction

Addition of the chimeric anti-CD20 monoclonal antibody, rituximab, to chemotherapy regimens revolutionized the treatment of B cell malignancies, significantly improving progression-free survival and, in some B cell malignancies, overall survival. Thus, chemoimmunotherapy has become the mainstay of therapy for this group of diseases. However, despite the impact of rituximab, several areas of unmet clinical need remain. Patients with Diffuse Large B Cell Lymphoma (DLBCL) who relapse less than 1 year after combination rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) therapy and those who have *MYC* gene rearrangements have particularly poor outcomes despite standard salvage chemotherapy and autologous stem cell transplant (auto HSCT) [1,2]. Patients with indolent B cell lymphomas who have clinically significant disease that is refractory to rituximab and subsequent lines of therapy, as well as follicular lymphoma patients who have early progression of disease after initial therapy R-CHOP, also need alternative treatment strategies [3]. Patients with Chronic Lymphocytic Leukemia (CLL) with high risk cytogenetic features have an inferior prognosis with standard rituximab, fludarabine, cyclophosphamide chemoimmunotherapy [4]. Those CLL patients who have progressive disease on kinase inhibitors or who are intolerant of these drugs also require therapeutic alternatives [5].

In the 1970s, allogeneic hematopoietic stem cell transplantation (allo HSCT) was demonstrated to be capable of maintaining complete remissions after salvage chemotherapy in patients with relapsed and, in some cases, refractory hematologic malignancies [6,7]. The mechanism behind allo HSCT is the transfer of T cell-mediated cellular immunity from the stem cell donor to the recipient. In the rituximab era, allo HSCT is still an option that can result in long term survival in relapsed B cell malignancies; however, the need to find a suitable donor, the occurrence of graft versus host disease (GVHD), and the high mortality rate in the initial two years following transplantation, limit allo HSCT as a feasible therapeutic option for many patients [8,9]. Given these drawbacks of allo HSCT, there has been unrelenting interest in devising ways to redirect a patient's own immune cells to target his/her malignancy.

Chimeric Antigen Receptor (CAR) modified T cell therapy is a novel form of cellular therapy in which a patient's own T cells are engineered to target their malignancy. The unique design of CAR modified T cells combines antibody-like antigen specificity and high affinity binding with T cell effector function. By using autologous T cells, GVHD is completely avoided. CD19 directed CAR T cells have already achieved remarkable success in the treatment of B cell Acute Lymphoblastic Leukemia (ALL) and other B cell malignancies. We believe that this therapeutic approach is a "game changer" for patients with relapsed and refractory B cell malignancies, and may replace the role

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Correspondence:
Jie Wang, Department of General Oncology, University of Pennsylvania, USA,
E-mail: Jie.Wang@uphs.upenn.edu

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for allo HSCT and, for some patients, auto HSCT in this group of diseases.

In this review, we summarize the structure and function of CAR T cells, describe the efficacy of this new therapeutic approach for CAR T cells directed against CD19 in B cell malignancies, discuss adverse reactions and appropriate management, and examine the potential future role for this breakthrough therapy.

CAR Structure and Function

A Chimeric Antigen Receptor (CAR) is a synthetic protein constructed from elements derived from 3 or more distinct human proteins - a single chain variable fragment (scFv) derived from a monoclonal antibody, a hinge and transmembrane domain, and intracellular signaling domain(s). This "chimera", when expressed in T cells, endows them with antibody-like specificity and affinity for a given antigen via the scFv.

The extracellular fragment of a CAR is composed of a scFv tethered to the transmembrane domain by a "hinge". The scFv comprises the variable heavy (V_H)and light (V_L) chains of the parent monoclonal antibody connected to each other by a short peptide "linker." The "hinge" is derived from the extracellular domain of CD8α, IgG1, IgG4, or CD28. It allows for scFv extension from the cell surface, providing structural flexibility to facilitate antigen binding. As the specific distance permitting productive antigen engagement is unique to each CAR and its target, the optimal hinge domain must be determined empirically [10,11]. The intracellular portion of a CAR is composed of a signal transduction domain, which activates T cell effector function upon antigen binding. CD3ζ is often chosen because it is sufficient for T cell activation even in the absence of the CD3 γ, δ, and ε chains normally present in the T cell receptor (TCR) signaling complex [12].

Analogous to activation via conventional TCRs, CAR antigen binding leads to immune synapse formation between T cell and target cell, resulting in phosphorylation of tyrosine residues of the immunoreceptor tyrosine activating motif (ITAM) of CD3ζ. This initiates a polarized cell-signaling cascade that results in antigen-dependent T cell activation, target cell killing, and CAR T cell proliferation.CAR T cells lyse the engaged target cell by releasing the contents of preformed cytotoxic granules, such as perforin and granzyme B [13]. Antigen activated CAR T cells also secrete cytokines that promote their own function and proliferation. These include IL-2, IFNγ, TNF, GM-CSF, IL-12, IL-4, IL-6, IL-10, and MIP1α. Thus, in a given patient, CAR T cell activation and proliferation are commensurate with tumor burden. Initially, there is rampant CAR T cell proliferation and target cell cytolysis, but as tumor cells die, the antigenic load decreases and cytokine levels fall, leading to contraction of the CAR T cell population [14].

Unlike conventional TCRs, CARs recognize antigen directly and independently of the major histo compatibility complex (MHC). MHC independence is a critical factor in the success of CAR T cell therapy. First, aberrant MHC expression is common in neoplasms including B cell malignancies [15,16]. Under selective pressure, tumor cells may down-regulate MHC expression, or undergo immune editing, leading to loss of MHC-peptide complexes [17]. The efficacy of CAR T cell therapy is unaffected by these changes. Second, the MHC-independence of CARs allows one CAR construct to be used across all HLA types. Third, CARs can be designed to recognize a variety of potential targets, including cell surface proteins, as well as carbohydrates, glycoproteins, and lipids, which are not presented in the context of MHC [18].

Choice of Antigenic Target for Lymphoma

Selecting an appropriate CAR target for a given disease is fundamental to clinical outcome. An ideal antigenic target should be uniquely and uniformly expressed on the target tissue. In the context of B cell malignancies, potential CAR targets include the B cell surface antigens CD19, CD20, CD22, CD37, and CD79. B cell antigens are a suitable choice because long-term B cell aplasia does not cause clinically unacceptable immune suppression. Initial preclinical studies clearly established the cytolytic activity of CAR T cells directed against B cell surface antigens upon co-culture with B cell lymphoma cells in vitro [19-21] and established their feasibility for use in vivo [22].

CD19-directed CAR T cells have had the most success in clinical trials for relapsed and refractory B cell malignancies. There are many versions of CD19-directed CAR T products in clinical trials today [23]. CD19 is an ideal target because it is constitutively expressed on all pre-B lymphocytes through their differentiation into terminal effector cells as well as their malignant counterparts, but is not expressed by other bone marrow cells or other non hematopoietic tissue [24].

The ideal form of cellular therapy would not only target malignant cells with minimal collateral damage to normal tissues, but would also have prolonged persistence and curative potential without the need for repeat infusions. Unlike allogeneic stem cell transplantation where donor $CD34^+$ stem cells, together with more mature T cells in most cases, are transplanted into the recipient, CAR modified T cells are created from fully differentiated T cells. Thus, the question arises, can CAR T cells persist long term in vivo? They can and do, but require a co-stimulatory signal.

Co-simulation and Persistence

Early versions of CARs were composed of an scFv antigen recognition domain linked to a single T cell signaling domain [12]. Clinical trials of these "first generation CAR T cells" had disappointing efficacy due to limited persistence. In a proof of concept clinical trial of CD20-directed first generation CAR modified T cells in 7 patients with refractory B cell lymphomas, the infused T cells had a limited persistence of 5-21 days *in vivo*. There was only modest improvement with administration of adjuvant IL2 [25]. In another trial of first generation CD19- and CD20-directed CARs, the infused CAR T cells were not detectable beyond 1 week by quantitative PCR for detection of recombinant plasmid despite the administration of low dose IL-2 [26]. Poor CAR T cell persistence was not explained by the cell dose [26].

Since physiologic T cell responses require one or more co-stimulatory signals in addition to TCR signaling, it was hypothesized that delivery of an activation signal via the TCR alone in the absence of a co-stimulatory signal, was responsible for a poor CAR T cell proliferative response and induction of energy or apoptosis [27,28]. To test this hypothesis, CAR modified EBV-specific Cytotoxic T Lymphocytes (CTLs) that received optimal co-stimulation upon viral engagement of their native TCR were compared with CAR T cells without native virus specificity. Indeed, the virus specific CTLs had greater in vivo persistence and clinical efficacy [29,30]. Likewise, "second generation CAR T cells", engineered to possess

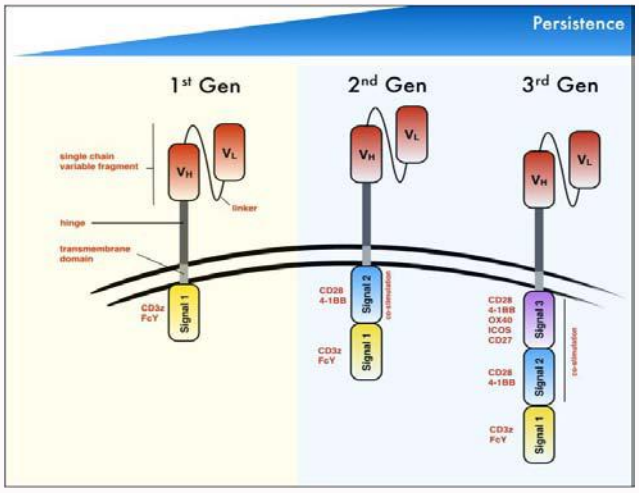

Figure 1: Schematic diagram of 1st, 2nd, and 3rd generation chimeric antigen receptors.

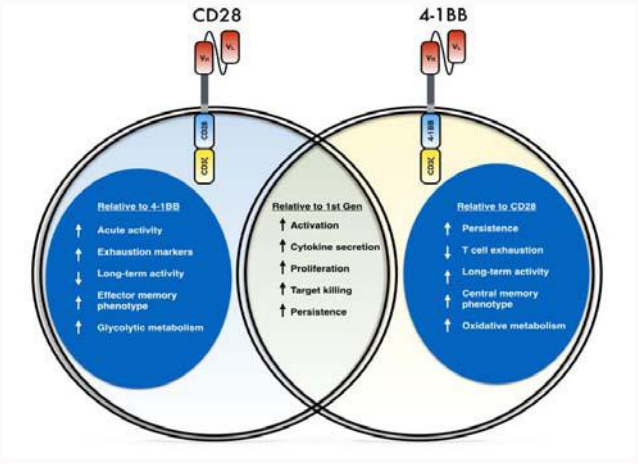

Figure 2: Venn diagram summarizing the functional differences between CD28 and 4-1BB co-stimulated CAR T cells. Both CD28 and 4-1BB co-stimulated CARs demonstrate enhanced functionality relative to 1st generation CD3ζ-only CAR T cells.

a single combined chimeric antigen receptor and co-stimulatory domain, have improved activity *in vivo* [31] and are capable of multiple sequential rounds of expansion and antigen specific target cell lysis [32] (Figure 1). Unlike CAR modified virus specific CTLs, co-stimulation in second generation CARs is dependent upon tumor antigen alone.

By simultaneously infusing six non-Hodgkin lymphoma (NHL) patients with both a first and second generation CD19-directed CAR T cell product, Savoldo et al. [33] convincingly show that having a co-stimulatory domain enhances CAR T cell survival and proliferation in human subjects. Third generation CARs that incorporate two distinct co-stimulatory domains (Figure 1) have not yet been shown to have superior function or persistence compared to second generation CARs.

A number of co-stimulatory domains have been studied and preclinical studies show that they may confer different levels of persistence and efficacy to CAR T cells [34,35]. The optimal co-stimulatory domain has not yet been determined, but the two most commonly used co-stimulatory domains in clinical trials are derived from CD28 and 4-1BB. CD28 is expressed on T cells and engages in native TCR co-stimulation by binding CD80 and CD86 on antigen presenting cells. It is considered to be the classic "second signal" of T cell activation. CD28 co-stimulation leads to intracellular signaling via the PI3K/AKT, PKCθ, LCK, and RAS pathways, ultimately resulting in greater T cell function through induction of enhanced cytokine secretion, T cell proliferation, cell cycle progression, and survival [36]. In contrast, 4-1BB (CD137) is a co-stimulatory receptor on T cells that is normally upregulated upon collaborative signaling by the TCR complex and CD28. Upon binding to its ligand 4-1BBL, 4-1BB enhances T cell function through a TRAF dependent signaling cascade [37,38].

Preclinical studies suggest that the 4-1BB co-stimulatory domain confers increased CAR T cell persistence and reduced susceptibility to PD-1 inhibition and T cell exhaustion compared to CD28 [39-41]. The underlying mechanisms for these differences relate to the ability of 4-1BB containing CARs to take on the phenotype of central memory T cells, and the propensity for CD28 containing CARs to induce immune checkpoint receptor upregulation, e.g. PD1, TIM3, and LAG3 [40,42]. In addition, Kawalekar et al. [40] recently demonstrated that the difference in persistence between CD28 and 4-1BB CAR T cells is due in part to metabolic reprogramming–CD28 enhances glycolytic metabolism while 4-1BB enhances oxidative metabolism. CD28 and 4-1BB co-stimulatory domains likely confer different pharmacokinetics to the CAR T cell product, with CD28 containing CARs having an enhanced acute response and 4-1BB CARs having greater persistence and long-term activity [32]. The differences between CD28 and 4-1BB as co-stimulatory domains are summarized in Figure 2. There have been no clinical trials that directly compare CD28 and 4-1BB containing CAR T cells, but longer persistence has been reported in the 4-1BB CARs in human subjects compared to CD28 containing CARs [14,43-47]. Nevertheless, both CD28 and 4-1BB containing CAR T cells have shown capacity for remarkable clinical efficacy.

Clinical Trials

Use of 2nd and 3rd generation CARs in B cell malignancies have led to dramatic and durable remissions in precursor B cell ALL, CLL, and B cell NHLs. The majority of trials in the US using second generation CAR constructs were conducted at four research institutions: Memorial Sloan Kettering Cancer Center (MSKCC), Baylor College of Medicine (BCM), National Cancer Institute (NCI) and the University of Pennsylvania (UPenn)-Children's Hospital of Philadelphia (CHOP) group. With the exception of trials conducted at UPenn-CHOP, most reported trials used CD28 as the co-stimulatory domain. These trials show that successful engraftment, expansion, and persistence of CAR T cells are critical to clinical success. Figure 3 summarizes the cumulative clinical response rates in precursor B cell ALL, CLL, and B cell NHLs to CD19-directed CAR T cells in the published literature. Multicenter trials are currently in progress.

Precursor B cell acute lymphoblastic leukemia

CD19-directed CAR T cells can achieve clinical Complete Remission (CR) in up to 90% of patients with relapsed and refractory B cell ALL [43,45,48,49]. These trials demonstrated that robust T cell expansion and persistence is critical to clinical efficacy. CAR T cells can also induce deep molecular remissions [14,43] and can traffic to the CNS [48]. While some institutions have used CAR T cells to achieve CR in order to bridge patients to allo HSCT, long term follow up at UPenn-CHOP shows that durable remissions are possible with

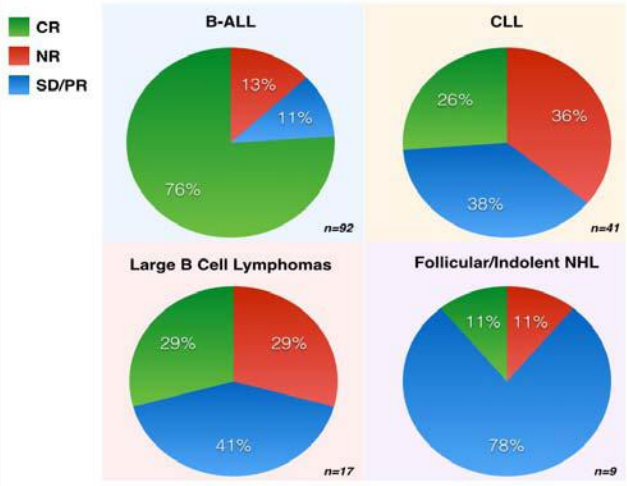

Figure 3: Cumulative rates of Complete Responses (CR), Non-Responses (NR), and Stable Disease (SD)/Partial Responses (PR) in the published literature, combining anti-CD19 CAR clinical trial results for B-ALL [14,43-45,48,49,60-62], CLL [44,46,47,52,58,60,62,63], large B cell lymphomas [33,48,52,62], and follicular/indolent NHL [33,52,53,63].

CD19-41BB CARs, without bridging to allo HSCT [45,50].

CD19-directed CAR T cells for relapsed or refractory B cell ALL show remarkable clinical efficacy and can rapidly induce molecular remissions. Five patients with relapsed ALL treated at MSK with CD19-28ζ CAR T cells all achieved tumor eradication and MRD negativity [43]. Four eligible patients went on to undergo allogeneic stem cell transplantation per protocol and durable remissions were achieved. Subsequently, an expanded cohort of 16 patients were treated and all but 2 patients achieved a CR, with 12 patients achieving MRD negative CR [14]. Treatment failures were correlated with poor *in vivo* expansion of the T cells. Seven eligible patients underwent allo HSCT and 2 patients electively declined further therapy. One patient died from allo HSCT related complications. The others had durable remissions with follow up as long as 24 months. The success of CD19-28ζ CAR T cells as a bridge to allo HSCT was also demonstrated by the NCI in a cohort of children and young adults [48]. Fourteen of 21 patients achieved CR with 12 of these patients achieving MRD negativity prior to proceeding to allogeneic stem cell transplant. At a median follow up of 10 months, all patients achieving CR remained disease-free.

While achieving CR allowed patients to undergo allo HSCT, this approach did not permit evaluation of CAR T cell therapy as definitive therapy. Given the treatment related toxicities of allo HSCT and that many patients may be either ineligible for or decline allo HSCT, it is important to evaluate the curative potential of CAR T cells as a definitive therapy. At UPenn-CHOP, infusion of CD19-41BB CAR T cells resulted in long term remissions without subsequent allogeneic stem cell transplant, proving the durability of this treatment approach. After two children with refractory B ALL achieved rapid MRD negative CRs, an expanded cohort of 30 children and adults were treated. Ninety percent (27/30) achieved complete morphologic remission at 1 month after infusion, 22 of whom were MRD negative [49]. Importantly, even in patients with a high burden of disease with greater than 50% bone marrow involvement, the CR rate was 82%. Maude et al. [49], reported that 15 out of 19 patients had sustained remissions of up to two years. These patients did not undergo allogeneic stem cell transplantation due to patient choice, lack of suitable donor, or history of prior allo HSCT, thus allowing for longer follow up of the efficacy of the CAR T cell therapy. Remarkably, the infused T cells were detectable in the peripheral blood for up to 11 months by flow cytometry and 2 years by qPCR.

Chronic lymphocytic leukemia

CD19-directed CAR T cells can result in durable and deep remissions in CLL, regardless of high risk cytogenetic features. Three patients with chemotherapy refractory CLL were treated with CD19-4-1BBζ CAR T cells at the University of Pennsylvania. Two patients, including one with del (17p), achieved complete remission; the third patient had a partial response. Remarkably, the patient with del (17p) had an ongoing remission at 10 months and FISH became negative for deletion TP53 in 198/200 cells [47]. The CAR T cells demonstrated in vivo persistence of greater than 6 months and CAR T cells of central memory phenotype were established. In an updated study, 8 of 14 patients with relapsed and refractory CLL responded to CD19-4-1BBζ CAR T cells (CTL019). Molecular remissions were achieved, as determined by deep sequencing of the immunoglobulin heavy chain (IGH) locus, and response was not affected by del (17p). In the first two patients achieving CR, B cell aplasia was sustained for over four years suggesting long term CAR T cell persistence. To date, all but one patient achieving CR have remained in CR.

CD19-28ζ CAR T cells can also induce long lasting remissions in heavily pretreated CLL patients with a high disease burden [51,52]. A patient with progressive CLL after 3 prior therapies achieved a CR lasting more than 15 months following CD19-28ζ CAR T cell infusion. Pre-treatment, 96% of his peripheral blood B cells were CLL cells and 50-60% of his bone marrow was involved by CLL. In a subsequent study, 3 out of 4 patients with CLL achieved CRs with CD19-28ζ CAR T cell infusion lasting 14-23+ months with follow up ongoing at time of publication [52].

Non-Hodgkin lymphoma

CD19-directed CAR modified T cells have also been effective in the treatment of relapsed and refractory B cell non-Hodgkin lymphomas. Kochenderfer et al. [52] at the NCI first reported the case of a patient with progressive stage IVB follicular lymphoma after multiple lines of therapy who achieved a partial response lasting 32 weeks following infusion of CD19-28ζ CAR T cells. A subsequent clinical trial conducted at the NCI included four patients with NHL - 3 patients with follicular lymphoma and one with splenic marginal zone lymphoma [51]. This time, a course of IL2 was given following infusion of CD19-CD28ζ CAR T cells. Aside from one patient who died due to influenza, partial response was achieved in the patients with follicular lymphoma, lasting upwards of 8 to 18 months with follow up ongoing at time of publication. The patient with splenic marginal zone lymphoma had a partial response lasting 12 months, and was enrolled on a subsequent CAR T cell study, was reinfused with CAR T cells, and achieved a second partial response lasting greater than 22 months [52].

Remarkably, lasting remissions can also be achieved in chemotherapy refractory diffuse large B cell lymphoma (DLBCL) patients. Out of 7 evaluable patients with relapsed DLBCL, three patients had primary mediastinal B cell lymphoma, one patient had DLBCL transformed from CLL, and the remainder had DLBCL NOS. At the time of the report, four patients had complete remissions lasting upwards of 6 - 22 months, 2 patients had partial responses (1 month - 6+ months), and one had stable disease at one month follow up [52]. This study demonstrated that anti-CD19 CAR T cells can be

efficacious in the treatment of refractory DLBCL.

At the University of Pennsylvania, Schuster et al. [53] are conducting a phase IIa clinical trial to evaluate the safety and efficacy of CAR T cells directed against CD19 (CTL019) in patients with relapsed or refractory CD19+ NHL including DLBCL, follicular lymphoma, and mantle cell lymphoma. Preliminary results were presented at the American Society of Hematology Annual Meeting in December 2015 (Abstract # 0183). At that time of the report, 22 patients were evaluable for response, including 13 patients with DLBCL, 7 patients with follicular lymphoma, and 2 patients with mantle cell lymphoma. Fifty-four percent (7/13) of patients with DLBCL had an objective response, whereas all patients with follicular lymphoma (7/7) had an objective response. Two patients with mantle cell lymphoma have been treated so far with one CR. At the median follow up of 11.7 months, PFS was 43% for DLBCL and 100% for follicular lymphoma.

Toxicities

Cytokine release syndrome

Cytokine Release Syndrome (CRS) is a potentially life threatening immunologic response that has been observed after CAR T cell infusion. Our understanding of CRS in the context of CAR T cell therapies for B cell malignancies largely derives from the experience in ALL and CLL. CRS is associated with large scale immune activation and release of pro-inflammatory cytokines, including IL6, IL2, IFNγ, and TNF. The clinical manifestations can range from mild to severe, and symptoms include fever, tachycardia, hypoxia, and hypotension. CRS can manifest as Macrophage Activation Syndrome (MAS) [14,55] and may clinically mimic sepsis. Davila et al. [14] proposed criteria for the diagnosis of severe CRS (sCRS) secondary to CAR T cells. These include fevers for at least three consecutive days, two cytokine maximum fold changes of at least 75 or one cytokine maximum fold change of at least 250, hypotension requiring a vasoactive pressor, hypoxia, or neurologic changes. Some critical complications of CRS that have occurred in ALL and CLL patients include respiratory failure requiring mechanical ventilation, cardiac arrest, macrophage activation syndrome, acute renal failure, and death [45,48,56].

Fortunately, not all patients exhibit sCRS. It is therefore important to identify patients at high risk of sCRS in order to provide timely and appropriate care. Predicting onset before overt clinical manifestations is challenging because cytokine measurements are not routinely available in most hospital laboratories. Using CRP levels to identify patients with sCRS has been proposed [14] but this awaits prospective validation. High dose corticosteroids can reverse the symptoms of CRS, but may result in the decreased expansion and efficacy of CAR modified T cells [14]. The IL-6 receptor blocker tocilizumab and TNF inhibitor etanercept are generally preferred because they do not compromise T cell proliferation [14,45] and are effective treatments.

In ALL, the severity of cytokine mediated toxicities is correlated with the presence of greater tumor bulk at the time of CAR T cell infusion [14,43,48,57]. In a clinical trial of CD19-CD28ζCAR T cells for refractory ALL patients, the two patients with highest tumor burden had the greatest CAR T cell expansion and experienced the highest cytokine elevations, whereas patients with only minimal residual disease at time of CAR T cell infusion demonstrated relatively modest cytokine elevations [43]. The correlation between severity and tumor bulk is less well established in CLL and NHL and NHL patients with primarily extra medullary disease may be less susceptible to severe CRS. In CLL, severity of illness has also been correlated with cytokine elevation [48,51] and the occurrence of CRS has been associated with clinical response [58].

The onset of CRS is concurrent with T cell proliferation and typically occurs within a week of CAR T cell infusion [48,51,58], but delayed onset sometimes occurs. Elevations are seen in IL2, sIL2R, IFNγ, TNF, and IL6, among other cytokines, and timing of cytokine elevation is correlated with clinical symptoms of CRS [45,49,51]. The use of diverse CAR constructs bearing different co-stimulatory domains may affect the kinetics of the pro-inflammatory cytokine response as well as the cytokines released. CD28-containing CAR modified T cells, such as used at the NCI and MSKCC, elicit earlier cytokine responses compared to the 4-1BB containing construct used by the UPenn-CHOP group [14]. This observation is most likely due to differential kinetics of expansion of the two CAR constructs, as CD28-containing CAR T cells expand more rapidly than those bearing the 4-1BB co-stimulatory domains [14,43,46]. It has also been suggested that 4-1BB containing CARs are less likely to trigger IL2 and TNF secretion compared to CD28 CARs [39]. Clinical trial experience in the treatment of CLL patients supports this notion [46,47]. The two different CAR constructs, however, have not been shown to result in different clinical toxicity profiles. Further research into different co-stimulatory domains may lead to elucidation of a CAR with the greatest therapeutic index.

Neurologic toxicities

CAR T cells can penetrate the Central Nervous System (CNS) and can eradicate leukemic cells in the cerebrospinal fluid (CSF). However, CNS trafficking may also contribute to neurologic toxicities. Symptoms are usually self-limited and include delirium, aphasia, confusion, hallucinations, and seizures [14,48,57]. The mechanism(s) underlying neurotoxicity are not fully understood; however, the potential for rare irreversible neurotoxicity is recognized. In one study, higher concentrations of CAR T cells in the CSF were correlated with the occurrence of neurologic symptoms [48]. Interestingly, only one patient who developed CNS toxicity had leukemia cells in the CSF by cytology. Since the CNS lacks CD19 expression [52,59,60], this finding suggests that CNS penetration may not be antigen driven. In another study, some patients who had neurologic toxicity did not have CAR T cells identified in the CSF [14]. This suggests that CAR T cell penetration of the CNS may not be the only mechanism for neurologic toxicity. Tocilizumab has been reported to be ineffective in the management of CAR T cell related CNS toxicity [52]; optimal management of severe CNS toxicity has not been determined and warrants further investigation. Fortunately, most cases of neurologic toxicity are self-limited and do not require specific intervention.

Conclusions

CAR T cells directed against CD19 have earned breakthrough therapy status by the FDA. Having shown efficacy in the treatment of relapsed and refractory precursor B cell ALL, DLBCL, CLL, and other B cell lymphomas, this treatment approach has already resulted in many long term remissions in patients who otherwise would not have had viable treatment alternatives.

CD19-directed CAR T cells have a real potential to address several areas of unmet clinical need in the rituximab era. Namely these are patients with 1) DLBCL who relapse within 1 year of R-CHOP chemotherapy, 2) DLBCL with *MYC* rearrangement and so called "double hit" DLBCL, 3) CLL with poor risk cytogenetic features

and refractory to or intolerant of kinase inhibitors, and 4) multiply relapsed and progressive indolent B cell lymphomas. Patients with these conditions are unlikely to respond to further chemotherapy with or with stem cell transplantation, but may achieve durable remissions following CD19-directed cellular therapy.

As a cellular therapy, CAR T cells provide an alternative to allo HSCT in B cell malignancies. Like allo HSCT, CAR T cells exhibit prolonged in vivo persistence and can induce long lasting remissions with a single infusion. For example, some patients in the ALL trials achieved long lasting remissions without subsequently undergoing allo HSCT. While allo HSCT is associated with significant morbidity and high treatment related mortality in the first 2 years, therapy with CAR T cells is generally well tolerated and treatment related toxicities are usually reversible. Since CAR T cells can be manufactured from the patient's own T cells, this treatment approach does not cause GVHD. CAR T cells offer an attractive option for patients who are medically unfit for allo HSCT, for those who do not have a suitable stem cell donor, and for those unwilling to accept the risks of allo HSCT. It is anticipated that clinical trials comparing CAR modified T cells to allo HSCT or high-dose chemotherapy with auto HSCT will be forthcoming.

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