

Review Article

Chimeric Antigen Receptor Therapy as a Means of Cellular Immunotherapy in B Cell Malignancies

M Frances Wright and Olalekan O. Oluwole MD*

Department of Medicine, Vanderbilt University Medical Center, USA

*Corresponding author

Olalekan O. Oluwole, Hematology Oncology Division, Department of Medicine, Vanderbilt University Medical Center, 1301 Medical Center Drive (3927 TVC), Nashville TN, 37232-5505, USA, Tel: 615 9368422; Fax: 615 9361812; Email: olalekan.oluwole@vanderbilt.edu

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Abstract

Chimeric antigen receptor T cells (CARs) are a form of cellular immunotherapy whereby a patient's T cells are re-engineered to express a chimeric tumor antigen-specific T cell receptor, leading to activation and expansion upon recognition of target antigen-bearing cells. Some patients with B cell malignancies, chemotherapy may have limited efficacy in achieving the disease control necessary for undergoing hematopoietic stem cell transplant, which is often the only curative option. However, numerous phase 1 and ongoing phase 2 clinical trials have demonstrated the efficacy of CD19-targeted CARs at achieving disease control in relapsed and refractory patients in a range of B cell malignancies, with response rates as high as 60-90%. Side effects associated with CAR therapy may be severe but are manageable and include cytokine release syndrome, neurotoxicity, and B cell aplasia. In this article, we present a review of the CAR construct, early and ongoing CAR trials in B cell malignancies, the presentation, monitoring, and management of major side effects associated with CAR therapy, and future directions for CAR research and development.

ABBREVIATIONS

CAR: Chimeric Antigen Receptor; FDA: Food and Drug Administration; APC: Antigen Presenting Cell; HLA: Human Leukocyte Antigen; CLL: Chronic Lymphocytic Leukemia; ALL: Acute Lymphoblastic Leukemia; MRD: Minimal Residual Disease; DLBCL: Diffuse Large B Cell Lymphoma; CRS: Cytokine Release Syndrome; CRP: C Reactive Protein; TNF: Tumor Necrosis Factor; MAS: Macrophage Activation Syndrome; HLH: Hemophagocytic Lymphohistiocytosis; DLBCL: Diffuse Large B Cell Lymphoma; IVIG: Intravenous Immunoglobulin's; EEG: Electroencephalogram; GVHD: Graft Versus Host Disease; LP: Lumbar Puncture; HLA: Human Leukocyte Antigen; CD: Cluster of Differentiation; IL: Interleukin; CNS: Central Nervous System; RNA: Ribonucleic Acid; DNA: Deoxyribonucleic Acid; PD-1: Programmed Cell Death Protein 1

INTRODUCTION

Cellular immunotherapy in the treatment of B-cell malignancies arose because existing salvage chemotherapy options are of limited efficacy and are associated with significant morbidity and mortality, including severe or prolonged myelosuppression, cardiac toxicity, and other organ dysfunction [1]. Patients with relapsed B-cell malignancies who have chemotherapy-sensitive disease are often required to undergo autologous, and in some cases, allogeneic stem cell transplant

for disease control [2,3]. However, only a fraction of patients ultimately qualify for transplant, either because their disease is not sufficiently chemotherapy-sensitive, or because they have suffered complications with salvage chemotherapy that preclude further treatment. Prognosis in either instance is poor. On the other hand, patients who are able to qualify for and undergo allogeneic stem cell transplant, for example, for acute lymphoblastic leukemia, have significant disease-free and overall survival compared to salvage chemotherapy alone [4,5]. For many patients, the primary barrier to transplant is inadequate disease control. The success of CAR trials in the setting of B-cell malignancies, including their role as a bridge to transplant, represents a major advance for immunotherapy in the treatment and eventual eradication of select B cell malignancies. In this review, we present an overview of cellular immunotherapy using chimeric antigen receptor (CAR) technology. We also present some of the recently concluded clinical trials and enumerate ongoing trials. Lastly, we hypothesize about the future of CAR therapy, and cellular immunotherapy in general, in the treatment of hematologic malignancies.

THE CAR CONSTRUCT

The part-human, part-murine chimeric antigen receptor construct is analogous to a monoclonal antibody attached to the intracellular signaling domain of a T cell receptor. It is designed to initiate intracellular signaling upon binding its target cell surface

antigen, precipitating T cell activation and clonal expansion in a manner that is independent of Human Leukocyte Antigen (HLA) presentation [6,7]. This design allows for target selection according to tumor cell antigen expression and is capable of inducing a potent antigen-specific immune response.

Several experiments in the 1980s and 1990s paved the way for the development of the essential components of the CAR construct [8,9]. In order to make the CAR construct, a single-stranded DNA fragment is delivered into the mature T lymphocyte using a gamma retro viral or lent viral vector. The CAR transgene comprises a single chain variable fragment (scFv) for antigen recognition, the CD3 zeta chain (CD3z) derived from the T cell intracellular signaling cascade, and at least one costimulatory domain (typically, CD28z or 4-1BBz) [10-12]. The scFv is, in effect, a synthetic monoclonal antibody; it is composed of the variable portions of the heavy and light chains linked by a hinge domain (Figure 1).

EARLY CLINICAL DATA

The first generation CARs, consisting of only an scFv domain linked to a CD3z endo domain, were able to bind to the specified target, but they were unable to multiply and persist in the host sufficiently to mount a sustained and effective immune response. The CAR construct was intended to render T cell activation independent of antigen presenting cells (APC), but these early CARs importantly lacked the costimulatory signal normally generated through interaction of the activated T cell and the APC (CD28 on the T cell and CD80/86 on the APC) necessary for a robust and sustained immune response. Thus, second generation CARs incorporated a costimulatory domain (CD28z or 4-1BBz) into the CAR endo domain in sequence with the CD3z. These CARs were able to mount a robust antitumor response and persisted to ensure successful eradication of the defined target, including malignant cells, and they resulted in clinically meaningful response rates. Preclinical studies suggested even greater antitumor activity with "third-generation" CARs, which incorporate an additional costimulatory domain in sequence with the CD3z and CD28z signaling moieties [6,13]. Ultimately, however, they were not proven to convey increased efficacy,

and thus most current trials employ second generation CARs [14]. An illustration of the first, second and third generation CAR constructs is shown in Figure 2.

SELECT COMPLETED TRIALS USING CAR T THERAPY

Several non-randomized, mostly small phase 1 and single-institution clinical trials have provided valuable insights into the efficacy and safety of CAR therapy. These trials have also informed strategies to predictably enhance as well as control CARs, leading to the successful development of single arm phase 2 clinical trials.

Chronic lymphocytic leukemia (CLL)

One of the earliest human experiments using CAR therapy was performed in patients with relapsed, refractory CLL. In the trial reported by Brentjens et al., adult patients with refractory CLL with high disease burden were treated with the CD19-CD28z construct. The first four patients had no objective response and subsequently died of either progressive disease or infection. Authors concluded that the lack of clinical response was due to the omission of lympho depleting therapy; therefore, the subsequent cohort of four patients was pre-treated with cyclophosphamide. This group's CARs underwent more robust expansion, and some of the patients achieved meaningful clinical responses that lasted 2-6 months [15].

Summary: This study supported the argument that lympho depleting chemotherapy plays an important role in creating the appropriate milieu for CAR proliferation without harmful effects on the host's unmanipulated T cells, which may account for the difference in response rates between the two cohorts. Some of the theories are that lympho depleting chemotherapy depletes the host T cells including T regulatory cells which may interfere with CAR T cell proliferation.

Acute lymphoblastic leukemia (ALL)

Several early CAR studies were done in the setting of acute lymphoblastic leukemia. Most were small, single-institution phase 1 trials, and their treatment regimens varied greatly.

In a study of young patients reported by Maude et al., in 2014 [16], 30 children and young adults were treated with the CD19-4-1BBz construct. Patients received different lympho depleting chemotherapies per physician choice, and CAR dosage ranged from 0.8-21 x 10⁶ cells per kg body weight. Treatment was highly successful, with 90% of patients achieving complete remission and 73% minimal residual disease (MRD) negative status. Adverse side effects were common; cytokine release syndrome (CRS) occurred in all patients (with no CRS-related deaths reported), and 43% of patients suffered neurotoxicity. Overall survival (OS) at six month follow-up was 78.6%.

In another study reported by Davila et al. [17], sixteen adults with relapsed, refractory ALL were treated with the CD19-CD28z construct. Median patient age was 50 years, and all patients underwent lympho depleting pre-treatment with cyclophosphamide, 1.5-3.0 g/m², and CAR infusion at a dosage of 3 x 10⁶ cells per kg body weight. Treatment was highly efficacious, with 88% of patients achieving complete remission

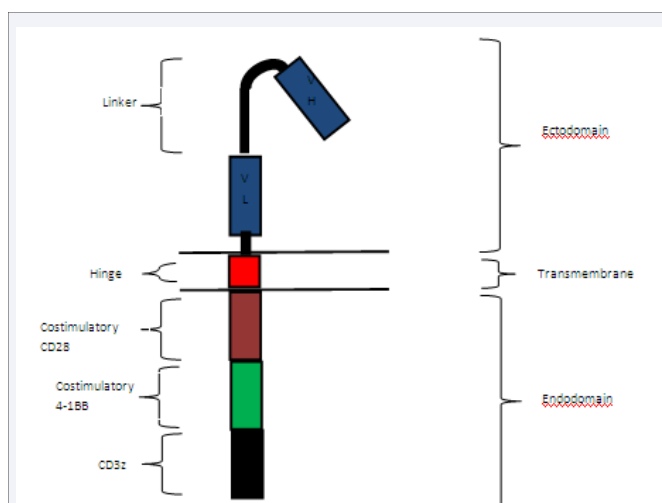


Figure 1 Components of the CAR construct.

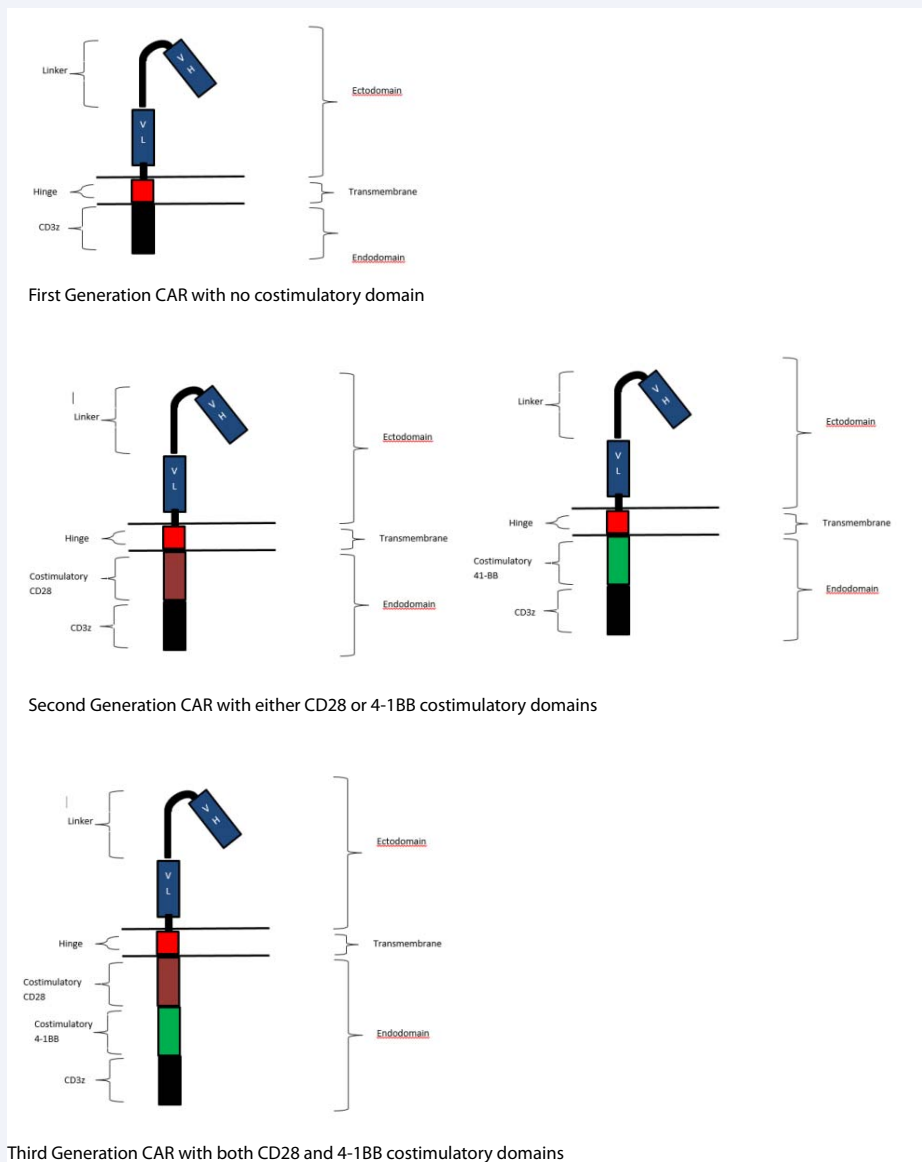


Figure 2 Schematic Depiction of Chimeric Antigen Receptor.

and 75% MRD negative status. CRS occurred in all patients, and neurotoxicity affected 31% of patients. No deaths were reported.

In a third study reported by Lee et al. [18], 21 children were treated with the CD19-CD28z construct. CAR dosage ranged from 0.03-3.6 x 10⁶ cells per kg body weight. Lympho depletion chemotherapy consisted of fludarabine 25 mg/m² per day on days -4, -3, and -2, and cyclophosphamide 900 mg/m² per day on day -2. Treatment resulted in a 60% complete remission rate, with 57% of patients achieving MRD negative status. CRS occurred in all patients, and neurotoxicity affected 29% of patients. In the 9.7-month follow-up period, event-free survival was 51.6%.

Summary: These three trials showed that CAR therapy could be safe and effective for patients spanning a wide age range and that complication such as CRS and neurotoxicity were generally treatable. Furthermore, these studies paved the way for multicenter phase 2 trials in pediatric and adult patients. Some of the ongoing clinical trials are listed in Table 1.

Other lymphoid malignancies

Few studies have been completed in the setting of Diffuse Large B Cell Lymphoma (DLBCL). Kochenderfer et al. [19], presented a study of CAR therapy in patients with DLBCL (7 patients) and other B cell malignancies (8 patients). All received the planned dose of CARs (1-5 x 10⁶/kg), even though many were significantly lymphopenic. Of the seven patients with DLBCL, four achieved CR, two partial remissions (PR), and one stable disease. Of the patients with indolent lymphomas, all achieved either CR or PR. All but two patients had features of CRS or neurotoxicity. Authors concluded that CD19 CAR therapy in the setting of DLBCL and other B cell malignancies can be associated with significant response rates and a manageable toxicity profile. Porter et al. [20], also reported a series of 14 patients with CLL who received CAR therapy dosed at 0.14-11 x 10⁸ cells per kg body weight. At follow-up (median duration 19 months, range 6-53 months), treatment had resulted in 28.5% CR and 28.5% PR;

Table 1: Ongoing Clinical Trials of CAR T Cell Therapy in B Cell Lymphoma.

Clinical trial identifier	Phase	Title/Indication	Age (years)	CD19 CAR type	Gene transfer	Conditioning	Lead site
NCT02028455	I/II	CD19+ Leukemia	1-26	19-41BB	Gamma retrovirus	Not stated	Seattle
NCT02529813	I	Lymphoid Malignancies	1 - 80			Flu 25 or 30 mg/m ² Days -5 to -3 Cy250 or 300 or 500 mg/m ² Days -5 to -3	M.D. Anderson Cancer Center
NCT02030834	II	Refractory CD19+ Lymphomas	≥ 18	19-41BB	Lentivirus	Not stated	University of Pennsylvania
NCT02374333	I	Relapsed or Refractory CD19+ Leukemia and Lymphoma Previously Treated With Cell Therapy	1 - 24	19-41BB	Lentivirus	Not stated	University of Pennsylvania
NCT02640209		CART+ Ibrutinib In Patients With Relapsed Or Refractory CD19+ Chronic Lymphocytic Leukemia	≥ 18	19-41BB	Lentivirus	Not stated	University of Pennsylvania
NCT02050347	I	Relapsed CD19+ Malignancies Post-Allo HSCT	Any age	19-28	Gamma retrovirus	cyclophosphamide and fludarabine	Baylor College of Medicine
NCT01865617	I/II	CD19+ hematologic malignancies	≥ 18	19-41BB	Lentivirus	Not stated	Fred Hutchinson Cancer Research Center
NCT02146924	I	Acute Lymphoblastic Leukemia	≥ 18	19-28	Lentivirus	Not stated	City of Hope Medical Center
NCT02926833	I/II	DLBCL	≥ 18	19-28	Gamma retrovirus	cyclophosphamide and fludarabine	Multicenter

Abbreviations: CAR: Chimeric Antigen Receptor; DLBCL: Diffuse Large B Cell Lymphoma; r/r: Relapse Refractory; Cy: Cyclophosphamide; Flu: Fludarabine

43% of patients did not achieve a meaningful response. All those who achieved CR were MRD negative, and authors concluded that there was potential for CAR therapy to eradicate CLL in patients who achieve MRD negative status.

There are also a few case series that document the effectiveness of CAR therapy in the treatment of multiple myeloma using CARs that target CD19, CD138, or B cell maturation antigen (BCMA) and the results have been variable ranging from partial to complete responses [21-24]. More carefully conducted phase 1 clinical trials are ongoing with CARs targeting BCMA.

One published report of a phase 1 trial of CD30 directed CAR therapy in 18 patients with relapse refractory Hodgkin lymphoma suggested that the therapy was well tolerated. Objective responses were noted in 39% of patients and the median progression free survival was 6 months. Toxicity profile was tolerable. Authors opined that the CD30 CAR therapy should be further studied in Hodgkin lymphoma [25].

ONGOING CLINICAL TRIALS

A detailed list of ongoing clinical trials is displayed in Table 1. The role for these early phase trials is to harmonize the treatment schedule of CAR therapy and to test the hypothesis that this innovative way of treating patients is feasible outside of highly

specialized research laboratories. It remains to be seen whether CAR therapy could transition to the community setting.

SIDE EFFECTS OF THERAPY

The use of chimeric antigen receptor therapy has been associated with specific side effects whose pathophysiologies are often inherent to the mechanisms of CAR functioning. The most notable of these side effects are cytokine release syndrome (CRS), neurotoxicity/encephalopathy, and B cell aplasia.

Cytokine release syndrome

Cytokine release syndrome (CRS) is a reversible but potentially life-threatening condition thought to result from excessive pro inflammatory cytokine release and accompanying immune cell activation following rapid CAR activation and T cell expansion [16-19,26,27]. It is common in patients receiving CAR therapy (64-100% of patients in four major B-ALL and CLL trials were affected) and can present within days to weeks after CAR infusion [13,14,16-18,27]. Its presentation ranges in severity from mild, flu-like symptoms to high fevers, hypotension requiring volume resuscitation, capillary leak syndrome with hypoxia requiring supplemental oxygen or mechanical ventilation, and shock with multi-organ failure. Lab findings may be similar to those seen in hemophagocytic lympho histiocytosis

Table 2: Select Phase 1 Clinical Trials of CAR T Cell Therapy with at least 10 patients.

Author	Institution	Sample Size	Disease Type	CAR Type	Gene Transfer	Median Age (years)	Lymphodepleting Chemo	CAR T cell Dose	Response
Davila et al. [17],	MSKCC	16	ALL	19-28	Gammaretrovirus	50	Cy	3 x 10 ⁶ /kg	88% achieved CR, 75% MRD negative. 44% bridges to allo-SCT
Maude et al. [16],	UPENN	30	ALL	19-41BB	Lentivirus	14	Physician Choice	0.8 – 21 x 10 ⁶ /kg	90% achieved CR, 73% MRD negative. OS 78.6% at 6 months.
Lee et al. [18],	NCI	21	ALL	19-28	Gammaretrovirus	13	Flu 25mg/m ² Cy 900mg/m ²	0.03 – 3.6 x 10 ⁶ /kg	68% achieved CR, 57% MRD negative. EFS 51.6% at 9.7 months
Porter et al. [20],	UPENN	14	CLL	19-41BB	Lentivirus	67	Flu/Cy 21% Bendamustine 43% Pen/Cy 36%	0.14 - 11x10 ⁸	28.5% achieved CR, 28.5% PR and 43% did not respond

Abbreviations: ALL: Acute Lymphoblastic Leukemia; CLL: Chronic Lymphocytic Leukemia, CAR: Chimeric Antigen Receptor; CR: Complete Remission; SD: Stable Disease; ORR: Objective Response Rate; EFS: Event Free Survival; OS: Overall Survival; NA: Not Applicable; MRD: Minimal Residual Disease; Cy: Cyclophosphamide; Flu: Fludarabine; Pen: Pentostatin, MSKCC: Memorial Sloan Kettering Cancer Center; UPENN: University of Pennsylvania; NCI: National Cancer Institute.

(HLH) and macrophage activation syndrome (MAS), including hyperferritinemia, hypofibrinogenemia, hypertriglyceridemia, and elevated C-reactive protein (CRP). Likewise, the cytokines characteristically elevated in CRS-IFN-gamma, TNF-alpha, soluble IL-2R alpha, IL-6, and IL-10-overlap with those implicated in HLH/MAS [13,27]. Daily monitoring of c- reactive protein (CRP) is recommended with CAR infusion to facilitate early identification of CRS; CRP in excess of 20 mg/dL during the time of CAR T cell expansion which occurs typically peaks between day 4-7 has been associated with high risk of severe CRS [17]. Off-label treatment of CRS with tocilizumab, an IL-6 receptor blocker that spares CAR function, has shown clinical efficacy for symptom reversal [14,17]. Corticosteroids may cause CAR ablation with an associated increased risk of disease recurrence and should be reserved for refractory cases of CRS [13,28]. CRS occurrence, but not severity, is thought to reflect CAR efficacy, while symptom severity tends to correlate with tumor burden at the time of CAR infusion [13].

Neurotoxicity

CAR-related neurotoxicity varies in presentation and severity and tends to be progressive but reversible. Reported manifestations include dysphasia, confusion, global encephalopathy, akinetic mutism, and delirium, ranging to seizures, cerebral edema and coma. Seizure prophylaxis is recommended prior to CAR infusion [27], and patients with severe symptoms may require intubation for airway protection. Patients often must undergo imaging, EEG, and/or LP to rule out other causes of their symptoms. The mechanism of CAR-mediated neurotoxicity is poorly understood, but a theory of general inflammatory injury is favored over direct CAR T-mediated toxicity, as symptoms do not appear to correlate with either CAR presence in the cerebrospinal fluid or central nervous system (CNS)-lymphoma [17,27].

B cell aplasia

CD19-targeted CARs are designed to eliminate all CD19-bearing cells, including normal B cells. B cell aplasia is therefore termed an “on-target, off-tumor” toxicity and is considered

a surrogate marker for CD19CAR efficacy [29,30]. Notably, hematopoietic stem cells, lacking CD19, are spared by CD19 CAR therapy and continue to manufacture B lymphocytes [31]. It follows that the duration of B cell aplasia corresponds with CAR persistence [32], the determinants of which are incompletely understood but thought to vary with tumor type, patient characteristics, conditioning regimen, and CAR construct [31]. In CD19-targeted CAR trials with high response rates in B-ALL, CARs have persisted for as little as one month to as long as two years [14]. Patients become severely hypogammaglobulinemic and can be managed with monthly infusions of intravenous immunoglobulins (IVIG) to decrease infection risk. Clinical monitoring of immunoglobulin levels has been used to identify IVIG requirement and as a proxy for detection of CAR depletion [32], which, if the patient is not in remission, may indicate tumor escape, poor CAR functioning, or a host immune response against the CAR.

FUTURE DIRECTIONS

CD19-directed CAR therapy has delivered on efficacy; response rates in the relapse setting are significantly higher than expected, especially considering that patients have more chemotherapy-resistant disease at the time of relapse. However, this efficacy has for the most part been restricted to very early-phase clinical trials in a population that is subject to selection bias. It remains to be seen if these results are reproducible in ongoing phase 2 clinical trials and whether the described toxicities will make for a manageable safety profile. Once a tolerable safety profile is established in phase 2-3 multicenter trials, regulatory approvals will be sought to confirm the position of CAR therapy in the relapse setting. Given the range of prognoses for adult and pediatric patients with B-ALL, the future of CARs as first-line therapy is unclear and may vary depending on disease subtype. CARs will most likely emerge as an upfront treatment option for patients with poor prognoses for whom we do not currently have reliable therapies.

In the meantime, there are possible avenues for expanding the use of current CD19-targeted CARs, both in terms of re-treating

patients who have already received CAR therapy and enlarging the pool of eligible patients. For example, at the present time, active CNS lymphoma is a contraindication to most CAR therapies. There is concern that the phenomenon of neurotoxicity, which is poorly understood, may occur with greater severity in patients with known CNS lymphoma; thus, such patients have been excluded from clinical trials. However, as our understanding of CARs and the pathophysiology of their side effects improves, we may be able to develop specific protocols that would allow for treatment of patients with CNS involvement from systemic lymphoma, as well as those with primary CNS lymphoma.

Response to CD19CARs in different B cell malignancies has been variable, ranging from 80-90% in B-ALL to 45-76% in CLL [3,29,30]. The rapidity with which CD19CARs induce response and relative lack of definable antibodies to the CAR construct suggest that patients who tolerated therapy but only achieve durable response can be retreated with CARs. Determinants of response, however, remain unclear. Alteration of tumor antigen expression may explain why some patients fail to respond, become resistant to, or relapse after CAR therapy. As with other targeted therapies, tumor cells may escape CARs by down regulating or eliminating expression of the target antigen or through expansion of an antigen-negative sub clone. For example, antigen escape has been cited as a common cause of relapse in multiple trials of CD19-targeted CARs for B-ALL [31,33,34]. In an attempt to prevent this phenomenon, investigators have developed CARs that can recognize multiple antigens. In preclinical studies, so-called "bispecific" CARs were activated upon binding either CD19 or CD20, another pan-B cell marker, and showed robust antitumor response against both CD19-positive and CD19-negative lymphoma cell populations [34,35]. In the event that CD19-negative relapse does occur following CD19CAR therapy, follow-up treatment with CARs targeted to CD22, yet another pan B cell marker, have proven capable of inducing complete remission [31]. Similarly, in the setting of mature B cell malignancies, CARs targeted to the kappa light chain are showing promise in patients with CD19-negative relapse after CD19CAR therapy. An advantage to this approach is the persistence of lambda-B cells, such that patients are spared the B cell aplasia and severe hypogammaglobulinemia associated with CD19-targeted therapy [35].

Strategies for improving CAR specificity and decreasing toxicities have addressed multiple steps in CAR generation, including CAR gene delivery, mechanism of expression, structure, and co expression of other genes or CARs. Transfection of donor T cells with an inducible "suicide gene" enables rapid termination of graft versus host disease (GVHD) in patients receiving donor lymphocyte infusions after stem cell transplant for leukemia; in the context of CARs, a similar mechanism could provide an "off switch" to be employed in the case of severe adverse effects [2,10]. Alternatively, transfection of T cells with CAR mRNA, rather than DNA, allows for transient CAR expression and decreases the theoretical risk of mutagenesis associated with DNA integration into the genome [14,29,36]. Because the scFv portion of the CAR is typically of murine origin, patients may sometimes develop anti-mouse antibodies against the construct, with adverse impacts on CAR T functioning and persistence. Fully-humanized CARs have demonstrated efficacy, but self-tolerance to many target antigens, including CD19, makes selection of a suitable target difficult [37].

Preclinical and limited clinical data have suggested concurrent checkpoint inhibition may augment the efficacy of CAR therapy, both in hematologic malignancies and solid tumors [28,38-40]. Tumor cells are capable of evading immune system detection by exploiting the inhibitory T cell receptor programmed cell death protein-1 (PD-1), which normally functions as a feedback regulator on T cell response. By disrupting tumor cell PD-L1 engagement of PD-1 on T cells, PD-1 inhibitors like nivolumab and pembrolizumab prevent tumor-mediated T cell suppression. Combination PD-1 inhibitor/CAR therapy may therefore enhance CAR efficacy by averting CAR inactivation by tumor cells. Phase 1 and 2 trials combining CARs with pembrolizumab or an investigational PD-L1 monoclonal antibody, such as atezolizumab or durvalumab, are currently underway in patients with non-Hodgkin lymphomas as stated in Table 1 [39]. Other philosophically similar approaches, such as CARs that have manipulated to express non functioning or modified PD-1 receptors, have also been associated with increased CAR activation [8]. Finally, combination CAR therapy with ibrutinib, a tyrosine kinase inhibitor, has been shown to decrease PD-1 expression by T cells and PD-L1 expression by CLL cells in patients with CLL [41].

Natural killer (NK) cells obtained from umbilical cord appear to have activity in acute myeloid leukemia (AML) and are being studied as a way of treating AML in the relapse setting [42-45]. There is evidence that NK cells can expand in the human body, secrete cytotoxic cytokines, and immunologically target acute leukemia cells. Meaningful responses have been described in mouse models [42]. Human studies are either planned or underway [42-45].

CARs have had limited success in the treatment of solid tumors. Difficulties in this setting include tumor architecture, which may prevent CARs from encountering many neoplastic cells, immunosuppressive tumor microenvironment, and selection of a target antigen that is widely expressed by the tumor and not by normal tissue [14,36]. Nevertheless, CAR trials for a range of solid tumors, including lung, breast, ovarian, colon, prostate, renal cell carcinoma, mesothelioma, glioma, and neuroblastoma, have been completed or are underway. The majority of these trials employ the standard CAR construct, targeted to a tumor-specific antigen such as CAIX (e.g., for renal cell carcinoma) [46]. However, the challenges solid tumors pose to traditional CARs have spurred CAR design modifications in the hopes of improving efficacy in this setting [36]. One example are the so-called "fourth-generation" CARs, whose activation additionally triggers pro-inflammatory cytokine release and innate immune system recruitment; this strategy is posited as a potential solution to solid tumor cells' relative inaccessibility. Additionally, as innate immune cells may recognize and destroy tumor cells that have lost target antigen expression, these CARs may forestall tumor escape or relapse [47].

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