CAR-T treatment for hematological malignancies

Shebli Atrash ,[1] Kulsum Bano,[1] Bradley Harrison,[1] Al-Ola Abdallah[2]

[1]Department of Hematologic Oncology and Blood Disorders, Levine Cancer Institute, Charlotte, North Carolina, USA
[2]Department of Internal Medicine, Division of Hematological Malignancies and Cellular Therapeutics, University of Kansas Medical Center, Kansas City, Kansas, USA

Correspondence to
Dr Shebli Atrash, Levine Cancer Institute, Charlotte, NC 28204, USA; Shebli.Atrash@Atriumhealth.org

Accepted 25 February 2020
Published Online First 21 March 2020

To cite: Atrash S, Bano K, Harrison B, *et al*. *J Investig Med* 2020;**68**:956–964.

ABSTRACT

Chimeric antigen receptor (CAR)-T-cell therapy has sparked a wave of optimism in hematological malignancies, reflected by the successful results of early clinical trials involving patients with pre-B-cell acute lymphoblastic leukemia, B-cell lymphomas and multiple myeloma. CAR-T-cell therapy is considered to be a novel immunotherapy treatment that has the potential for curing certain hematological cancers. However, as use of CAR-T-cell therapy has grown, new challenges have surfaced. These challenges include the process of manufacturing the CAR-T cells, the mechanisms of resistance that underlie disease relapse, adverse effects and cost. This review describes the published results of clinical trials and expected developments to overcome CAR-T resistance.

INTRODUCTION

Hematological malignancies contribute to a major burden of new cancer cases detected each year, with close to 178 000 new cases of lymphoma, leukemia and multiple myeloma (MM) detected each year.[1] Encouragingly, over the past three decades, improvements in cancer survival rates have been most rapid for hematological malignancies in comparison to other malignancies. Treatment protocols are changing particularly with the advent of targeted cellular immunotherapies to manage hematological malignancies. Here, we will review the utilization of these targeted therapies, particularly chimeric antigen receptor T-cell (CAR-T) therapy, for the treatment of hematological malignancies; the rationale behind target selection and the toxicities associated with CAR-T therapy.

Like other forms of immunotherapy, the mainstay of CAR-T therapy is the activation of a T-cell response against a malignancy. CAR-Ts are a form of genetically modified autologous immunotherapy. CARs are recombinant proteins, each composed of an antibody-derived extracellular single-chain variable fragment (scFv) linked to the intracellular T-cell signaling domains of the T-cell receptor.[2] This adoptive transfer of engineered T cells that express CARs can be used to target specific tumor-associated antigens (TAAs) in an human leukocyte antigen (HLA)-independent manner; thus, this therapy could be used in patients of all HLA types. CAR-T therapy has shown incredible success and promise in treating relapsed/refractory leukemias and lymphomas in short periods of time. This success has catapulted CAR-T therapy into the spotlight and made it more accessible in general clinical practice.

CAR-Ts were first developed in 1993 by Esshar *et al*.[3] However, first-generation CARs had limited efficacy in vivo due to their short half-lives, limited expansion and poor antitumor efficacies. Over the course of almost a decade, second-generation CARs that used co-stimulatory domains (eg, CD28, 4-1BB (CD137) and OX40 (CD134)) were produced, overcoming the shortcomings of first-generation CARs with improved persistence and antitumor effects.[4] Third-generation CARs involved the incorporation of multiple co-stimulatory domains.

B-cell malignancies have garnered significant interest as a potential indication for therapy with CARs due to the presence of CD19 and CD20, which are B-cell-specific antigens that have been deemed as ideal targets for CARs to act on due to certain inherent properties described in the 'target selection' section below. Consequently, following multiple preclinical and clinical trials, CD19-directed CAR-T (CAR-T19) therapy (tisagenlecleucel-t) was Food and Drug Administration (FDA) approved for treatment of acute lymphoblastic leukemia in August 2017, followed by the approval of CAR-T19 therapy—axicabtagene ciloleucel (axi-cel)—for the treatment of large B-cell lymphomas in October 2017. Another antigen of interest that has been extensively studied for the treatment of MM is the B-cell maturation antigen (BCMA), or CD269. This antigen is specifically expressed on the surfaces of plasmablasts and plasma cells but not on other classes of B cells, hematopoietic cells or normal cells.[5]

To produce CAR-Ts for clinical use, T cells are collected from the patient by leukapheresis, activated, modified, expanded and then reinfused to the patient after inducing lymphodepletion using lymphodepleting chemotherapy.

The original T-cells are transduced with the CAR via a viral vector. However, this is a costly process that requires manufacturing of the virus, with complicated quality control process, and carries a risk of insertional oncogenesis. Another method for modifying the T cells is by using non-viral transposon transfection, also known as 'sleeping beauty' methods.[6] It is a simple, cut-and-paste process to translocate the transgene/transposon (the CAR gene) into the T cells (figure 1).

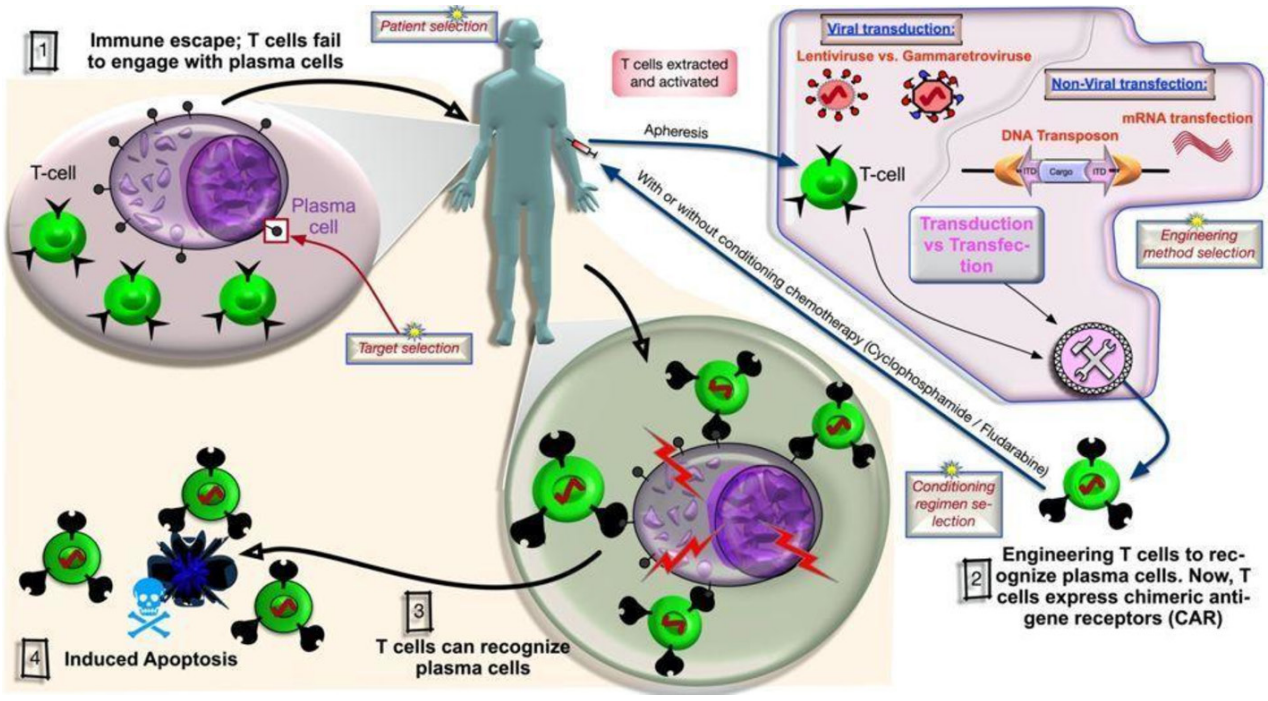

Figure 1 Chimeric antigen receptor T-cell (CAR-T) manufacturing process. (1) Plasma cells in relapsed/refractory myeloma escape immune surveillance by T cells. Patients undergo apheresis to select T cells. (2) The next step is to engineer T cells to recognize plasma cells. This engineering process could be done with many available methods with a viral vector (lentivirus or gamma virus) or without a viral vector (DNA transposon system or RNA transfection). (3) The selection of a conditioning regimen to deplete host T cells. Options include using cyclophosphamide, using fludarabine, or not using any chemotherapy. T cells can now recognize plasma cells. (4) Apoptosis induction and clinical response. Multiple factors may be involved in the variations in efficacy and toxicity between the different constructs seen in clinical trials (marked with stars). These may include 1) factors having to do with patient selection; 2) target selection and degree of tumor burden and/or target expression; 3) aspects intrinsic to the CAR construct, such as variations in single-chain variable fragment (scFv) sequencing or co-stimulatory molecules or differences in transduction mechanisms and vectors and 4) potentially, differences in conditioning regimens used for lymphodepletion.

As expected, CAR-T therapy comes with a unique set of complications, the most common of these being cytokine release syndrome (CRS), neurotoxicity and B-cell aplasia. These complications will be discussed in detail below.

Target selection

One of the primary aspects of CAR-T therapy is the selection of an appropriate target for the CAR-T to act on. Target selection involves the selection of a TAA, which is selectively presented on the malignant cell in question. An ideal target antigen would be one that remains stable and consistently presented throughout the neoplastic process and is only present on malignant cells and not on non-malignant cells.

CD19

CD19 meets most of the aforementioned requirements. It is a B-cell-specific antigen that is expressed on both mature and developing B cells, absent on hematopoietic stem cells and consistently present throughout the course of the malignant B-cell differentiation. As a result, CD19 garnered a tremendous amount of interest as a CAR-T target for B-cell neoplasms. CD19 CAR-T therapy for B-cell neoplasms has truly heralded the breakthrough of cellular therapeutics.

CD19 is absent on plasma cells, however, targeting plasma cells precursors with CD19 CAR-T showed clinical benefits in early phase trials.[7]

B-cell maturation antigen

BCMA is a CAR-T-cell target that has been explored in MM. Functionally, it helps regulate B-cell maturation, is increasingly present throughout the plasma cell differentiation process and correlates with prolonged plasma-cell continued survival in mouse models.[8] However, despite its role in B-cell maturation, humoral response and germinal center formation were unimpaired in BCMA (−/−) mice, suggesting that BCMA inhibition may allow for the selective targeting of plasma cells without compromising memory B cells and humoral immunity mechanisms.[9]

BCMA carries particular promise as a CAR-T target because it is expressed on plasma cells with limited expression elsewhere and is notably absent on major organs, hematopoietic stem cells and normal T cells.[5 10 11] Increasing expression of BCMA was detected along the spectrum from normal plasma cells to monoclonal gammopathy of undetermined significance to smoldering MM to MM.[12] Furthermore, plasmacytoid dendritic cells (pDCs) have been shown to play a role in MM progression and plasma cells resistance.[13] BCMA is also notably expressed in pDCs

in MM, suggesting an additive benefit of BCMA targeting.[14] In total, higher serum BCMA levels were correlated with disease progression and inversely correlated with overall survival, and recent studies trialing anti-BCMA CAR-T therapy have shown promising results.[15 16]

Other targets

Several ongoing trials directing CAR-T toward other targets in myeloma might be considered as treatment options; some of those targets have proved promising in early phase clinical trials, and others are still in preclinical phase.

CD38 was considered as a target because of its high expression on plasma cells. However, CD38 is also expressed on normal hematopoietic cells, such as red blood cells, natural killer (NK) cells and other tissues, increasing the likelihood of 'on-target, off-tumor' toxicity.[17 18] A study to evaluate the safety and efficacy of anti-CD38 CAR-T in relapsed/refractory MM (RRMM) patients is ongoing (NCT03464916).

CD138 is another target that is highly expressed on plasma cells. However, it is also expressed on normal tissues, such as epithelial cells, potentially increasing 'on-target, off-tumor' toxicity. CD138 is highly expressed on MM cells and is involved in their development and proliferation.[19] In a clinical report on five patients treated with CD138-directed CAR-T, no excess off-target effects were observed.[20] A phase I clinical trial with CD138-directed CAR-T is ongoing (NCT03672318). CAR-Ts against κ light chains have been developed and tested in a clinical trial with no myeloma response.[21]

Other targets that have shown encouraging preclinical activity and are currently undergoing clinical trials include: 1) signaling lymphocyte activation molecule F7, which is widely expressed on plasma cells as well as subsets of normal B and T cells, NK cells, monocytes and dendritic cells and is already a therapeutic target of the monoclonal antibody elotuzumab[22]; 2) GPRC5D, which is expressed on plasma cells as well as some normal cells, such as hair follicle and lung tissue cells (expression is variable, and the expression on plasma cells is 500–1000 times that found on normal cells)[23] and 3) NKG2D receptor, which activates NK cells and T-cell subsets after binding to a group of ligands that is expressed on infected cells and a variety of tumor cells, including MM. Importantly, the expression of NKG2D has not been observed on normal, healthy tissues.[24 25]

On-target, off-tumor effects

Cytokine release syndrome

CRS is a potential complication of CAR-T therapy, characterized by a clinical spectrum ranging from low-grade fever and constitutional symptoms to potentially life-threatening hemodynamic instability, hypoxia and renal failure. CRS differs in part from autoimmune toxicity, in which antigenic sites are incidentally expressed and targeted on host tissue, colloquially referred to as 'on-target, off-tumor' effects.[26] Rather, CRS, while incompletely understood, is theorized to present as a function of initial on-target activation with subsequent widespread cytokine release in the setting of extensive bystander lymphocyte, macrophage and neutrophil activation.[26 27] Furthermore, markers of endothelial activation such as von Willebrand factor, Ang-2 and other Weibel-Palade body products are notably elevated in severe CRS, physiologically accounting for the capillary leak, hypotension and coagulopathy often observed in these patients.[28] Clinically, CRS is frequently graded according to severity with treatment recommendations varying by grade. According to the American Society for Transplantation and Cellular Therapy, grading is delineated by post-CAR-T fever (grade 1) plus low-flow oxygen (grade 2), with progression to need for either one vasopressor or high-flow oxygen (grade 3) versus multiple vasopressors and/or positive pressure or mechanical ventilation (grade 4)[29] (table 1).

On average, most patients developed an initial fever 1–4 days following CAR-T transfusion,[27 30] with observations of more severe CRS occurring, on average, 1 day post-transfusion and with less severe iterations occurring, on average, 4 days post-transfusion.[31] However, the onset of fever depends on the construct of CAR-T. For example, the onset of fever occurs between 6 and 9 days after infusing LCAR-B38M CAR-T,[32] which is BCMA directed CAR-T cells. In addition to the CAR-T dose, the co-stimulatory signal of the CAR-T, for example, CD28 >4-1BB, may lead to increased CAR-T-cell expansion in vivo and higher toxicity, as seen in the ZUMA-1 trial.

Some factors, such as disease burden at the time of infusion and CTL019, have been shown to predict predisposition to and severity of CRS.[31 33] CRS severity has also been retrospectively associated with elevated serum interferon-γ, tumor necrosis factor levels, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), granzyme B and interleukin (IL)-1β.[32 34]

Non-specific markers of inflammation, such as ferritin and C reactive protein (CRP), were elevated in CRS and exhibited limited positive predictive value in terms of predicting disease onset[27]; CRP may also have trend-based clinical utility in identifying the peak of the inflammatory cascade.[26] Low fibrinogen levels were widely observed in all grades of CRS, whereas more significant transaminitis, renal injury and coagulopathy were observed in more severe grades of the syndrome.[30]

Clinicians must have a high index of suspicion of CRS in the post-transfusion setting so that treatment can be initiated promptly. IL-6 has been implicated as a central driver in CRS, and IL-6 blockade with tocilizumab has been shown to ameliorate CRS symptomatology without significant inhibition of CAR-T expansion.[31] Tocilizumab recently gained FDA approval for treatment of CRS as well.[34] Steroids have also significantly dampened observed toxicities associated with CRS,[33 35 36] and patients who received methylprednisolone in the midst of CRS still demonstrated antitumor response to CAR-T therapy.[37] Initial results assessing the use of prophylactic tocilizumab have indicated a reduction in the incidence of severe CRS in patients receiving tocilizumab on day 2, post-transfusion; however, additional studies regarding prophylactic use and more definitive CRS treatment regimens are still ongoing.[38]

Neurotoxicity

Neurotoxicity is a well-known complication of CAR-T therapy. Previously known as CAR-T cell-related encephalopathy syndrome, it is currently referred to as immune

Table 1 Grading and management of CRS/neurotoxicity from CAR-T therapy

Cytokine release syndrome (CRS)		
Grade	Symptoms	Management
1	Fever only	Onset <72 hours: consider tocilizumab 8 mg/kg±dexamethasone 10 mg every 24 hours Onset ≥72 hours: supportive care
2	Fever with hypotension (not on vasopressors) and hypoxia (requires only nasal canula)	Onset <72 hours: consider tocilizumab 8 mg/kg AND dexamethasone 10 mg every 24 hours Onset ≥72 hours: consider tocilizumab 8 mg/kg±dexamethasone 10 mg every 24 hours
3	Fever with hypotension (requires a vasopressor) and hypoxia (requires high flow nasal canula '>6 L/min' or non-rebreather mask) or grade 4 transaminitis	Consider tocilizumab 8 mg/kg AND dexamethasone 10 mg every 12 hours to every 24 hours
4	Fever with hypotension (requires multiple vasopressors) and hypoxia (requires positive pressure like CPAP, BiPAP or intubation)	Consider tocilizumab 8 mg/kg AND dexamethasone 20 mg every 6 hours
Immune effector cell-associated neurotoxicity syndrome*		
Grade	Symptoms	Management
1	7–9 points	Seizure prophylaxis; dexamethasone 10 mg every 8–12 hours
2	3–6 points	Seizure prophylaxis; dexamethasone 10 mg every 8–12 hours
3	0–2 points; any clinical seizure	Seizure prophylaxis; dexamethasone 10 mg every 6–8 hours
4	Unarousable; life-threatening	Seizure prophylaxis; dexamethasone 20 mg every 6 hours

Adopted from the ASBMT Consensus. Multiple other grading systems are available like the CTCAE 5.0,[88] Penn criteria,[89 90] MSKCC criteria[91] and CARTOX criteria.[92]
*Encephalopathy is graded by CARTOX-10 criteria: orientation: year, month, city, hospital, president (5 points). Ability to name three objects (3 points). Ability to write a standard sentence (1 point). Attention: ability to count down from 100 by intervals of 10 (1 point).
BiPAP, bilevel positive airway pressure; CAR-T, chimeric antigen receptor T-cell; CPAP, continuous positive airway pressure.

effector cell-associated neurotoxicity syndrome (ICANS). The pathogenesis of ICANS is not completely clear. However, multiple hypotheses have been put forward based on preclinical and clinical studies. Increased blood-brain barrier permeability is thought to be a cause of ICANS, as evidenced by elevations in CSF proteins.[39] This may be secondary to excessive cytokine release in the cerebral circulation, as evidenced by the presence of high levels of cytokines in the CSF during neurotoxicity. It is interesting to note that ICANS, very much like CRS, has been seen to develop in patients with higher numbers of CAR-Ts due to the greater expansion of these cells.[40]

The prevalence of ICANS has varied from study to study, with a prevalence rate of 23%–67% for patients with lymphoma and 40%–62% for those with leukemia. ICANS can present with a wide spectrum of neurological signs and symptoms, ranging from headache and confusion to seizures and myoclonus with rare cases of progression to diffuse cerebral edema and even obtundation requiring intubation.

Gust *et al* studied neurological toxicities in 133 adults with refractory B-cell acute lymphoblastic leukemia (B-ALL), non-Hodgkin's lymphomas (NHL) or chronic lymphocytic leukemia (CLL) who received lymphodepletion chemotherapy followed by infusion of CD19 CAR-T. The multivariable analysis showed that pre-existing neurological comorbidities, cyclophosphamide and fludarabine lymphodepletion, higher infused CAR-T cell dose and higher burden of malignant CD19+ B cells in marrow were associated with an increased risk of neurotoxicity.[39]

Several markers have been proposed to correlate CAR-T therapy with ICANS, such as elevated levels of ferritin, GM-CSF and a cytokine called monocyte chemoattractant protein-1.[39]

The treatment of ICANS includes a high dose of corticosteroids. However, some researchers have proposed that corticosteroid use may negatively affect the persistence of CAR-Ts and have proposed other modalities of management, such as GM-CSF neutralization, which have yet to be studied adequately.[41] Tocilizumab, which is used for severe CRS, has not been shown to be effective in treating ICANS.[42]

Other complications

Another, on target, well-described side effect with CD19 CAR-T cells is B-cell aplasia, which makes patients more susceptible to viral infections, as shown below in the 'CAR-T for lymhoma and acute lymphoblastic leukemia/lymphoma' section.

Resistance mechanisms

Understanding the mechanisms of resistance to CAR-T therapy will assist in optimizing the potential of this novel treatment to improve patient outcomes. The mechanisms of resistance to CAR-T therapy can be summarized as follows: a) CAR-T factors, b) the tumor microenvironment and c) tumor factors.[43]

Expansion, persistence and tumor cytotoxicity are the three main characteristics of CAR-Ts that influence treatment efficacy. T cells from patients with cancer are often deficient in terms of intrinsic cytotoxicity.[44] T-cell exhaustion refers to a state of dysfunction characterized by a decrease in effectors and increased expression of inhibitory receptors, usually induced by chronic stimulation, as it is in cancer.[45 46] The activation of IL-6/signal transducer and activator of transcription-3 signaling pathways promotes

central memory T-cell differentiation, which may play an important role in regulating the proliferation of CAR-Ts.[46]

Studies on the role of the tumor microenvironment in CAR-T therapy are rare. Some studies have shown that specific components of the inflammatory tumor environment, such as prostaglandin E2 produced by tumor cells in a mouse model, can affect the antitumor activity of T cells depending on IL-6, chemokine (C-X-C motif) ligand 1 (CXCL1) and G-CSF.[47] Cancer-associated fibroblasts, myeloid-derived suppressor cells and M2 subtypes of tumor-associated macrophages in the tumor microenvironment have been reported to restrict infiltration of cytotoxic T lymphocytes (CTLs).[48]

Antigen escape can occur as a potential mechanism of relapse post-CAR-T therapy. Tumor cells downregulate the targeted antigen expression or express a different epitope that is not targeted by the CAR-Ts. This has been observed in CD19-directed CAR-T therapy as well as myeloma.[49 50] Antigen escape after CD19-CAR-T is reported in about 17% of cases.[51] CD19-negative relapsed B-ALL can be due to lineage switching (conversions of leukemic cell lineage) or genetic event like SRSF3-involved alternative messenger RNA splicing of exon 2 of CD19, or other mutations in exons 2–6, which resulted in the loss of the targeted epitope in the membrane and led to immune-escape phenomena.[52–54]

Tumors can exhibit trogocytosis, which refers to decreased antigen expression on target tumor cells and, in fact, transfer of the antigen to T cells, which mediates CAR-T-induced fratricide of T cells.[55] A strategy of dual CAR-T (two different CAR-T products) or bispecific CAR-T can be used to overcome this form of resistance.[56 57]

CAR-T for acute lymphoblastic leukemia/lymphoma

ALL accounts for less than half of 1% of all cancers in the USA. In 2020, it is expected to have about 6150 new cases.[1] Five-year overall survival for pediatric ALL is about 80%–90%. However, the prognosis is much worse for relapsed disease, with 5-year survival rates of only 30%–50% after the first relapse, and <20% after subsequent relapses.[58]

CD19-targeted CAR-T therapy has shown incredible promise for the treatment of B-cell ALL. In 2013, Grupp *et al* first reported two cases of children with relapsed/refractory pre-B-cell ALL who were treated with CD19-targeted CAR-T (CTL019), later to be called tisagenlecleucel, with complete remission observed in both patients.[59] Subsequently, in a pilot study published in 2018 by the same group, the ELIANA trial showed positive responses to CD19-targeted CAR-T (CTL019), with 82% overall response and a median overall survival of 19 months.[60] About 88% of patients had a grade 3 or 4 adverse event. Out of 75 patients, 58 (77%) had CRS with median time to onset of 3 days. Intensive care unit admissions were reported in 47% of cases for management of CRS. About 89% of patients reported adverse events of special interest, which included CRS, cytopenia that did not resolve by day 28, infections, neurological events and the tumor lysis syndrome. Neurological events occurred in 40% of patients within 8 weeks after infusion. No grade 4 events or cerebral edema were reported.[60]

FDA approval was granted to tisagenlecleucel (a CD19-targeted CAR-T) in August 2017 for the treatment of relapsed/refractory B-cell ALL in patients up to 25 years of age.[61]

A phase I dose escalation trial in a National Cancer Institute study reported safety results on 19 dosed patients.[62] CRS, fever and hypokalemia were the most common non-hematological grade 3 side effects.[62]

Target identification for T-cell ALL has posed challenges in that leukemic cells exhibit the same antigens as normal T cells and in that T-cell aplasia is not a complication that may be tolerated, in contrast to the B-cell aplasia seen with B-ALL treatment, which can be treated.[31] CAR-T therapy for ALL is associated with a side-effect profile similar to that associated with other uses of this therapy. CRS is observed in nearly all patients that are treated with CD19 CAR-T but typically responds to tocilizumab (table 2).

CAR-T for lymphoma

Aggressive B-cell NHL, including diffuse large B-cell lymphoma (DLBCL), are potentially curable in 50%–60% of patients with first-line combination chemoimmunotherapy.[63] Approximately 40%–60% of patients with relapsed or refractory DLBCL respond to second-line chemotherapy; 50% of these patients proceed to undergo autologous hematopoietic stem-cell transplantation, and of these, approximately 30%–40% remain progression-free 3 years after transplantation.[64–68] A retrospective study reviewed the outcomes of 636 patients with primary refractory DLBCL or a relapse of DLBCL <12 months after autologous transplantation. The rate of response to the next line of therapy was 26%, with a complete response (CR) rate of 7%; the median overall survival duration was 6.2 months.[69] These poor outcomes reinforce the need for new therapeutic options for patients with relapsed or refractory DLBCL.

Tisagenlecleucel is an anti-CD19 CAR-T agent with a 4-1BB co-stimulatory domain. High response rates, to CD19-based CAR-T therapy, have been observed among adult patients with relapsed or refractory DLBCL. The JULIET trial enrolled 93 patients, in the efficacy analysis set, with relapsed/refractory DLBCL, overall response rate was 52% and 40% of patients showed CR and 12% showed partial response (PR). The rates of ORR and CR

Table 2 Summary of pivotal clinical trials using CAR-T therapy for all

Results of CAR-T trials for ALL						
Study	**Enrolled**	**Infused**	**Population**	**CR %**	**EFS**	**OS**
ELIANA trial; tisagenlecleucel[60]	92	75	Children and adults	81	@1 year 50%	@1 year 76%
MSK; 19–28z CAR-T[91]	83	53	Adults	83	6.1 months	12.9 months
NCI[62]	21	19	Phase I, MTX was 1×10^6 cells			

ALL, acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T-cell; CR, complete response; EFS, event free survival; MTX, methotrexate; OS, overall survival.

were 38% and 32%, respectively, at month 3 and 33% and 29%, respectively, at month 6. The JULIET trial used two different lymphodepleting regimens (fludarabine 25/m^2×3 days cyclophosphamide 250 mg/m^2×3 days or bendamustine 90 mg/m^2×2 days), for white cell count was >1000 cells per cubic millimeter within 1 week before tisagenlecleucel infusion. Response rates did not differ substantially according to the type of lymphodepleting therapy received.[70] Durable responses were observed for up to 18.4 months after infusion. The median progression-free survival duration has not been reached for patients who showed CR. The estimated rate of progression-free survival at 12 months was 83% among patients who showed CR or PR at 3 months. The median overall survival duration among patients who received infusions was 12 months. The most common adverse events of any grade were CRS (58%), anemia (48%), fever (35%), decreased neutrophil count (34%), decreased platelet count (33%), decreased white cell count (33%), diarrhea (32%), infections (20%), neurological events (12%) and febrile neutropenia (15%).[70]

Axicabtagene ciloleucel (axi-cel) is an anti-CD19 CAR-T agent with a CD28 co-stimulatory domain. The ZUMA-1 trial was a landmark study that eventually led to the FDA approval of CAR-T therapy for the treatment of large B-cell lymphomas.[71] In a ZUMA-1 trial with 111 enrolled patients, axi-cel was successfully administered to 101 of these patients (91%).[71] The ORR was 82%, and the CR rate was 54%. With a median follow-up time of 15.4 months, 42% of the patients continued to show response and 40% continued to show CR. The overall rate of survival at 18 months was 52%. The most common grade 3 or higher adverse events that occurred during treatment were neutropenia (78%), anemia (43%) and thrombocytopenia (38%). Grade 3 or higher CRS and neurological events occurred in 13% and 28% of the patients, respectively. Three of the patients died during treatment.[71]

Both, axicabtagene ciloleucel and tisagenlecleucel, gained FDA approval for treatment of relapsed/refractory DLBCL.[72 73] It is important to note the difference between the two CAR-T product and clinical trials design. axicabtagene ciloleucel used a CD28 co-stimulatory signal with retrovirus-based vector delivery, whereas, tisagenlecleucel used 4-1BB co-stimulatory signal with lentivirus-based vector delivery.

Lisocabtagene maraleucel (liso-cel; JCAR017) is another CD19-directed 4-1BB CAR-T product. The TRANSCEND-NHL-001 Study included two cohorts, the FULL dataset includes all patients in the DLBCL cohort (ie, excludes MCL) and the CORE dataset includes de novo DLBCL or transformed from follicular lymphoma without prior allogenic transplant.[74] CRS was seen in 35% of patients, and a single patient (1%) developed grade 3–4 CRS. Neurotoxicity developed in 19% of patients, and 12% of patients developed grade 3–4 neurotoxicity. The median onsets of CRS and neurotoxicity were 5 and 10 days, respectively. Nineteen patients (21%) received tocilizumab and/or dexamethasone. Ninety-one patients were treated and evaluable for safety and 88 were treated and evaluable for efficacy. The best ORRs in the FULL and CORE populations were 74% (65/88) and 80% (52/65), respectively; the best CRs were 52% (46/88) in the FULL population and 55% (36/65) in the CORE population. A higher rate of durable response with double dose of 1×10^8 cells CAR-T was observed in the CORE population, with a 6-month ORR and CR of 50% and 50% (7/14), respectively, vs 40% (8/20) and 30% (6/20) at dose level 5×10^7 cells (table 3).[64]

Table 3 Summary of CAR-T results for DLBCL

Co-stimulatory domain/vector	ZUMA-1: axicabtagene ciloleucel[71 93]	JULIET: tisagenlecleucel[70]	TRANSCEND: lisocabtagene maraleucel[74]
	CD28/Retroviral	41BB/Lentiviral	41BB/Lentiviral
Best ORR	82%	53%	80%
Best CR	58%	40%	59%
6 months ORR	41%	37%	47%
6 months CR	36%	30%	41%
CRS all grades	94%	58%	37%
CRS grade 3/4	13%	23%	1%
Neurotoxicity all grade	87%	21%	23%
Neurotoxicity grade 3/4	28%	12%	13%
Outpatient treatment	No	Yes (26%)	Yes

CAR-T, chimeric antigen receptor T-cell; CR, complete response; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; ORR, objective response rate.

CAR-T for chronic lymphocytic leukemia

Treatment of CLL has dramatically improved over the years due to the development of effective chemoimmunotherapy (CIT) regimens.[75] Monoclonal antibodies (rituximab, ofatumumab and obinutuzumab) and targeted therapies (ibrutinib, acalabrutinib, venetoclax and idelalisb) play major roles in the treatment of patients with CLL.[76–82]

Despite improvements in care, CLL is incurable and patients usually relapse after initial treatment. Experience in the use of CAR-Ts to treat CLL is limited, but safety and efficacy data are encouraging, suggesting that it may be possible to use CAR-Ts in populations of patients with CLL with particularly unfavorable prognoses. Liso-cel was used in an open-label phase I/II study of patients with relapsed/refractory CLL.[83] All patients received ibrutinib prior to the study; 56.5% had progressed on ibrutinib and received therapy with venetoclax, and 91% were refractory to or had relapsed on ibrutinib. Liso-cel was successfully manufactured in 96% of patients. Twenty-two were evaluable for efficacy, with an ORR 82% and a CR rate of 45.5%, a PR rate of 36% and stable disease reported in 14%. The most common grade 3 or higher adverse events were thrombocytopenia (70%), anemia (96%), neutropenia (56.5%) and leukopenia (43.5%). Two patients (8.6%) had grade 3 CRS and five (21.7%) had grade 3 or higher neurological events.[83]

A randomized phase II study of two CTL019 (CD19-targeting CAR) doses in R/R CLL. Twenty-eight patients treated at stage I were randomized to receive high doses or low doses; 11 patients received high doses with an ORR of 54.5% and a CR rate of 36.3%, and 13 patients received low doses with an ORR of 30.7% and a CR rate of 7.6%.[84] Both doses showed similar toxicity, so the higher dose was chosen for stage II. Twenty-one patients were treated with higher doses and 17 were evaluable for response (11 from stage I and 6 from stage II). The ORR was 53%, with 35% having achieved CR and 17.6% having achieved PR. All 35 patients were evaluable for toxicity and 19 had delayed CRS. Seven patients (20%) had grade 3 or higher CRS. The

dose of CAR-T was not associated with CRS development or severity.[84]

CAR-T for multiple myeloma

Early efforts to use CAR-T therapy for MM have demonstrated promising results. The aggregate of published CAR-T therapeutic trials targeting BCMA noted an objective response in an average of 75.9% of patients with a median duration of progression-free survival of 8.29 months.[16] As anti-BCMA trials were conducted and relapses were observed, multiple different markers were subsequently explored as potential targets as well. Several additional theoretical targets have recently been investigated, including CD138, CD19, NK cell ligands and kappa light chains; cohorts were small, but treatment efficacy ranged from no response to as high as 80% PR or very good partial response with multiple complete remissions observed.[85] Further investigative works targeting a litany of other CD receptors, G-protein signaling mechanisms, NK cell receptors and carbohydrate antigens are in process as well.[85] A number of these trials should conclude phase II testing by the year's end, and several more are slated to begin phase III testing over the course of 2019 as well.[86]

Despite recent advancements and excitement surrounding potential new targets under investigation, barriers to long-term durable responses still exist. Antigen loss, or the downregulation or loss of the target antigen on tumor cells, remains a principle obstacle to the longevity of CAR-T-mediated responses.[87] The process of BCMA antigen transfer from the tumor cell to the CAR-T itself with subsequent recognition and destruction of fellow CAR-Ts has also been described.[55] Furthermore, suboptimal CAR-T persistence and continued long-term efficacy remain additional barriers to durable remission as well.[43]

Lastly, with the increasing effort and investment in CAR-T therapy for myeloma comes augmented costs as well as swelling patient bases. The European Myeloma Network has indicated a need for a more robust registry of patients undergoing CAR-T therapy as well as a need for expert-level consensus on appropriately managing escalating costs (table 4).[86]

Table 4 Summary of pivotal CAR-T trials for relapsed/refractory multiple myeloma with expected approval in the next few months

Summary of pivotal CAR-T trials for myeloma		
Trial	**KarMMa-1**[16]	**CARTITUDE-1**[94]
Product	BB2121	LCAR-B38M
ORR	85%	91%
CR or better	45%	6 out of 21 patients
MRD negativity	15 patients were MRD-negative at the 10^{-5} sensitivity level	10 patients were MRD-negative at the 10^{-5} sensitivity level
CRS	26%	88%
PFS	11.8 months	NA

*Multiple other ongoing clinical trials in early/newly diagnosed myeloma are likely to be reported soon.
CAR-T, chimeric antigen receptor T-cell; CR, complete response; CRS, cytokine release syndrome; MRD, minimal residual disease; NA, not available; ORR, overall response rate; PFS, progression-free survival.

CONCLUSION

The outstanding outcomes of immunotherapy have sparked major interest in the treatment of DLBCL, ALL, CLL and MM. CAR-T therapy is an innovative approach to overcoming conventional drug resistance and has demonstrated the ability to selectively extirpate malignant cells. CAR-Ts are genetically modified cells, lymphocytes or NK cells that specifically target selective antigens. Currently, CAR-T therapy is approved for the management of relapsed/refractory DLBCL and ALL, and it is likely to gain approval for relapsed refractory MM and CLL.

CRS, ICANs and prolonged immune suppression are all unique adverse events that can occur after CAR-T therapy and require a special attention for early detection and management. Finally, understanding the mechanisms of resistance to CAR-T therapy is the first step to cultivating better CAR-T constructs.

Twitter Shebli Atrash @AtrashShebli

Contributors BH, KB, A-OA and SA helped writing the manuscript. SA did the final review and editing.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Obtained.

Provenance and peer review Commissioned; externally peer reviewed.

ORCID iD
Shebli Atrash http://orcid.org/0000-0003-4547-7534

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