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R E V I E W

Axicabtagene ciloleucel (KTE-C19), an anti-CD19 CAR T therapy for the treatment of relapsed/ refractory aggressive B-cell non-Hodgkin's lymphoma

Michael D Jain[1,2]
Christina A Bachmeier[3]
Vania H Phuoc[2]
Julio C Chavez[2,4]

[1]Department of Blood and Marrow Transplantation and Cellular Immunotherapy, Moffitt Cancer Center, Tampa, FL, USA; [2]Department of Oncologic Sciences, University of South Florida, Tampa, FL, USA; [3]Department of Pharmacy, Moffitt Cancer Center, Tampa, FL, USA; [4]Department of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

Abstract: Adoptive T-cell immunotherapy is a rapidly growing field and is shifting the paradigm of clinical cancer treatment. Axicabtagene ciloleucel (axi-cel) is an anti-CD19 chimeric antigen receptor T-cell therapy that was initially developed at the National Cancer Institute and has recently been commercially approved by the US Food and Drug Administration for relapsed or refractory aggressive non-Hodgkin's lymphomas including diffuse large B-cell lymphoma and its variants. The ZUMA-1 Phase I and II clinical trials formed the basis of the US Food and Drug Administration approval of this product, and we discuss the particulars of the clinical trials and the pharmacology of axi-cel. In addition, we review the CD19 chimeric antigen receptor T-specific toxicities of cytokine release syndrome and neurotoxicity, which remain the challenges to the safe delivery of this important therapy for aggressive B-cell lymphomas with poor prognosis.

Keywords: axicabtagene ciloleucel, CAR T, non-Hodgkin's lymphoma, immunotherapy

Epidemiology of aggressive B-cell lymphomas

Aggressive B-cell lymphoma is a subtype of B-cell non-Hodgkin's lymphomas (NHLs) that encompasses a clinically and molecularly heterogeneous group. In this group, diffuse large B-cell lymphoma (DLBCL) is the most common with ~22,000 cases a year and it represents 30%–35% of all lymphomas.[1] Within DLBCL itself, there is additional heterogeneity and this is reflected in the World Health Organization 2016 classification that further subtypes of DLBCL to include high-risk histologies such as high-grade B-cell lymphomas not otherwise specified (NOS) and high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements, known as double (DHL) or triple hit lymphomas (THL).[2] Primary mediastinal B-cell lymphoma (PMBCL) and transformed follicular lymphoma (TFL) are less frequent, but important variant subtypes that are also described.

Clinical indicators of prognosis in DLBCL

The prognosis of aggressive B-cell lymphomas is based on clinical, molecular and genetic factors as well as responsiveness to induction chemotherapy. The International Prognostic Index (IPI), based on clinical features found at initial diagnosis (age, stage, extranodal disease, performance status and lactate dehydrogenase elevation) is historically the most widely used and is a powerful prognostic system.[3] The revised

Correspondence: Julio C Chavez
Department of Malignant Hematology, Moffitt Cancer Center, 12902 Magnolia Drive FOB3, Tampa, FL 33612, USA
Tel +1 813 745 4294
Email julio.c.chavez@moffitt.org

version of the IPI score extended the predictive value of the IPI into the rituximab era (ie, when the anti-CD20 rituximab became widely added to chemotherapy due to overall survival [OS] benefits).[4] An enhanced scoring system, the National Comprehensive Cancer Network-International Pronostic Index (NCCN-IPI) score, stratifies newly diagnosed DLBCL patients in four risk groups based on the same prognostic factors used in the IPI score, but with better discrimination of the low- and high-risk subgroups. Patients in the high-risk subgroup category per the revised version of the IPI score and the NCCN-IPI score have a 5-year OS of 54% and 33%, respectively.[5,6] Overall, the prognostic scores highlight that a proportion of patients do not have successful outcomes with standard DLBCL treatment.

Molecular and genetic factors predictive of poor prognosis in DLBCL

Molecular profiling studies and next-generation sequencing have improved the understanding of DLBCL and its heterogeneity, and may prospectively identify cases with high-risk features.[7,8] Based on the cell of origin using gene expression profiling studies, DLBCL can be classified as activated B-cell (ABC) subtype and germinal center B-cell (GCB) subtype (with 10% of cases being unclassifiable). ABC and GCB have distinct phenotypes and clinical characteristics, and with upfront chemotherapy, the ABC–DLBCL subtype has inferior outcomes after standard R-CHOP.[9–11] Widespread use of gene expression profiling or next-generation sequencing remains limited due to cost and complexity, while immunohistochemistry methods are more practical for most pathology laboratories. By immunohistochemistry, the Hans classifier uses three markers (CD10, BCL6 and MUM1/IRF4) and classifies DLBCL as GCB or non-GCB, and seems to be a useful tool to differentiate DLBCL by the cell of origin.[12,13] That said, RNA-based diagnostic testing to discriminate ABC and GCB subtypes appears to have higher accuracy, and recent advances allow testing in standard formalin-fixed paraffin-embedded biopsies rather than requiring fresh or frozen material.[14]

Another high-risk subgroup of DLBCL patients is characterized by the chromosomal translocation of the proto-oncogene c-MYC that promotes uncontrolled cell growth, unregulated cell division and extranodal dissemination of lymphomas.[15] IgH-MYC rearrangements detected by fluorescent in situ hybridization or cytogenetics can be present in 3%–17% of all DLBCL cases and have particularly poor outcomes when coupled with BCL2 and/or BCL6 rearrangements (DHL or THL).[16–18] Clinically, DHL and THL are characterized by advanced disease stage, highly elevated lactate

dehydrogenase and central nervous system involvement.[19,20] The optimal treatment of DHL has not been well defined, but it appears that frontline intensive regimens (such as dose-adjusted EPOCH-R with central nervous system prophylaxis) are associated with better outcomes.[20–22] Given its poor outcomes with standard chemotherapy, DLBCL patients with c-MYC rearrangements represent another unmet need.

Response to therapy as a prognostic indicator

The addition of rituximab to CHOP improved the OS of patients with DLBCL; however, despite this advancement, ~30%–40% of patients relapse or are refractory to this regimen.[23] In general, patients with refractory disease (less than partial response [PR] to initial treatment) or early relapse (relapse within a year from diagnosis or 6 months after the end of treatment) or progression within 2 years (event free survival 24) have poorer prognosis than those with late relapses.[24,25] The current standard for relapsed DLBCL consists of salvage chemotherapy followed by autologous stem cell transplantation (ASCT) with a proportion of patients achieving cure.[24,26] Approximately 50% of these patients who completed ASCT will ultimately relapse with minimal hope of cure despite international efforts at clinical trials and drug development in DLBCL.[24,27,28] Indeed, the prognosis of relapsed DLBCL after ASCT relapse is poor with a median OS of 10 months, and is particularly worse for those relapsing within 6 months of transplantation with a median OS of 5.7 months.[27,29]

A retrospective international multicenter study (SCHOLAR-1) was carried out using data from Phase III randomized clinical trials (the European trial CORAL and the Canadian Clinical Trials Group LY.12 trial) and two observational studies (MD Anderson Cancer Center and University of Iowa/Mayo Clinic) that evaluated patients who achieved stable disease (SD) or progressive disease as the best response at any point during chemotherapy, or if relapse occurred within 12 months of ASCT. The results of this pooled analysis showed an overall response rate of 26% with a median OS of 6.3 months.[30] Another large retrospective study of 15 US academic institutions assessed the risk factors of refractory DLBCL. Three subgroups were identified to have poor outcomes: primary progression (progressive disease with or within 6 months of chemoimmunotherapy [CIT]), residual disease (PR or SD after completion of CIT) and early relapse (relapse within 6 months of CIT and having achieved a complete response [CR]), with a 2-year OS of 18.5%, 30.6% and 45.5%, respectively.[31] Multivariate analysis demonstrated that ultra-high risk (UHR) features

in this cohort were primary progression, the presence of MYC rearrangement and intermediate-high or high NCCN-IPI score at the time of progression. The 2-year OS was very poor at 13.9% if any of these UHR factors were present (versus 57.6% in those without UHR features).[31] These poor outcomes represent a significant unmet need in which novel approaches are urgently needed. It is in the landscape of relapsed/refractory and UHR DLBCL that CD19 chimeric antigen receptor (CAR) T cells have been studied to date.

Pharmacology and mode of action of axicabtagene ciloleucel (axi-cel)

One mechanism by which the immune system is able to recognize and fight cancer cells is through activation of T-lymphocytes that use T-cell receptors to recognize tumor peptides presented on major histocompatibility complexes.[32] Once the T cells are activated, proliferation of T cells and cytotoxic granule secretion ensues, which leads to antitumor activity and lysis of tumor cells. However, tumors perform immune evasion by several mechanisms including increased expression of immune checkpoints (that limit T-cell activation and cause T-cell exhaustion) and effects of the tumor microenvironment. The design of CAR T cells is intended to enhance T-cell responses against tumor cells. The CARs are composed of an antibody-derived single-chain variable fragment linked to CD28 and CD3 zeta signaling domains. The combination of the specificity of a monoclonal antibody with the activation domain of T cells allows the CARs to deliver activated T cells with potent cytotoxicity against tumor-specific antigens and to target tumors independently of the major histocompatibility complex. Overall, CAR T engagement of CD19-expressing cancer cells results in T-cell activation, proliferation and secretion of inflammatory cytokines and chemokines resulting in tumor cell lysis.[33,34]

First-generation CARs utilized a signaling domain composed of CD3 zeta only and demonstrated weak proliferation ability and short survival, with short-lived antitumor activity. Co-stimulatory molecules, such as CD28, were introduced in second-generation CARs, and they proved to significantly improve signaling strength, expansion and persistence of CAR T cells.[35] There are other co-stimulatory domains that are effective in improving T-cell signaling; however, CD28 and 4-1BB are currently the most widely used in CAR T-cell clinical trials. The optimal co-stimulatory domain is currently unknown. In general, the CD28-based CAR T-cell construct exhibits a greater peak expansion, whereas CAR T cells using 4-1BB co-stimulation show greater longevity (persistence).[36] Further data are needed to confirm these findings and to find out whether the co-stimulatory domain used impacts clinical outcomes.

CD19 is a transmembrane glycoprotein that is expressed at all stages of differentiation of normal B cells. As a target, CD19 is expressed in over 95% of B-cell malignancies including chronic lymphocytic leukemia, B-cell NHL and acute lymphoblastic leukemia. Thus, CD19 is an attractive target for immunotherapeutic approaches with several companies and academic institutions developing pivotal trials with anti-CD19 CAR T cells.[37,38]

The development of axi-cel began with preclinical and clinical work at the National Cancer Institute (NCI). Initial studies demonstrated the proof of principle that anti-CD19 CAR T cells derived from human T cells can eradicate CD19-expressing malignant B cells, co-stimulation is required for CAR T-cell persistence and efficacy and finally CD19 CAR T cells can be clinically useful.[34,39] The NCI CD19 CAR T cells were then developed by Kite Pharma (now owned by Gilead), leading to axi-cel, an US Food and Drug Administration-approved CD19-directed cellular immunotherapy of autologous T cells genetically engineered to express CARs.[39]

Pharmacokinetics

As a cytoreductive agent targeting rapidly dividing cells, chemotherapy has the greatest impact immediately following initiation of therapy. The decrease in tumor burden is initially dramatic, but short-lived, resulting in a return to the pretreatment growth rate and necessitating multiple cycles of chemotherapy to provide a long-term antineoplastic effect.[40]

Axi-cel offers a unique mechanism of action and pharmacokinetic profile in the treatment of NHL. In contrast to cytoreductive therapy, the decrease in tumor burden is not as rapid, but sustained after a single dose infusion. Although CAR T cells have the potential to cause serious toxicities such as cytokine release syndrome and neurotoxicity, the targeted action of axi-cel against CD19 limits additional adverse effects related to the damage of healthy cells to on-target off-tumor effects. Due to the expression of CD19 on all nonmalignant B-cell lymphocytes, on-target prolonged suppression of B cells (B-cell aplasia) is one of the complications of CD19 CAR T-cell therapy.[41]

Axi-cel is a single-dose infusion containing a suspension of 2×10^6 CAR-positive viable T cells per kilogram body weight (maximum of 2×10^8 CAR-positive viable T cells) in ~68 mL.[42] Following administration, axi-cel exhibits a rapid expansion, with peak CAR T-cell levels occurring within 7–14 days. In the pivotal Phase II trial, ZUMA-1, of axi-cel in large B-cell lymphoma, CAR T cells remained detectable in
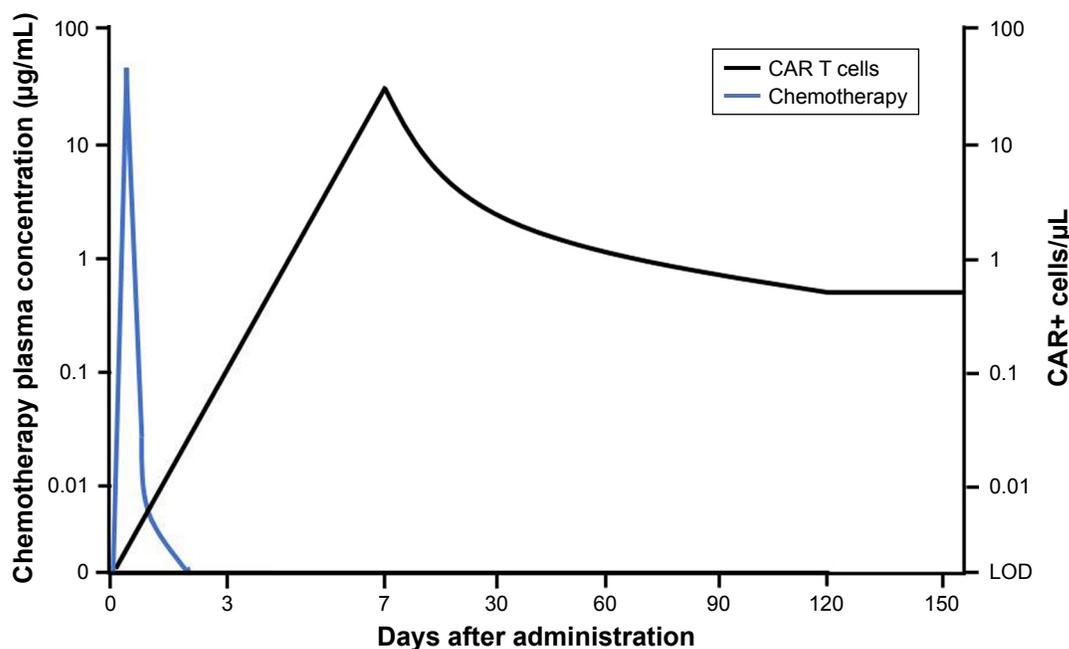
Figure 1 Comparative serum concentrations of chemotherapy and CAR T-cell expansion.
Notes: Traditional cytoreductive chemotherapy has the greatest impact immediately following initiation; however, it is short-lived, requiring multiple cycles for a long-term antineoplastic effect. In contrast, the pharmacokinetic profile of axi-cel leads to a slower initial decrease in tumor burden, but it is sustained after a single infusion due to potential persistence of CAR T cells in patients' blood over time.
Abbreviations: CAR, chimeric antigen receptor; LOD, limit of detection.

most patients at 180 days. The median CAR T area under the curve (AUC), defined as the cumulative level of CAR-positive cells/μL of blood over the first 28 days following axi-cel, was 462.3 cells/μL (range 5.1–14,329.3). Notably, CAR T expansion and peak cellular concentration within 28 days of infusion demonstrated a positive correlation with objective clinical response. The CAR T AUC was 5.4 times higher in patients with an objective response rate (ORR) versus patients without a response. Of the patients obtaining a CAR T AUC exceeding the median AUC, the ORR was 96%.[43,44] These results further emphasize the importance of CAR T expansion post-infusion, as observed in previous studies.[45,46] Peak expansion was also associated with grade 3 or higher neurologic events, but was not correlated with cytokine release syndrome[43] (Figure 1).

In contrast to cell expansion, the significance of cell persistence is unclear. CAR T-cell persistence and B-cell aplasia have been reported with axi-cel.[47,48] In ZUMA-1, three patients with an ongoing complete remission at 24 months still had detectable blood levels of CAR T cells.[43] However, recent data from earlier studies of NCI showed that long-term complete remissions are documented with limited persistence and recovery of normal B cells.[48] This is in contrast to patients with acute lymphoblastic leukemia in whom recovery of normal B cells may be associated with poor prognosis.[49,50]

Long-term persistence allows for continued disease assessment, however, at the cost of long-term B-cell aplasia.[51] The effect of CAR T-cell persistence is unknown with limited and conflicting data; determining the role and impact on disease response is an area of ongoing research.

Manufacturing

The general manufacturing processes of CAR T cells consist of T-cell collection (harvesting), T-cell selection (through magnetic beads) and activation (with an anti-CD3 antibody in the presence of interleukin [IL]-2), introduction of the CAR gene into the activated T cell through a vector (lentivirus or retrovirus), CAR T-cell expansion and CAR T-cell product formulation.[52,53] In order to streamline the process and improve the support for large global trials (or even widespread clinical use), some key steps were taken for the manufacturing of axi-cel, such as removal of human serum from the culture media, thus reducing the infection risk, and excluding the use of magnetic beads for T-cell selection. In addition, there was a decrease in the duration of ex vivo expansion, thus minimizing the risk of T-cell exhaustion.[52,54] This process led to the production of predominantly $CD3^+$ T cells with $CD8^+$ T cells in 57% and $CD4^+$ T cells in 43%, with the majority being effector T cells and central memory T cells (T_{CM}).[54] The process was

highly successful and the expected time of axi-cel production was <2 weeks.[43,54]

Efficacy of axi-cel
Lymphoma response criteria and trial design

Results of axi-cel in DLBCL and variants have mainly been reported in terms of patients who received infusion of the CAR T cells, although an intent-to-treat analysis is also reported.[43,44] Although the lymphoma response criteria have been updated, in ZUMA-1, the Cheson 2007 criteria were used, which allows a comparison with prior clinical trials.[55,56] By these criteria, the ORR is defined as the rate of patients attaining a CR (disappearance of measurable disease on computed tomography scan or residual masses that are positron emission tomography negative) or a PR (50% decrease in tumor burden with ongoing positron emission tomography avidity). However, for the purposes of clinical outcome in aggressive lymphoma, attaining CR is the most important and it is further important that these CRs are durable and that relapse does not occur.

Axi-cell was initially tested outside of the NCI in the Phase I ZUMA-1 study; this was the first multicenter study of CAR T-cell therapy in refractory aggressive NHL including DLBCL, TFL and PMBCL. This study included seven patients and showed durable responses with axi-cel with an ORR and CR rate of 71% and 57%, respectively.[47] These encouraging results led to the multicenter Phase II portion of ZUMA-1. Key eligibility criteria for this study were aggressive B-cell lymphomas (DLBCL, PMBCL and TFL), refractory disease with no response to last therapy or relapse within 12 months of autologous hematopoietic stem cell transplantation, prior anti-CD20 monoclonal antibody and anthracycline and an Eastern Cooperative Oncology Group (ECOG) of 0–1. The primary endpoint was ORR in patients with >6 months follow-up post-axi-cel infusion. The secondary endpoints were duration of response, OS, safety and levels of CAR T cells and cytokines. The CAR manufacturing success was 99%. The conditioning regimen consisted of cyclophosphamide 500 mg/m^2 and fludarabine 30 mg/m^2 ×3 days followed by infusion of KTE-C19 or axi-cel at a dose of 1–2×10^6 CART cells/kg. The conditioning regimen and doses were determined in previous studies.[57]

Efficacy of axi-cel on ZUMA-1

Administration of axi-cel was highly efficacious in 101 patients with refractory/relapsed DLBCL, who were considered to have a poor prognosis with a median OS of ~6 months and no available standard therapies.[30] The median time

to response was 1 month, which is the timing of the first response assessment in the trial protocol of ZUMA-1. However, the range was 0.8–6.0 months, which indicates that patients not showing response at the 1-month assessment could convert and be seen to respond in subsequent months. Indeed, 11 of 35 patients in PR and 12 of 25 patients with SD at 1 month subsequently converted to CR. That said, patients who never attained CR (maximum response of PR) had a median duration of response of 1.9 months only, consistent with the usual situation in aggressive lymphoma where PR is not an adequate depth of response[44] (Table 1).

Including all variants of DLBCL enrolled, the ORR of axi-cel was 82% with a CR rate of 54%. The expected ORR of conditioning doses of fludarabine and cyclophosphamide alone without CAR T cells is not known. In general, fludarabine-based regimes are not considered to have significant antitumor activity in aggressive lymphomas.[58–60] Therefore, these results suggest that axi-cel was active in the majority of patients due to the anti-lymphoma effects of the cellular product.

Although no statistical test was applied, the CR rate of the variants of DLBCL (TFL and PMBCL) was numerically higher than that for de novo DLBCL (DLBCL, CR rate 49%; TFL/PMBCL, CR rate 71%).[43,44] Beyond this, the subgroup analysis did not identify clinical or disease-related risk factors that predicted response. The caveat of the subgroup analysis is that it is underpowered and may not identify true differences. Nonetheless, typical DLBCL risk factors such as age, stage, IPI risk score or bulky disease >10 cm did not obviously predict CAR T failure per multivariate analysis.[43]

The earliest patients treated with the CD19 CAR T construct at the NCI (the same construct as axi-cel) have attained long-term durable CRs up to 56 months.[48] In ZUMA-1, the updated analysis was conducted after a minimum follow-up of 1 year, with 42% of patients still in CR. In general, very few relapsed occurred if patients remained in CR at 6 months post-CAR T infusion, but long-term follow-up will be required to determine the curative potential of this therapy.

Efficacy comparison of axi-cel to other CD19 CAR T products in DLBCL

When comparing the efficacy of axi-cel to other CD19 CAR T-cell products including tisagenleucel (tis-gen) and lisocabtagene maraceucel (liso-cel), it is important to note some key differences in trial design. For example, tis-gen was tested in both DLBCL and follicular lymphoma, and not all DLBCL patients (12 patients – 86%) met the criteria for refractory disease.[61] Conversely, the variants TFL and

Table 1 Highlights of the ZUMA-1 trial of axicabtagene ciloleucel, a CD19 CAR T-cell product, in DLBCL and variants

Highlights of the Zuma-1 clinical trial with axicabtagene ciloleucel (Yescarta) in DLBCL and variants	
Product	Autologous T cells expressing a CAR: • Antibody fragment to target CD19 • Intracellular domains of CD3 zeta (to activate T-cell receptor signaling) and CD28 (for co-stimulation and persistence)
Inclusion/exclusion highlights	• DLBCL, TFL and PMBCL all allowed. Richter transformation from CLL, indolent lymphoma and mantle cell lymphoma was not included • Chemorefractory disease: stable disease or progressive disease as the best response to the most recent regimen (second line or greater), or progression within 12 months of autologous stem cell transplant • No history of CNS lymphoma or current involvement
Manufacturing	• Median time from leukapheresis to delivery 17 days • Successful CAR T production in 99% of patients
Conditioning and dose	• Days −5 to −3: Fludarabine 30 mg/m^2 and cyclophosphamide 500 mg/m^2 daily • Day 0: 2×10^6 CAR T cells/kg
Bridging while awaiting cell infusion	• Chemotherapy not allowed • Ten of 111 patients with successful manufacturing did not proceed to CAR T-cell infusion. Seven were due to adverse events or death prior to infusion
Efficacy	• Median time to response 1 month (range 0.8–6.0 months) • ORR 82%, CR 54%, 6-month PFS 49%. Relapses appear to be infrequent after 6 months • TFL and PMBCL have a higher CR rate than de novo DLBCL (71% versus 49%) • Historical cohort (SCHOLAR-1) ORR 26%, median OS of 6.3 months
Deaths	Of 101 patients receiving CAR T cells: • 37 died of disease progression • 2 died of CRS-associated events (cardiac arrest, HLH) • 1 died of pulmonary embolus
Toxicity	• CRS grade 3 or higher: 13% • Neurologic events grade 3 or higher: 28% • Toxicity management with tocilizumab or steroids did not appear to affect CAR T-cell efficacy
Biomarkers	• CAR T-cell efficacy was not affected by classic prognostic markers in DLBCL, such as IPI, cell of origin (ABC versus GCB) or number of prior lines of therapy • Expansion (peak CAR T-cell levels in the blood) was correlated not only with response, but also with neurologic events • Cytokine release was associated with CRS and neurologic events

Abbreviations: ABC, activated B-cell subtype of DLBCL; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CR, complete response rate; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell subtype of DLBCL; HLH, hemophagocytic lymphohistiocytosis; IPI, International Prognostic Index; ORR, objective response rate (includes complete and partial responses); OS, overall survival; PFS, progression-free survival; PMBCL, primary mediastinal B-cell lymphoma; TFL, transformed follicular lymphoma; CNS, central nervous system; ABC, activated B-cell subtype of DLBCL.

PMBCL (with high CR rates for axi-cel) were not included. The efficacy results of tis-gen were similar to those of axi-cel with 6 out of 14 DLBCL patients (43%) attaining CR.[61] Similarly, liso-cel (at the optimized dose level) showed a 6-month CR rate of 50%, in 14 patients with DLBCL or TFL only.[62] While cross-trial comparisons should be viewed with great caution for methodological reasons, overall, it seems that the efficacy of axi-cel is similar to that of the other two CD19 CAR T-cell products. This is despite substantial design and manufacturing differences among the three products (reviewed in Jain and Davila[63]).

Biomarkers of efficacy

After infusion into the patient, CAR T cells duplicate inside the patient. On ZUMA-1, CAR T-cell numbers rapidly increased, peaking at day +7 after the infusion.[64] The peak number of cells (known as peak expansion) was fourfold higher in responding patients compared to that in patients who

did not respond (p=0.002), highlighting that expansion is important to obtain a response. An identified determinant of CAR T expansion was the composition of the T-cell subsets that made up the manufactured axi-cel product.[65] Axi-cel product that had a higher proportion of naïve and T$_{CM}$ led to superior expansion after infusion compared to the product that had more effector and effector memory T cells. It is not well understood which clinical, biologic or manufacturing factors promote naïve and T$_{CM}$ retention in manufactured CAR T-cell products.

Safety and tolerability

While anti-CD19 CAR T-cell therapy offers a new therapeutic strategy for aggressive B-cell lymphomas, its growing use requires further education/training and prompt recognition and management of these unique toxicities. In the primary analysis of ZUMA-1, all 101 patients who received axi-cel encountered adverse events, with 95%

having grade 3 or higher related adverse events.[43] Two of the most common toxicities observed are cytokine release syndrome (CRS) and CAR T-cell–related encephalopathy syndrome (CRES). Close monitoring parameters, early identification and intensive supportive management minimize the life-threatening risks caused by this potentially curative therapy.

Cytokine release syndrome

CRS is the most common and well-described toxicity associated with CAR T-cell therapy, occurring in over 90% of patients at any grade, as reported in the ZUMA-1 clinical trial.[43] The activation of T cells upon recognition of tumor antigens by CARs results in the release of inflammatory cytokines and chemokines including IL-6, soluble IL-6R, IL-2, soluble IL-2Rα, interferon-γ, tumor necrosis factor α, IL-8, and IL-10, granulocyte-macrophage colony-stimulating factor and activated immune effector cells.[66]

Given the nature of the systemic inflammatory response, any organ system can be affected, including cardiovascular, pulmonary, gastrointestinal, hepatic, renal, nervous, integumentary, musculoskeletal and hematologic systems. The most common symptoms of CRS include pyrexia, hypoxia and hypotension, and the onset of CRS symptoms typically occurs within the first week after CAR T-cell infusion with a median onset at 2 days in the ZUMA-1 trial. However, CRS sometimes persists, with symptoms resolving by a median time of 8 days.[41,43] As the use of CAR T-cell therapy becomes widespread, more experience and knowledge with clinical use will lead to more detailed assessment systems, algorithms, preventive measures and supportive interventions.

Lee et al proposed the current consensus criteria for CRS grading as seen in Table 2, and organ toxicity assessment

Table 2 Grading of CRS

Grade 1	Grade 2	Grade 3	Grade 4
Fever (≥38.3°C)	Hypotension responsive to fluids or one low–dose vasopressor	Hypotension requiring multiple or high-dose vasopressors	Requiring mechanical ventilation
Constitutional symptoms	Hypoxia responsive to <40% FiO$_2$	Hypoxia requiring ≥40% FiO$_2$	Organ toxicity grade 4 excluding transaminitis
	Organ toxicity grade 2	Organ toxicity grade 3 Grade 4 transaminitis	

Abbreviation: CRS, cytokine release syndrome.

was graded as per the Common Terminology Criteria for Adverse Events (v4.0).[67] For management of grade 1 and 2 CRS, there should be assessment for infection and vigilant supportive care including intravenous fluids, antipyretics and analgesics as needed, with a close monitoring of cardiac complications and other organ function. Tocilizumab is an anti-IL-6 monoclonal antibody that can be considered in patients with identified CRS, and tocilizumab can result in rapid resolution of CRS toxicities without loss of CAR T-cell expansion or efficacy. The timing for tocilizumab administration has evolved in the last few years, especially since more experience has been gained with high-risk immunotherapy trials. It was initially recommended for grade 3 CRS, but the current consensus recommendation is to use it with grade 2 CRS and higher.[43,44] Tocilizumab doses of 4–8 mg/kg (maximum dose 800 mg) can be repeated as needed for patients with persistent signs/symptoms of CRS. However, if there is no improvement despite anti-IL-6 therapy with ongoing severe CRS grade 3 or higher, high-dose corticosteroids could be considered. The role of steroids is fundamental in the treatment of CRS (and neurotoxicity) and should be considered for severe cases after tocilizumab therapy failure or even concomitantly to anti-IL-6 therapy in special situations.[66,67] Although there is a theoretical risk of subsequent lymphotoxic effect and CAR T-cell suppression with steroid therapy, this has not been demonstrated in the ZUMA-1 study.[43,68] In the ZUMA-1 trial, tocilizumab and steroids (at various doses) were administered to 43% and 27% of patients, respectively, for the treatment of CRS and/or neurotoxicity[43] (Table 1).

CAR T-cell–related neurotoxicity

Grading scales for neurologic events in the ZUMA-1 trial followed the NCI Common Terminology Criteria for Adverse Events, version 4.03, and the grading of common neurotoxicities seen is displayed in Table 3. In this trial, 64% of patients experienced a neurologic event at any grade, with 28% having grade 3 or higher neurotoxicity.[43,44] The best described is CRES, which occurred in 34% of patients to any grade and 21% of patients at grade 3. Other grade 3 neurologic events frequently observed were confusional state, aphasia and somnolence, and early neurologic signs included word-finding difficulties, inattention, disorientation, agitation or seizures. Median onset of neurotoxicity occurred at day 5 post-CAR T-cell infusion, with the median time of resolution of neurologic symptoms being day 17. All patients who developed neurologic symptoms had complete resolution, except for four patients who died from either progressive disease or unrelated adverse events[43] (Table 1).

Table 3 Grading of the common neurotoxicity symptoms

Neurotoxicity grading assessment (CTCAE 4.03)	Grade 1	Grade 2	Grade 3	Grade 4
Encephalopathy	Mild limiting of ADL	Limiting instrumental ADL	Limiting self-care ADLs	Life-threatening consequences
Confusion	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
Dysphasia	Awareness of receptive or expressive characteristics; impaired ability to communicate	Moderate receptive/expressive characteristics; impaired ability to communicate spontaneously	Severe receptive/expressive characteristics; impaired ability to read, write or communicate intelligibly	N/A
Seizure	Brief partial seizure; no loss of consciousness	Brief generalized seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures
Somnolence	Mild, but more than usual drowsiness or sleepiness	Moderate sedation; limiting instrumental ADL	Obtundation or stupor	Life-threatening; urgent intervention indicated

Note: Neurotoxicity grading assessment of common adverse events from CAR T-cell therapy.
Abbreviations: ADL, activities of daily living; CAR, chimeric antigen receptor; CTCAE, Common Terminology Criteria for Adverse Events; N/A, not applicable.

In a review of 133 adults who received lymphodepletion chemotherapy followed by infusion of CD19 CAR T cells, 53 (40%) developed one or more grade 1 or higher neurologic adverse events. Grade ≥3 neurotoxicity was associated with more severe CRS, disseminated intravascular coagulation with subsequent coagulopathy, earlier peak of IL-6 concentration and evidence of endothelial activation with capillary leak.[69] Patients who developed severe neurotoxicity also showed increased cerebrospinal fluid protein levels, increased cells including CAR T cells, and inflammatory cytokines such as IL-6, interferon-γ and tumor necrosis factor α in the cerebrospinal fluid.[66,69] This suggests increased blood–brain barrier permeability associated with the pathophysiology of neurotoxicity after CAR T-cell infusion.

Monitoring and grading of CRES involves close neurologic assessments with ongoing evaluation for signs or symptoms of further complications including increased intracranial pressure and seizures. Grade 1 CRES involves supportive care with consideration of electroencephalogram, magnetic resonance imaging, funduscopic exam and neurology consultation, and grade 2 CRES adds to this management with anti-IL6 therapy, particularly if concurrent CRS occurs. More severe CRES grades 3 and 4 require intensive care unit admission, corticosteroids for symptoms not responding to anti-IL-6 and medical management of status epilepticus and increased intracranial pressure with consideration of neurosurgical evaluation if indicated.[66]

Other safety and supportive care considerations

Other commonly encountered and potential risks include cytopenias, B-cell aplasia, consumptive coagulopathies, neutropenic fever, infections and tumor lysis syndrome. Patients enduring B-cell aplasia may require intravenous immunoglobulin infusions for prolonged period of time.[66] Ongoing investigations are looking into preventive measures to limit toxicity experienced by patients receiving CAR T-cell therapy. Efforts include identifying baseline and treatment-related risk factors for development of CRS, which have been associated with higher marrow tumor burden, fludarabine/cyclophosphamide lymphodepletion and higher CAR T-cell dose.[70]

Prophylactic supportive care considerations have previously consisted of seizure prophylaxis with levetiracetam 750 mg orally every 12 hours for 30 days starting on the day of CAR T infusion, tumor lysis precautions per institutional guidelines and other institution-dependent algorithms for monitoring and symptom management.[66,67] As clinical utilization and experience grows, further standardized assessments, grading and adverse event management protocols can be developed for improved early recognition and expedited treatment.

Patient-focused perspectives

The results of ZUMA-1 (and other multicenter studies in CAR T-cell therapy for refractory lymphomas) met the primary endpoint of improving objective responses in a patient population with refractory aggressive B-cell lymphomas with otherwise no options of cure.[30,43,48] Thus, these data suggest that there is a significant patient benefit with CAR T-cell therapy. It is most likely to be accepted as a viable alternative for the disease with an otherwise very poor prognosis, despite the potential associated toxicity.

The toxicities of CAR T-cell therapy are unique; thus, strategies for recognition and management of CRS and CRES

are of paramount importance in order to minimize and/or speed up the recovery of patients receiving this type of high-risk immunotherapy. Conversely, patient education will become essential not only for understanding the efficacy and toxicity of CAR T-cell therapy, but also for the recognition of early toxicity signs. The development of guidelines and harmonization of the management of CAR T-cell therapy–related toxicities have been the focus of investigators recently.[66,67] In addition, the development of institutional guidelines and specialized immunotherapy centers with multidisciplinary support from different specialties and allied health providers may ensure the success of utilizing this therapy in a large scale for further benefiting patients.[71]

With the approval of axi-cel for the treatment of refractory DLBCL and since its clinical benefit will likely outweigh the risk associated with this therapy, a Risk Evaluation and Mitigation Strategies program has been established in order to mitigate the side effects related to CAR T-cell therapy. The Risk Evaluation and Mitigation Strategies program ensures that hospitals or cancer centers are adequately trained for the management of the toxicity and have the essential elements to treat toxicity (ie, availability of tocilizumab).

As this therapy will become widely available as the standard of care for refractory aggressive B-cell lymphomas, it is important to recognize that not all patients will have a favorable clinical condition/comorbidities that will make them candidates to receive this therapy. Honest discussion of the pros and cons of CAR T-cell therapy should occur and alternatives should be offered, including quality of life recommendations.

Conclusion

The development of CAR T-cell therapy represents the paradigm of personalized medicine and cancer immunotherapy. With the imminent widespread use (given the US Food and Drug Administration approval), one of the main focuses has been the optimization of CAR T-cell construct technology and improving the manufacturing process in order to increase its efficiency without affecting its activity and potency.[72] The understanding and management of CAR T-related toxicities represent another challenge and focus of research.

The dramatic responses seen with CAR T-cell therapy in B-cell lymphomas (and other hematologic malignancies) will likely shift the treatment paradigm of these conditions. This may include the use of this therapy in earlier stage of the disease or even as frontline therapy. However, the expected widespread availability CAR T-cell therapy will also come with a significant economic burden due to the high cost of the therapy and the care of patients developing related toxicities.

Several hurdles and setbacks have marked the path of CAR T-cell therapy from its initial preclinical and case reports to multicenter studies with promising results.[73] There is still much to do in the field, and it will depend on the collaboration, hope and vision of investigators, patients and supporters.

Disclosure

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