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# CAR T-cell therapy for B-cell lymphomas: clinical trial results of available products

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***Abstract*:** Adoptive cellular immunotherapy with chimeric antigen receptor (CAR) T cell has changed the treatment landscape of B-cell non-Hodgkin's lymphoma (NHL), especially for aggressive B-cell lymphomas. Single-center and multicenter clinical trials with anti-CD19 CAR T-cell therapy have shown great activity and long-term remissions in poor-risk diffuse large B-cell lymphoma (DLBCL) when no other effective treatment options are available. Two CAR T-cell products [axicabtagene ciloleucel (axi-cel) and tisagenlecleucel] have obtained US Food and Drug Administration approval for the treatment of refractory DLBCL after two lines of therapy. A third product, liso-cel, is currently being evaluated in clinical trials and preliminary results appear very promising. CAR T-cell-related toxicity with cytokine-release syndrome and neurotoxicity remain important potential complications of this therapy. Here, we review the s biology, structure, clinical trial results and toxicity of two commercially approved CAR T-cell products and others currently being studied in multicenter clinical trials in B-cell NHLs.

***Keywords:*** B-cell lymphoma, chimeric antigen receptor, cytokine-release syndrome, immunotherapy, refractory

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## Introduction

The use of chimeric antigen receptor (CAR) modified T cells targeting specific tumor-cell antigens has certainly changed the landscape of immunotherapy in cancer. This technology involves harnessing cytotoxic immune T cells in order to target specific tumor-cell antigens. In non-Hodgkin's lymphomas (NHLs), specifically in diffuse large B-cell lymphoma (DLBCL), targeting CD19+ malignant B cells has proven highly efficacious in the refractory-disease setting when no other available treatment options exist. As a result, two CAR T-cell products are nowadays approved for refractory DLBCL. Here, we extensively review the mechanism of action, efficacy and toxicity(ies) of available CAR T-cell products currently in clinical use for B-cell NHLs.

## Treatment overview of selected B-cell lymphomas

It is estimated that in 2019, there will be 74,200 diagnosed cases of NHL with approximately 19,970 disease-specific related deaths.[1] NHL is the seventh leading cause of new cancer cases and accounts for approximately 3% of cancer-related deaths in the United States.[1] Among all NHLs, DLBCL is the most common lymphoma subtype comprising 32.5% of all newly diagnosed cases, followed by follicular lymphoma (FL) with 17.1%, and mantle-cell lymphoma (MCL) representing 3–5%.[2]

### *Diffuse large B-cell lymphoma*

Over 25,000 new cases of DLBCL are diagnosed annually in the United States, representing an incidence rate of 6.9 per 100,000.[3] Addition of the anti-CD20 monoclonal antibody, namely rituximab, to the standard chemotherapy, R-CHOP, resulted in significant improvement in complete response (CR) rates, event-free (EFS) and overall (OS) survival in DLBCL.[4] Unfortunately, approximately 30–40% of cases relapse or progress after R-CHOP.[5] There are specific subgroups of patients who will have poor responses and outcomes to standard R-CHOP such as MYC-rearranged DLBCL, high-grade B-cell lymphomas

with MYC, BCL2 or BCL rearrangements, activated B-cell (ABC) DLBCL that could benefit from novel approaches.[6–11]

High-dose therapy followed by autologous hematopoietic cell transplantation (auto-HCT) is considered standard of care in patients with relapsed DLBCL that is sensitive to salvage chemoimmunotherapy, typically a platinum-containing base regimen.[12,13] Randomized controlled studies and registry data have shown better survival with auto-HCT *vis-à-vis* standard chemotherapy or chemoimmunotherapy.[12,14] Nonetheless, 40–50% of the cases will not be eligible for auto-HCT due to chemorefractory disease, and the other 50% who undergo the procedure are at risk of disease relapse postautografting.[12,14,15] Unfortunately, salvage therapies have limited efficacy in some relapsed/refractory settings such as primary progression, stable disease after frontline therapy and relapsed disease within 12 months from diagnosis, showing short-lasting objective response rates of only 26% (complete response rate of 7%) and an overall survival (OS) of 6.3 months.[16,17] In patients who ultimately receive an allogeneic HCT (allo-HCT), the 5-year OS ranges from 18–37%, based on two registry studies from the Center for International Blood and Marrow Transplant Research (CIBMTR).[18–20] This limited efficacy of allo-HCT is in large part due to the high nonrelapse mortality (NRM), which may exceed 40%, mainly when using myeloablative conditioning (MAC) regimens.[18,21,22]

### *Follicular lymphoma*

FL is a biologically heterogeneous disease that represents the most common type of indolent NHL in the Western world.[23,24] There are several prognostic tools or models that integrate clinical data, laboratory studies and even molecular data that stratify the disease in different risk subgroups with specific outcomes.[25–27]

Combination of conventional chemotherapy plus rituximab is considered the standard frontline treatment of patients with FL and other indolent lymphomas.[28] Treatment response is an important determinant of outcomes in patients with lymphomas, including FL subtype. Trotman and colleagues, in a pooled analysis from three multicenter studies evaluating six cycles of frontline rituximab-based chemotherapy for high-tumor-burden FL prior to response assessment with conventional contrast-enhanced computed tomography (CT) and positron emission tomography (PET) low-dose CT, demonstrated that achievement of CR was associated with good prognosis.[29–32] Duration of first remission (CR1) has shown as prognostic in a landmark study that used data from the National LymphoCare Study (NLCS) that showed disease progression within 2 years from initial therapy was associated with inferior 5-year OS (50% *versus* 90%) in patients with stage 2–4 FL treated with R-CHOP as frontline regimen.[33] A combined observational study from the NLCS and CIBMTR showed that early use of auto-HCT (defined as within 1 year of frontline induction failure) was associated with significantly reduced mortality [hazard ratio = 0.63; 95% confidence interval (CI) = 0.42–0.94, $p = 0.02$].[34]

Patients with FL relapsing after multiple lines of therapy are offered an allo-HCT with curative intent if deemed eligible for the procedure. Use of MAC regimens have been associated with high NRM exceeding 40%.[35,36] Availability of reduced-intensity conditioning regimens have expanded allo-HCT to patients with FL owing to a more favorable toxicity profile, a lower risk of NRM of 16% and encouraging 3-year OS exceeding 80%.[37,38] Although impressive, there are several limitations to universally offering allo-HCT to FL patients due to the fact that these patients tend to, generally, be of more advanced age and have associated comorbidities that may disqualify them from receiving the procedure.

### *Mantle-cell lymphoma*

MCL is a relatively rare entity accounting for approximately 3–5% of all NHL cases.[39,40] It is a distinct subtype of B-cell lymphoma which is diagnosed by detection of cyclin D1, immunophenotyping of cell surface antigens (CD5+, CD20+, CD23−), and molecular testing for the t(11;14)(q13;q32) by fluorescence *in situ* hybridization.[39] In line with prognostic tools available for other NHLs, the MCL International Prognostic Index (IPI; MIPI) has been developed.[41] MIPI segregates MCL patients into three distinct prognostic risk subgroups: low, intermediate, and high, with anticipated median OS of not reached, 51 months, and 29 months, respectively.[41]

High-dose therapy followed by auto-HCT is considered an optimal treatment strategy as frontline

consolidation for chemosensitive disease, particularly younger patients or even for older patients who have adequate organ function and good performance status. The Nordic MCL trial treated 160 consecutive patients, treatment naïve, younger than 66 years, in a phase II protocol with dose-intensified induction R-CHOP, alternating with rituximab plus high-dose cytarabine. Authors reported excellent outcomes with long-term efficacy.[42] For patients of more advanced age with or without associated comorbidities and poor performance status, practicing hematologists generally prescribe R-CHOP as the preferred frontline treatment choice; however, other regimens such as bendamustine and rituximab (BR) are also offered.[43,44]

For relapsed/refractory MCL, either ibrutinib or acalabrutinib have elicited excellent responses but cures are not anticipated and patients will eventually relapse.[45,46] Prognosis of relapsed/refractory MCL is generally poor after failing an auto-HCT. An analysis from the European Society for Blood and Marrow Transplantation (EBMT) showed a 5-year OS of 34% in patients who receive an allo-HCT at the expense of an NRM of 30%.[47] Patients who received an allo-HCT after a late relapse (defined as $>$ 12 months) from auto-HCT had superior OS when compared with those with earlier progression after autografting.[47] Newer and more effective therapies are needed for patients with relapsed/refractory MCL.

## Rationale for CAR T-cell therapy in B-cell lymphomas

The basic anatomy of a CAR structure consists of an antigen-recognition domain, usually a single-chain variable fragment (scFv) derived from a monoclonal antibody targeting the selected antigen (i.e. CD19); a hinge [usually derived from CD8 or immunoglobulin 4 (Ig4) molecules] that links the recognition site to the transmembrane domain which bridges the membrane; and finally, the intracellular domain that typically contains a CD3ζ chain critical for T-cell receptor (TCR) signaling. Second-generation CAR molecules contain a second costimulatory-signaling molecule, such as CD28 or 4-BB, that enhances T-cell activation and antitumor potency.[48–51] CD19 is a transmembrane glycoprotein involved in regulating activation of B cells in an antigen–receptor-dependent manner. CD19 is uniformly expressed at all stages of B-cell differentiation and it is carried during B-cell malignant transformation.[52] CD19 is expressed in over 95% of B-cell malignancies, such as chronic lymphocytic leukemia (CLL), B-cell NHL, and B-cell acute lymphoblastic leukemia (ALL). Although CD19 is expressed on normal, nonmalignant B cells, it is well established that patients can survive depleted B-cell levels resulting from chemotherapy or chemoimmunotherapy.

All these factors make CD19 an attractive target for immunotherapeutic approaches. Several companies and academic institutions have developed and continue developing pivotal trials with anti-CD19 CAR T-cell-directed therapies

## Overview of CAR T-cell products and manufacturing process

### *CAR T-cell biology*

CAR T-cells represent an autologous cellular immunotherapy using gene transfer to reprogram T cells to recognize and eliminate cancerous cells by targeting tumor-associated antigens. Although CAR T-cell therapies have been recently approved for wide commercial use, this is hardly a new concept, as earlier reports showed the feasibility of combining a monoclonal antibody originally developed the idea engineering T-cell-derived scFv region with TCR-associated activation domains from CD3ζ or CD3γ. This strategy combines antibody specificity with the homing, tissue penetration and target-cell destruction mediated by T lymphocytes.[53] The first-regeneration CARs delivered activated T cells against specific tumor specific antigens but demonstrated limited persistence and weak proliferation, leading to limited antitumor activity.[54] According to the known two-step process for T-cell activation, costimulation is necessary for complete stimulation; therefore, second-generation CAR T cells included costimulatory domains that led to a significant improvement in signaling strength, expansion and persistence.[55] The most widely used costimulatory domains are CD28 and 4-1BB, but other molecules such as OX40 and CD27 have also shown enhanced CAR T-cell function.[56] Second-generation CAR T cells, as we know them today, contain three components: an extracellular antigen-recognition domain, a transmembrane domain and an intracellular signaling domain (as discussed above).[50] The majority of the trials are utilizing second-generation CARs. In general, CD28-based CARs have a greater expansion but less persistence in contrast to 4-1BB based CARs

which appear to have longer persistence. It remains to be seen whether these properties have clinical implications pertaining to efficacy.[57]

Axi-cel (KTE-019) was approved by the US Food and Drug Administration (FDA) in October 2017 for treatment of adult patients with refractory/relapsed (R/R) large B-cell lymphoma after two or more lines of systemic therapy (including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma and DLBCL arising from FL). Tisagenlecleucel (also approved for patients up to 25 years of age with B-cell precursor ALL) was also approved in May 2018 for adult patients with R/R large B-cell lymphoma after two or more lines of systemic therapy. As opposed to axi-cel, tisagenlecleucel is not approved for primary mediastinal lymphoma. Another CAR T-cell product, liso-cel (JCAR017) is currently being studied in clinical trials with promising efficacy.

### *CAR T-cell manufacturing*

The CAR T-cell manufacturing process begins with T-cell harvesting by collecting peripheral mononuclear cells (PMBCs) through leukapheresis. The product is transferred to a good manufacturing practice facility where CD3+ T cells are separated (in other products, no CD3+-based separation occurs), then expanded and activated. Then, CAR gene transduction into the T cells ensues through a vector, typically using a replication-defective virus (lentivirus or retrovirus). The CAR T cells are expanded *in vitro* and then infused back to the patient.[50,58] Although all three aforementioned anti-CD19 CAR products use the same scFv region, FMC63; there are several differences, but it is unclear whether these variances affect function, safety and clinical efficacy. As mentioned, axi-cel contains a CD28 costimulatory domain, while tisagenlecleucel and liso-cel, contain a 41-BB costimulatory domain. Liso-cel is the only product manufactured in a controlled process that enables administration of a fixed ratio of CD4 and CD8 CAR T cells. The lower variability and defined cellular composition may lead to lower rates of toxicity; however, this remains to be elucidated[59] (Table 1).

### *Pharmacokinetics and persistence*

In order to achieve antitumor efficacy, CAR T cells must reach tumor cells, interact with their intended antigen, proliferate, kill tumor cells, attempt to escape inhibitory immune mechanisms and a hostile tumor microenvironment and persist over time in order to ensure durable tumor control.[60] The pharmacokinetics (PK) of CAR T cells usually refers to maximum concentration ($C_{max}$) or peak, area under the curve and persistence. The PK of CAR T cells may differ across CAR constructs. In general, within hours after CAR T-cell infusion there is a rapid initial decline that seems to be related to redistribution to tissues. This is followed by the development of a $C_{max}$ (peak) and expansion that usually occur 1–2 weeks after infusion; the peak and expansion are clinically related to response. This is followed by a phase of slower decline in the number of CAR T cells that can last over a period of weeks to even years.[61] In general, the expansion and persistence of CAR T cells are considered essential for its antitumor efficacy, thus key predictors of clinical response.[62–65] The upregulation of the inflammatory cytokines interleukin 15 (IL-15) and the granulocyte/macrophage colony-stimulating factor (GM-CSF) have been shown to contribute with CAR T-cell expansion and persistence. These cytokines rise a few days after lymphodepleting chemotherapy (especially when fludarabine and cyclophosphamide are used).[63,66] There are other cytokines that appear to mediate the cytotoxic effect of T cells such as IL-6, IL-10 and granzyme B.[66]

## Clinical efficacy of CAR T-cell therapy

### *Early studies in B-cell lymphomas*

Initial clinical trials of anti-CD19 CAR T cells for B-cell lymphoma were carried out in single institutions. These included a diverse population of refractory B-cell NHLs, including DLBCL, FL, primary mediastinal B-cell lymphoma (PMBCL), marginal-zone lymphomas (MZL) and transformed follicular lymphomas (TFLs). Two early reports of anti-CD19 CART cells were described in patients with indolent NHL.[67,68]

The NCI conducted the first CAR T-cell study, which demonstrated clinical activity in DLBCL using the CD3ζ-CD28 CAR T construct (later licensed as axi-cel by Kite Pharma, a Gilead Company). The prescribed lymphodepleting regimen consisted of a combination of cyclophosphamide (total dose of 60 mg/kg) followed by fludarabine 25 mg/m$^2$ daily for 5 days.[62] This study included nine patients with refractory CD19+ B-cell lymphoma [DLBCL (four), PMBCL (four)

**Table 1.** Characteristics of selected second-generation CAR products studied in B-cell lymphomas.

| | Axicabtagene ciloleucel | Tisagenlecleucel | Lisocabtagene maraleucel |
|---|---|---|---|
| US FDA indication for lymphoma | Adult DLBCL | Adult DLBCL | Not applicable** |
| Costimulatory domain | CD28 | 4-1BB (CD 137) | 4-1BB (CD 137) |
| scFv | FMC63 | FMC63 | FMC63 |
| Vector delivery | Retrovirus | Lentivirus | Lentivirus |
| Defined cells | No | No | Yes, CD4:CD8 fixed ratio |
| Lymphodepleting chemotherapy (×3 days) | Cy 500 mg/m$^2$<br>Flu 30 mg/m$^2$ | Cy 250 mg/m$^2$<br>Flu 25 mg/m$^2$* | Cy 300 mg/m$^2$<br>Flu 30 mg/m$^2$ |

*An alternate lymphodepletion regimen can be given prior to tisagenlecleucel consisting of: bendamustine 90 mg/m$^2$ IV daily for 2 days if a patient previously experienced grade 4 hemorrhagic cystitis or demonstrates resistance to a previous Cy-containing regimen.
**Lisocabtagene maraleucel is not approved for commercial use at the present time.
CAR, chimeric antigen receptor; Cy, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; FDA, US Food and Drug Administration; Flu, fludarabine; IV, intravenous; scFv, single-chain variable fragment.

**Table 2.** Patient characteristics in the three anti-CD19 CAR T-cell therapy multicenter trials in aggressive B-cell NHLs.

| Characteristics | ZUMA-1 (Neelapu *et al.*[77]) | JULIET (Borchmann *et al.*[80]) | TRANSCEND[1] (Abramson *et al.*[87]) |
|---|---|---|---|
| Patients enrolled (infused), *n* | 111 (101) | 165 (111) | 134 (114)[2]<br>CORE: 73 |
| Evaluable patients, *n* | 101 | 93 | 102 (CORE: 73) |
| Median age (range), years | 58 (23–76) | 56 (22–76) | 60 (20–82) |
| Age ⩾ 65 years | 24% | 23% | 33 % |
| Lymphoma subtypes | DLBCL, TFL, PMBCL | DLBCL, TFL | DLBCL, TFL (CORE)[3] |
| Double-hit lymphoma | NR | 27% | 22% |
| ⩾ 3 lines of therapy | 69% | 51% | 50% |
| Primary refractoriness | 26% | NR | 49% |
| Refractory to last therapy | 77% | 54% | 67% |
| Prior autologous HCT | 21% | 49% | 38% |

[1]Data presented from the CORE cohort.
[2]Twelve patients had a nonconforming product.
[3]The FULL cohort included: DLBCL transformed from CLL (Richter transformation) and MZL, PMBCL and follicular lymphoma 3B. CORE included only DLBCL and TFL.
CLL, chronic lymphoblastic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HCT, hematopoietic cell transplantation; MZL, marginal-zone lymphoma; NR, not reported; PMBCL, primary mediastinal B-cell lymphoma; TFL, transformed lymphoma.

**Table 3.** Multicenter studies with autologous anti-CD19 CAR T-cell therapy for aggressive B-cell lymphomas.

| Variables | ZUMA-1 (Locke *et al.*[76]) | JULIET (Schuster *et al.*[82]) | TRANSCEND (Abramson *et al.*[87]) |
|---|---|---|---|
| Patients enrolled (treated), *n* | 111 (101) | 165 (111) | 134 (114)<br>73 in CORE |
| Median follow up | 27.1 months | 19.3 months[1] | 12 months |
| Costimulatory domain | CD28 | 4-1BB | 4-1BB |
| CAR T dose (range) | $2.0 \times 10^6$ cells/kg | Median, $3.1 \times 10^8$ cells | DL1 $5.0 \times 10^7$ cells[2]<br>DL2 $1.0 \times 10^8$ cells |
| Lymphodepleting regimen | Flu 30 mg/m$^2$ × 3 days<br>Cy 500 mg/m$^2$ × 3 days | Flu 25/m$^2$x 3 days<br>Cy 250 mg/m$^2$ x3 d or B 90 mg/m$^2$ × 2 days | Flu 30 mg/m$^2$ × 3 days<br>Cy 300 mg/m$^2$ × 3 days |
| Efficacy | | | |
| Best ORR (CR) | 82% (54%) | 52% (40%) | 80% (59%) |
| 6-month ORR (CR) | 41% (36%) | 33% (29%) | 47% (41%) |
| Ongoing ORR (CR) | 39% (37%) | NR | NR |
| mDOR | 11.1 months | Not reached | 9.2 months |
| 12-month PFS | 44% | 66% | NR |
| 18-month PFS | 40% | 64% | NR |
| 12-month OS | 59% | 49% | 63% |
| 18- month OS | 53% | 43% | NR |

[1]Median time from infusion to data cutoff.
[2]Six patients received double dose of DL1.
B, bendamustine; CR, complete response; Cy, cyclophosphamide; Flu, fludarabine; mDOR, median duration of response; NR, not reported, ORR, overall response rate.

and DLBCL transformed from CLL (one)] CAR T-cell manufacturing was successful in all patients. There were five CRs and two partial responses (PRs) out of the seven evaluable patients. Three patients still had ongoing CR at the last reported follow up.[62] The duration of response (DoR) ranged from 38 to 56 months, in patients with ongoing responses in a long-term follow-up report.[69]

A larger report from the NCI included 22 aggressive B-cell lymphoma patients (DLBCL = 13, TFL = 3, PMBCL = 2, FL = 2, MCL = 1 and Richter transformation (RT) = 1). In this study, a low-dose conditioning chemotherapy (cyclophosphamide 300–500 mg/m$^2$ and fludarabine 30 mg for 3 days) was considered to have a lymphodepleting action and was associated with less hematologic and nonhematologic toxicity. In DLBCL patients, the overall response rate (ORR) and CR rates were 68% and 47%, respectively. The median duration of remission was 12.5 months and the 12-months progression-free survival (PFS) was 63.3%.[63]

Investigators at the Fred Hutchinson Cancer Research Center (FHCRC) developed CAR T cells using a 4-1BB as costimulatory domain. A phase I clinical trial using this CAR construct, and a predefined 1:1 CD4:CD8 ratio was conducted based upon strong preclinical data. Specifically, CAR T cells manufactured using purified CD4+ or CD8+ central memory ($_{CM}$) or naïve ($_{N}$) T cells in a specific 1:1 CD4:CD8 ratio were more potent in eliminating CD19+ tumor cells as compared with those manufactured from effector memory ($_{EM}$) T cells in mouse models. Thirty-four patients with various refractory or relapsed B-cell NHLs including *de novo* DLBCL (11), TFL (11), MCL (4), and FL (6) were treated.[70] Patients with

**Table 4.** Comparison of efficacy and toxicity of patients treated with axi-cel outside clinical trials ('real-world experience') with ZUMA-1.

| Characteristics | ZUMA-1 (Locke *et al*. 2018) | Nastoupil *et al*.[88] (ASH 2018) | Jacobson *et al*.[89] (ASH 2018) |
|---|---|---|---|
| Patients enrolled (infused), *n* | 111 (101) | 295 (274) | NR (104) |
| Median age (range), years | 58 (23–76) | 60 (21–83) | 63.8 (21–80) |
| Median follow up | 27.1 months | 3.9 months | 5.6 months |
| Double-hit lymphoma | NR | 23% | 24% |
| ⩾ 3 lines of therapy | 69% | 75% | NR |
| Primary refractoriness | 26% | 35% | NR |
| Refractory to last therapy | 77% | 42% | 91% |
| Prior autologous HCT | 21% | 33% | 27% |
| Bridging chemotherapy | 0 | 55% | 40% |
| **Efficacy** | | | |
| Best ORR (CR) | 82% (58%) | 81% (57%) | 71% (44%) |
| Median PFS | 5.9 months | 6.18 months | 5.6 months |
| 6-month OS | 78% | 72% | NR |
| **Toxicity** | | | |
| CRS all grades (3–4) | 93% (13%) | 92% (7%) | 94% (16%) |
| Neurotoxicity all grades (3–4) | 65% (31%) | 69% (33%) | 76% (39%) |
| Tocilizumab use | 45% | 63% | 67% |
| Steroids use | 29% | 55% | 64% |
| Grade 5 AEs | 4% | 3%[1] | 7%[2] |

[1]A total of 7 nonrelapse mortalities due to: infection ($n = 5$); hemophagocytic lymphohistiocytosis ($n = 1$); cerebral edema ($n = 1$).
[2]A total of 7 nonrelapse mortalities due to: CRS ($n = 2$); neurotoxicity ($n = 1$); infection ($n = 2$); cardiovascular ($n = 2$).
AE, adverse event; ASH 2018, 60th Annual Meeting of the American Society of Hematology; axi-cel, axicabtagene ciloleucel; CR, complete response; CRS, cytokine-releasing syndrome; HCT, hematopoietic cell transplantation; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

relapsed postauto- and postallo-HCT were also included. Results were encouraging, with ORR and CR rates for the whole group of 63% and 33%, respectively. In a subgroup of aggressive lymphomas (DLBCL and TFL) the ORR and CR rates were 67% and 38%, respectively. This CAR construct is now licensed by JUNO Therapeutics for development as JCAR017.

Another 4-1BB CART (CTL019) construct with significant antilymphoma action was developed at the University of Pennsylvania. Preliminary results confirmed its efficacy in patients with a variety of B-cell NHLs, including DLBCL, FL, and MCL.[71,72] The updated analysis included 38 patients with DLBCL ($n = 23$) and FL ($n = 15$); however, 10 DLBCL patients could not be infused for a variety of reasons (rapid disease progression = 4, inability to manufacture CAR T cells = 5, and consent withdrawal = 1). The lymphodepleting chemotherapy included several regimens that were chosen as per physician discretion. The

**Table 5.** Toxicities in the three largest multicenter studies with anti-CD19 CAR T-cell therapy for aggressive B-cell lymphomas.

| Study | ZUMA-1 (Locke, 2018) | JULIET (Schuster)[82] | TRANSCEND[1] (Abramson *et al.*)[87] |
|---|---|---|---|
| No patients enrolled (treated) | 111 (101) | 165 (111) | 134 (114) |
| **Cytokine-release syndrome**[2] | | | |
| Time to onset, median, range | | | |
| Duration, median, range | 2 days (1–12) | 3 days (1–9) | 5 days (2–12) |
| Grade (all) | | | |
| Grade 3 or 4 | 8 days (NR) | 7 days (2–30) | 5 days (NR) |
| Tocilizumab use | 93% | 58% | 37% |
| Vasopressors use | 13% | 23% | 1% |
| Steroid treatment | 43% | 16% | 21% |
| ICU admission | 17% | 6% (high dose) | NR |
| | 27% | 11% | 17% |
| | NR | NR | NR |
| **Infections** | | | |
| All grades | 35%[3] | 34% | NR |
| Grade 3 or 4 | 31%[3] | 20% | NR |
| **Neurotoxicity**[2] | | | |
| Time to onset, median (range) | 5 days (1–17) | NR | 10 days (3–23) |
| Duration, median, range | 17 days (NR) | NR | 11 days (NR) |
| All grades | 64% | 20% | 23% |
| Grade 3 or 4 | 28% | 11% | 13% |

[1]Reported from the full cohort data.
[2]Grading was performed using the Penn criteria.
[3]Febrile neutropenia.
ICU, intensive care unit; NR, not reported.

median CTL019 dose was $5.79 \times 10^6$ (range: $3.08–8.87 \times 10^6$) CAR T cells/kg. Among DLBCL patients, the ORR and CR rates were 50% and 43%, respectively. The median PFS was 3.2 months; and the PFS at last follow up (median 28.6 months) was 43%. There were no significant differences in outcomes between GCB/non-GC, double-hit status or transformed FL subgroups.[72,73] The median DoR was not reached with 86% of responding DLBCL patients maintaining an ongoing response at the time of the last follow up.

*Multicenter studies in aggressive B-cell lymphomas.* The early single-center studies showed significant antilymphoma activity in aggressive B-cell NHLs and led the design of multicenter studies that included several academic institutions in association with pharmaceutical companies.

*Axicabtagene ciloleucel (KTE-C19).* The first multicenter trial to evaluate CAR T-cell therapy for refractory DLBCL used the NCI CD3ζ/CD28 CAR construct (KTE-19, now axi-cel)

with a streamlined closed-manufacturing process. The ZUMA-1 clinical trial consisted of a phase I and a phase II portion that evaluated the efficacy of axi-cel in refractory high-grade B-cell lymphoma. The cell dose and conditioning chemotherapy previously tested at the NCI were confirmed safe in seven patients with refractory DLBCL (as defined per SCHOLAR-1: best response as SD to last systemic therapy or progressed within 12 months of prior autologous transplant).[16] No bridging chemotherapy was allowed (prior to conditioning chemotherapy or prior to CAR T-cell infusion). The lymphodepleting regimen entailed cyclophosphamide 500 mg/m$^2$ and fludarabine 30 mg/m$^2$ $\times$ 3 days followed by infusion of axi-cel at a dose of 1–2 $\times$ 10$^6$ CAR T cells/kg[16,74] The objective response was 71% with four patients achieving CR (57%) at 1 month evaluation. Three patients had ongoing CR at 12 months post axi-cel infusion. Reversible grade 3 neurotoxicity (NT) and cytokine-release syndrome (CRS) were reported among this cohort. One fatality occurred in a patient who experienced grade 4 CRS and grade 4 encephalopathy, and died of intracranial bleeding, which was considered unrelated to axi-cel. This patient appeared to have had a high inflammatory state prior to chemotherapy and CAR T-cell infusion. For the phase II portion of the trial, changes in the safety evaluation were made and included baseline C-reactive protein (CRP) assessment and delaying CAR T-cell infusion in patients with fevers until appropriate work-up was completed.

The pivotal phase II portion of the ZUMA-1 had similar eligibility criteria as the phase I, with two cohorts: cohort 1 for DLBCL and cohort 2 for PMBCL and TFL.[75] The primary endpoint was ORR in patients with more than 6 months follow up postaxi-cel infusion, as compared with historical controls. Secondary endpoints were DoR, OS, safety, and levels of CAR T cells and cytokines. A total of 111 patients were enrolled. Seventy percent were refractory to at least three lines of therapy and 21% relapsed within 12 months of auto-HCT. Ten patients could not receive axi-cel for various reasons [serious adverse events (SAEs) prior to conditioning regimen = five, nonmeasurable disease = two, no product available = one, and SAE postconditioning regimen = two].

The 101 patients that received axi-cel infusion were the prespecified intent-to-treat analysis cohort. The CAR T-cell manufacturing success was 99%. The median time from apheresis to axi-cel delivery was 17 days. The study met the primary endpoint compared with the historical cohort (SCHOLAR-1) with an ORR of 83% and CR of 54% (in comparison with the a prespecified ORR of 20%, $p < 0.0001$) representing a eightfold higher CR rate in comparison with SCHOLAR-1. The latest data with a median follow up of 27.1 months was presented at ASH, 2018.[76] The ongoing ORRs and CRs were 39 and 37%, respectively. Overall objective responses remained consistent across patient and disease-specific variables, such as advanced stage, age, bulky disease, high IPI score or refractory subgroups [R/R postautohematopoietic stem-cell transplantation (HSCT) or higher than second line of therapy]. The median DoR was 11.1 months in all responders and was not reached in those achieving CR. The PFS at 12,18 and 24 months was 44%, 40%, and 39%, respectively. The 12, 18 and 24-month OS was 60%, 53% and 51%, respectively. An initial PFS plateau was seen at 6 months postaxi-cel infusion; however, there were 10 patients that exhibited disease progression beyond 6 months. The median PFS and OS were 5.9 months and not reached, respectively.[76] Of note, 23 out of 61 patients with either PR (11) or SD (12) converted into CR with no additional intervention. The median time of conversion of PR to CR was 64 days (49–424).[76,77] Based on these results, axi-cel was approved by the FDA for R/R high-grade B-cell lymphoma, TFL and PMBCL after two preceding lines of therapy.

*Tisagenlecleucel (CTL019).* The JULIET trial is a phase II multicenter global study in patients with refractory DLBCL utilizing CTL019, the anti-CD19 CAR using a 4-1BB costimulatory domain developed by scientists from the University of Pennsylvania and was initially studied in single-center trials.[73] Interim results were presented at the American Society of Hematology 59$^{th}$ annual meeting in 2017 and the European Hematology Association meeting in 2018.[78–80] CART cells were manufactured centrally; however, in contrast to ZUMA-1, cryopreserved apheresis products were utilized and bridging chemotherapy was allowed per clinician discretion for patients with rapidly progressive disease. There were two regimens utilized, as lymphodepleting chemotherapy consisted of fludarabine 25 mg/m$^2$ and cyclophosphamide 250 mg/m$^2$ for 3 days or bendamustine 90 mg/m$^2$ for 2 days. Key eligibility criteria included aggressive B-cell lymphoma (DLBCL or TFL), relapse after autologous HSCT or ineligible for HSCT,

or refractory after two lines of therapy. Similar to the ZUMA-1 trial, the primary endpoint was ORR and CR rates.

The update analysis had a data cutoff of 21 May 2018. A total of 167 patients were enrolled and 115 patients were infused with tisagenlecleucel (4 patients were not infused by data cutoff).[81,82] Fifty patients could not be infused with CTL019 due to inability to manufacture CAR T cells ($n$ = 12) and change in disease/patient status ($n$ = 38). The median age of study subjects was 56 (22–76) years. The median dose of transduced cells was $3.0 \times 10^8$ ($0.1–6 \times 10^8$). In this study, 51% had refractory disease with at least three lines of therapy and 49% had prior auto-HCT. A total of 92% patients received bridging chemotherapy. The median time from infusion to data cutoff was 19.3 months. In the 99 evaluable patients (⩾3 months of follow up) the best ORRs and CRs were 54% and 40%, respectively. The 12- and 18-month relapse-free survival was 64 %. The 12- and 18-month OS in all patients were 48% and 43%, respectively. The median DoR in responders was not reached. The median OS for all patients and CR patients was 11.1 months and not reached, respectively. Responses were similar across different subgroups (postauto- HCT, double-hit lymphoma, refractory/relapsed status, age, etc.). Similar to ZUMA-1, conversion into CR was observed in 15/28 (54%) patients who originally achieved PR.[80,82] Outpatient infusion of CTL019 was feasible and was given to 26 patients, and 20 (77%) of those remained as outpatients for more than 3 days.[78] No deaths were attributed to CLT019, but three patients died within 30 days of infusion (all due to disease progression).

*Lisocabtagene maraleucel (JCAR017).* The 4-1BB CAR T-cell construct using a defined CD4:CD8 T-cell ratio and developed at the FHCCR was tested in the multicenter TRANSCEND-001 study.[83,84] This study was divided in two groups: the FULL and CORE cohorts. The FULL cohort included patients with R/R DLBCL, TFL, FL grade 3b, MCL, RT, DLBCL arising from MZL and PMBCL. The CORE dataset included only R/R DLBCL and TFL. The initial analysis included three cohorts with different dose levels (DL): DL-1S was $5 \times 10^7$, and DL-2S was $1 \times 10^8$. A small cohort of patients received double dose ($n$ = 6) of JCAR017 at $5 \times 10^7$ that was administered 14 days apart (cohort no longer open). The conditioning regimen consisted of fludarabine 30 mg/m$^2$ and cyclophosphamide 300 mg/m$^2$ daily for 3 days. Bridging therapy was allowed for disease control. After the preliminary analysis, the DL-2S was determined for the expansion phase.[85,86]

In the updated analysis, 134 patients were enrolled and 114 patients infused with liso-cel. Twenty patients were not infused due to rapid disease progression/death ($n$ = 13), consent withdrawal ($n$ = 5) or inability to manufacture ($n$ = 2). Out of the infused patients, 12 had a nonconforming product, thus 102 patients were evaluable in the FULL cohort (CORE cohort = 73).[87] In the CORE cohort, the median age was 60 (20–82) years and, at least 50% were DLBCL cases refractory to three or more lines of therapy, and 38% had failed a prior auto-HCT. Preliminary analysis was reported previously.[84,85] The updated analysis has a median follow up of 12 months reporting the best ORR (CR) rates in the FULL and CORE cohorts of 75% (55%) and 80% (59%), respectively. The 6-month ORR and CR rates in the CORE cohort were 47% and 41%, respectively. Responses rates were not affected by high-risk DLBCL characteristics such as double-hit lymphoma status, chemorefractory disease or prior auto-HCT failure. The median DoR was not reached in CR patients in the FULL and CORE cohorts, confirming findings of prior reports.[84,85] The 12-month OS was 63% in all responders and 89% those achieving CR. A total of 93% patients with CR as best response at 6 months had ongoing response at data cutoff (Tables 2 and 3).[87]

*Clinical activity of CAR T-cell therapy for DLBCL outside clinical trials.* With the approval of axi-cel and tisagenlecleucel for the treatment of refractory DLBCL, there was growing interest in reporting the efficacy of this therapy in real clinical practice and outside clinical trials. In an effort to replicate the results of the ZUMA-1 trial with axi-cel, an extraordinary effort was carried out by 23 US cancer centers with experience and with certification to treat patients with CAR T-cell therapy. To date, there are no reports of tisagenlecleucel in DLBCL outside clinical trials.

The first study was reported by Nastoupil and colleagues[88] and included 295 patients, with 274 patients treated. The final CAR T-cell product did not meet FDA specifications in seven patients.

The median time of axi-cell manufacturing was 21.5 days. As opposed to ZUMA-1 trial, around 55% patients received any form of bridging therapy (chemotherapy, targeted therapy or radiation). General characteristics included a median age of 60 (21–83), stage III/IV in 83%, performance status (PS) 0–1 in 81%, three or more lines of therapy in 75% of cases and relapsed postauto-HCT in 33%. Interestingly, 43% of patients (124/286) would have not been eligible for the ZUMA-1 trial (such as platelets $<$ 75 000, ejection fraction $<$ 50%, prior allo-HCT, among other factors). The overall efficacy was similar as to the ZUMA-1 trial with 3-month ORR and CR rates of 81% and 57%, respectively.

The second study, presented by Jacobson and colleagues,[89] included 108 patients infused with axi-cel; of those, 104 were evaluable for efficacy. The median age was 63.8, PS 0–1 in 90% of cases, prior auto-HCT in 27%, prior allo-HCT in 3%. About 52% of the evaluable patients received bridging chemotherapy after apheresis; 60% of patients would have not met criteria for the ZUMA-1 clinical trial. In the 95 patients evaluable for response, the best ORR and CR rates were 71 and 44%, respectively. Similarly, about 50% of patients who initially had a PR, achieved CR at a later time.

These two reports concluded that the efficacy of axi-cel in refractory disease could be replicated outside the strict eligibility criteria of clinical trials. It should be highlighted that this therapy needs to be offered in centers with experience and capability of administering high-risk immunotherapy and cellular therapies (Table 4).

*CAR T-cell therapy for indolent lymphomas.* Although trials using anti-CD19 CAR T cells focus mainly on aggressive B-cell lymphomas, the first patients to receive this type of therapy were those having indolent NHLs. The first-generation anti-CD19 CAR T-cells (without costimulation) reported no clinical efficacy in FL cases.[67] The first case successfully treated with anti-CD19 CAR T cells with CD28 as the costimulatory domain was reported by the NCI in a refractory FL patient achieving long-term remission.[68] A subsequent NCI study with CD28 anti-CD19 CAR T cells reported PR of 100% in five indolent NHL patients (four FL and one MZL) with 75% having ongoing responses at the time the study was published.[90] These patients received a conditioning regimen consisting of cyclophosphamide 60 mg/kg $\times$ 2 days and fludarabine 25 mg/m$^2$ $\times$ 5 days.[90]

In the 32 patients treated with the 4-1BB CAR T-cell construct (1:1 CD4/CD8 ratio) from FHCRC, there were 5 evaluable FL patients and the reported ORR and CR rates were 80% (4/5) and 40% (2/5), respectively.[70]

The largest data in FL to date come from the CTL019 CAR T cell from the University of Pennsylvania that included 14 FLs. These FL patients had relapsed within 24 months of initial diagnosis and remained refractory to least two lines of therapy.[71,91] Patients received a variety of conditioning regimens such as bendamustine 70 mg/m$^2$ $\times$ 2 days, cyclophosphamide, radiation plus cyclophosphamide and fludarabine–cyclophosphamide. This trial included FL patients with poor prognosis features, including prior multiple therapies (median of five), relapsed postauto-HCT (21%) and allo-HCT (one patient). The updated analysis showed a 3-month ORR and CR of 79% (11/14) and 71% (10/14), respectively. The median PFS was not reached and 70% of FL patients were disease free at a median follow up of 28.6 months.[73,91]

These data support anti-CD19 CAR T-cell therapy as a promising alternative therapy in poor-risk FL, despite the low number of patients; and it may have a curative potential given the long-term ongoing responses. The ZUMA-5 is a dedicated indolent B-cell NHL trial that is currently enrolling patients [ClinicalTrials.gov identifier: NCT03105336].

*CAR T-cell therapy for MCL.* The experience with autologous anti-CD19 CAR T-cell therapy in MCL is limited to a few cases in single-center clinical studies. The 4-1BB CAR T-cell trial at the FHCRC with fixed CD4:CD8 ratio included four patients with MCL that received doses of 2 $\times$ 10$^5$–10$^7$ CARs/kg. The ORR was 25% (no CR).[70] The NCI-based CD28-CAR T-cell trial reported a long-term CR ($>$17 months) in an MCL.[63] The University of Pennsylvania (U Penn)-based CTL019 CAR T-cell trial included two patients with MCL having 50% ORR (no documented CR).[71] Albeit limited experience, there seems to be promising activity of anti-CD19 CAR T cells in refractory MCL. We are eagerly awaiting the

preliminary results of the ZUMA-2 clinical trial of axi-cel in patients with ibrutinib-refractory MCL [ClinicalTrials.gov identifier: NCT02601313].

## CAR T-cell-therapy-related toxicity

The toxicities related to CAR T-cell therapy were initially described in the earlier studies in B-cell lymphomas and ALL.[62,70,92,93] There are two main categories of toxicity: CRS and neurotoxicity or CAR T-cell-related encephalopathy syndrome (CRES). Organ damage can accompany CRS (renal failure, cardiac dysfunction, liver dysfunction, etc.).[94,95]

### *Cytokine-release syndrome*

CRS is an excessive inflammatory response caused by overactivation of immune-effector cells that leads to significant release and elevation of inflammatory cytokines such as IL-1, IL-2, IL-6, IL-10, IL-15, interferon (IFN)-γ and tumor necrosis factor (TNF). This occurs typically in patients that receive CAR T-cell therapy.[94–96] Patients, with this inflammatory response, present with a variety of symptoms, such as fevers, general malaise, hypotension, and hypoxia. In severe cases, irreversible organ damage and death can occur.[95,96]

The most important factors for successful treatment of CRS are early identification and accurate grading in order to guide optimal management. Grading is based on hemodynamic instability, degree of hypoxemia, organ damage and presence of comorbidities. Patients with grade 3 and 4 CRS (and sometimes grade 2 in patients with important comorbidities) usually require aggressive measures, such as vasopressors, management in the intensive care unit, anticytokine therapy and steroids. As IL-6 is a key player in the etiology of CRS, the administration of tocilizumab (anti-IL-6 receptor) and siltuximab (anti-IL-6 antibody) have become standard approaches for the CRS management.[92,95,96]

There are clinical factors that correlate with the development of CRS, such as disease burden (specifically in ALL) and dose of CAR T cells.[62,70,80,92,93] Increased levels of TNF (TNF-alpha), IL-2R, IL-6, IFN-γ, IL-10, IL-15, and ferritin have demonstrated association with severity of CRS.[62,70] Peak CRP levels have been shown to directly correlate with CRS severity and can be used as a surrogate marker for early treatment/supportive care.[92] Recent preclinical work in mouse models helped clarify further the potential etiology of CRS and NT. In two separate studies, the role of monocytes/macrophages and cytokine production upon interaction with CAR T cells, especially as a source of inflammatory cytokine production and kinetics (notably, IL-1 elevation preceded the IL-6 rise), showed the role of IL-1 blockade in potentially preventing CRS and NT.[97,98]

### *Neurotoxicity*

NT is another common complication of CAR T-cell therapy that is less understood than CRS. Symptoms of NT can sometimes overlap to those seen in CRS. Patients have a variety of symptoms such as confusion, obtundation, tremors and headaches. Other symptoms such as aphasia, cranial nerve abnormalities and seizures have been described. As in CRS, early identification and adequate grading is strongly recommended. Tools such as mini mental-status evaluation have been used commonly to grade NT. A more specific (and simplified) defined criterion for measurement of NT, also known as CRES, was recently developed.[94] The role of anti-IL-6 therapy is unclear and does not seem to have a beneficial role in treatment of NT, thus the mainstay treatment of CRES is steroids. Neurology evaluation with brain imaging, cerebrospinal fluid (CSF) examination and electroencephalogram (EEG) assessments is usually recommended to rule out other causes.

Additionally, NT appears to be cytokine driven.[62] The ZUMA-1 described how elevated levels of IL-2, ferritin and GM-CSF were significantly associated grade $\geq 3$ NT.[99] Baseline higher tumor burden, elevated lactate dehydrogenase (LDH), CRP and ferritin were associated with neurotoxicity and CRS in the JULIET trial.[80] The initial report of biomarkers in the TRANSCEND study demonstrated an eightfold increased risk of CRS and neurotoxicity with elevated LDH ($>500$/μl) and tumor burden ($>50\,cm^2$).[100] Disruption of the blood–brain barrier (BBB), endothelial activation and increased IL-1 levels have been recently described as potential drivers of NT.[98,101]

In general, the vast majority of CAR T-cell-related toxicities resolve within few weeks as reported in both single-center and pivotal multicenter studies; however, CAR T-cell fatalities have also been reported.[62,70,74,90] While, the symptoms and signs

of each type of toxicity may overlap, it is important for the clinician to recognize these potential complications, as they could be life threatening and, in certain circumstances, lead to death. The diagnosis and management of CRS and CRES have been extensively discussed in other publications.[94–96]

The multicenter ZUMA-1, JULIET and TRANSCEND trials have reported CAR T-related toxicity with some differences in frequency, timing and severity. These variations are possibly due to differences in patient population, disease subtypes (and clinical presentation), use of bridging chemotherapy, use of different toxicity grading systems and differences in CAR T-cell constructs. The Lee criteria[96] were used to grade CRS in ZUMA-1 and TRANSCEND studies while the U Penn Criteria were used for severity stratification of CRS in the JULIET trial.[102] Table 5 describes the frequency and features of CAR T-related toxicities in the three multicenter studies in NHL.

Toxicities such as CRS and NT were also described in the outside clinical trials (real clinical experience). In general, the rates of toxicity were somewhat similar to what it was seen in clinical trials, except for one of the reports that showed lower CRS grade $\geqslant$ 3. Another interesting finding was that higher rates of tocilizumab and steroid use were reported. We believe that this underscores the fact that centers may treat CRS and NT more aggressively or that there is a better knowledge that steroids or tocilizumab does not affect efficacy of CAR T cells. Table 4 describes a comparison of toxicities seen in ZUMA-1 and outside clinical trial experience.

## Challenges in CAR T-cell therapy: potential interventions

### *Overcoming resistance*

Availability of CAR T-cell therapy has changed the treatment landscape of refractory DLBCL. Unfortunately, about 50–60% of patients will not achieve a CR or will relapse after CAR T-cell therapy. Thus, understanding the mechanism of relapse after CAR T-cell treatment is paramount. One mechanism is CD19 immunological antigen escape, as CD19-negative B-cell malignancy relapses have been reported.[103] Inability to express CD19 in B-cell malignancies due to epitope/antigen loss of the CD19 through splicing/mutation mechanisms have been described as resistance mechanism or relapse in patients receiving CD19-directed therapy (such as CARs or bispecific antibodies).[77,104,105] Another potential mechanism is increased activation of the programmed cell-death 1/programmed cell-death ligand 1 (PD-1/PD-L1) pathway that has been seen in relapsed DLBCL post CAR T-cell therapy.[77]

One approach to overcome resistance is by targeting a different antigen. Anti-CD20 CAR T cells showed modest activity in earlier studies but with greater efficacy once costimulation with 4-1BB was added.[67,106] An ORR and CR rate of 80–83% and 17–50% were described in two different trials, respectively.[107,108] Another target, CD22, has shown antilymphoma activity in preclinical studies.[109] Although these results are focused on CD22+ B-cell ALL, there are ongoing trials for refractory CD22+ B-cell NHLs [ClinicalTrials.gov identifiers: NCT02315612, NCT02794961].[110] Bispecific CARs targeting CD19 and CD20 antigens for B-cell malignancies have been developed (CD19-OR-CD20 CAR) with significant preclinical activity, even in CD19-negative tumor cells.[111] Hossain and colleagues,[112] from Stanford University, presented preliminary data on CD19/CD22 bispecific CAR T cells in nine patients (five DLBCL and four B-cell ALL) that showed clinical activity (one CR and two PRs in the DLBCL cohort) and a tolerable toxicity profile. PD-1 inhibition has become an attractive approach, with reported success in case reports.[113,114] A trial of atezolizumab (anti PD-L1 inhibitor) plus axi-cel (ZUMA-6) was presented and included 12 patients with three different cohorts. The clinical activity was promising with an ORR of 92% (CR 58%). Of note, albeit in its early phase, there was a higher grade 3 NT (50%) in comparison with the reported NT of ZUMA-1.[115,116] Other checkpoint inhibitors such as pembrolizumab and durvalumab are being studied [ClinicalTrials.gov identifiers: NCT03310619, NCT03630159]. Other agents with the potential to improve activation, expansion, and persistence such as utomilumab (4-1BB agonist), ibrutinib and avadomide (CC-122, an immunomodulator) are also being tested in combination with different CAR T-cell products [ClinicalTrials.gov identifiers: NCT03331198, NCT03310619, NCT03704298].

### *Patient selection/timing*

With two available CAR T-cell products for refractory DLBCL after at least two preceding lines of therapy (excluding patients with primary central nervous system lymphoma) there is no guidance on

the FDA label with regards to (a) specific condition(s) where one product is to be favored over another. Early referral to centers certified to prescribe CAR T-cell therapies is strongly encouraged to avoid toxicities from ineffective chemoimmunotherapies.[117] Another issue is the time from apheresis to infusion of CAR T cells that can range between 2–5 weeks, depending of the CAR construct and preauthorization (by private insurances). This time could be critical for patients with an otherwise aggressive and refractory disease. Readily available CAR products are donor-derived CAR T cells (allogeneic) that seem feasible and safe.[118,119] Donor-derived CAR T cells were initially reported by the NCI in CD19+ B-cell malignancies, including DLBCL, ALL and CLL with clinical efficacy. The CAR T-cell production took 8 days. Interestingly, no cases of acute graft *versus* host disease (GVHD) were reported but two cases of chronic GVHD were.[120] In order to further minimize the risk of GVHD, another approach is to suppress the TCRs by genome editing: by disrupting the expression of the alpha or beta TCR chains using different technologies; the transcription-activator-like effector nucleases being one of the best known methodologies.[121]

### *Cost/Financial toxicity*

The excitement of this promising therapy for DLBCL has been tempered by its hefty cost of $375,000 for the CAR T-cell product alone without accounting for cost of hospitalization and treatment of complications such as CRS and CRES, which could amount to additional hundreds of thousands of dollars. It is important to consider these factors for pharmacoeconomic analysis in order to determine pricing-, coverage- and outcome-based reimbursement, as well as to the added value to society.[122]

## Conclusions

CD19- targeted CAR T-cells represent the new standard of care for patients with DLBCL that are refractory to at least two prior lines of therapy. While this represents a significant addition to the treatment armamentarium of DLBCL, approximately 50% of cases will continue to succumb to their disease. As a result, future research must focus on identifying disease-, treatment- or patient-related factors that can help successfully predict treatment outcomes. For patients who fail to achieve early CR (defined as within 90 days), early therapeutic interventions with immune modulators or checkpoint inhibitors, or others, represent interesting questions that will need to be studied in ongoing and future clinical trials.

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The authors declare that there is no conflict of interest.

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