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Review Article

CAR-T Cell Therapy in Large B Cell Lymphoma

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Abstract. Large B-cell lymphomas (LBCLs) are among the most frequent (about 30%) non-Hodgkin's lymphoma. Despite the aggressive behavior of these lymphomas, more than 60% of patients can be cured with first-line chemoimmunotherapy using the R-CHOP regimen. Patients with refractory or relapsing disease show a poor outcome even when treated with second-line therapies.
CD19-targeted chimeric antigen receptor (CAR) T-cells are emerging as an efficacious second-line treatment strategy for patients with LBCL. Three CD19-CAR-T-cell products received FDA and EMA approval. CAR-T cell therapy has also been explored for treating high-risk LBCL patients in the first-line setting and for patients with central nervous system involvement.
Although CD19-CAR-T therapy has transformed the care of refractory/relapsed LBCL, about 60% of these patients will ultimately progress or relapse following CD19-CAR-T; therefore, it is fundamental to identify predictive criteria of response to CAR-T therapy and to develop salvage therapies for patients relapsing after CD19-CAR-T therapies. Moreover, ongoing clinical trials evaluate bispecific CAR-T cells targeting both CD19 and CD20 or CD19 and CD22 as a tool to improve the therapeutic efficacy and reduce the number of refractory/relapsing patients.

Keywords: CAR-T; Large cell B-lymphoma; Salvage therapy.

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Introduction. Chimeric antigen receptors (CARs) are engineered receptors that enable T lymphocytes with the capacity to specifically recognize an antigen and induce a cytotoxic reaction against tumor cells based on their expression of this antigen.

Antitumor adaptive therapies represent a key strategy in the treatment of tumors. Some of these immunotherapies were based on the use of genetically engineered cells. Particularly, two different types of immunotherapies have been developed using genetically modified T lymphocytes: (i) T-cell receptor-engineered cells enabled to recognize specific membrane antigens in a HLA-restricted manner; (ii) CAR-T transduced T lymphocytes that interact with specific membrane antigens in a HLA-unrestricted manner and antibody-specific manner.

The molecular architecture of a CAR molecule implies four components: i) an extracellular target antigen-binding domain (ABD); ii) a hinge region; iii) a transmembrane region; iv) one or more intracellular

signaling domains. ABD is the part of the CAR molecule that confers specificity in antigen recognition and is usually derived from the variable heavy (V_H) and light (V_L) chains of monoclonal antibodies, connected through a flexible linker to generate a single-chain variable fragment (scFv). The hinge component is a spacer region that extends the ABD from the ABD to the transmembrane region and confers sufficient flexibility to avoid steric hindrance. The transmembrane region is required to anchor the CAR molecule to the cell membrane of T lymphocytes. The intracellular signaling domains play an important role in the modulation of CAR-T cell activity; a large part of CARs is based on the activation of T lymphocytes using CD3-derived immunoreceptor tyrosine-based activation motifs.[1]

The procedure for generating CAR-T cells evolved over time with 5 different CAR-T generations from the first procedures in late 1990 to the most recent developments.[1] The first generation of CAR-T was based on the CD3-ζ intracellular domain, in the absence of costimulatory domains; the second generation of CAR-T cells contained a costimulatory domain, such as CD28, in the intracellular domain; the third generation was based on the presence of multiple costimulatory domains; the fourth generation involved the production of T cell redirected for general universal cytokine-mediated killing (TROCKs), a property obtained through IL-12 production, either constitutive or after CAR-T activation; the fifth generation also included a STAT3 binding site required for generation of three activation signals acting on the cell signaling, costimulatory and cytokine signaling domains.[1] Compared to the first generation, the consistent advantages of second- and third-generation CAR-T cells consisted of enhanced proliferation, cytotoxicity, and lifespan *in vivo*.[1] The last generations of CAR-T cells showed superior *in vivo* persistence and enhanced antitumor effects in leukemia and solid tumor models compared to initial CAR-T cell generations and are expected to demonstrate superior antitumor effects with reduced toxicity in the clinic.[2]

The key role of CD19 targeting by CAR-T for the therapy of B-cell malignancies. The human CD19 antigen is a B-lymphocyte antigen belonging to the immunoglobulin superfamily, whose expression is restricted to the B-cell lineage starting from the early stages of B cell development corresponding to heavy chain immunoglobulin rearrangement to the late stages of B cell differentiation; CD19 expression increases during B cell differentiation.[3-4] On the cell membrane of B-lymphoid cells, CD19 forms a transduction complex with CD21, CD81, and Leuk-13.[5] Furthermore, CD81 regulates the expression of CD19 during B cell development.[6]

FDA and EMA approved CAR-T cell therapies for B lymphoid malignancies. Four CAR products are commercially available for patients with B cell lymphomas: Axicabtagene ciloleucel (Axi-Cel), Brexacubtagene autoleucel (Brexa-Cel), Lisocabtagene maroleucel (Liso-Cel) and Tisagenlecleucel (Tisa-Cel); two for B-ALL: Brexa-Cell and Tisa-Cel. All these products are based on a second-generation CAR construct (see **Figure 1**) and involve the presence of an intracellular component containing a T-cell activation domain (CD3ζ) and a costimulatory domain (CD28 in Axi-Cell and Brexa-Cel, 4-1BB in Tisa-Cel and Liso-Cel).

The structure of Axi-Cel and Brexa-Cel is identical, but their manufacturing process is different in that the procedure of production of Brexa-Cel also implies a step of removal of malignant cells from the apheresis sample; both these CAR genes are delivered to T lymphocytes using a gammaretrovirus. The CAR gene used for Tisa-Cel and Liso-Cel is delivered using lentiviruses; particularly, Liso-Cel is delivered to a defined CD4/CD8 T cell composition.

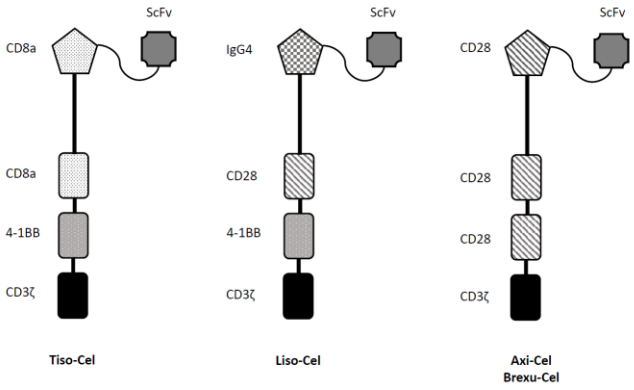

Figure 1. Schematic representation of CD19-CAR constructs currently available commercially and used for the therapy of DLBCL patients. All these products have a second-generation CAR construct, consisting of an antigen-binding domain, a hinge region, a transmembrane region, a costimulatory domain and a T-cell activation domain.

Clinical studies on CAR-T cells in DLBCL. Large B cell lymphomas are among the most frequent (about 30%) non-Hodgkin's lymphoma. Gene expression studies, based on cell-of-origin, have identified two main subtypes of DLBCLs with distinct clinical and molecular features: activated B-cell (ABC) and germinal center B cell (GCB).[7] Analysis of genomic alterations has shown an additional heterogeneity of ABC and GCB subgroups. Within GCB-DLBCLs, lymphomas with *EZH2* mutations and *BCL2* translocations have a poor outcome. Lymphomas harboring both *MYC* and *BCL2* and/or *BCL6* rearrangements are identified as high-grade B-cell lymphomas double-hit or triple-hit (HGBL-DH and HGBL-TH) and show an aggressive clinical-biological phenotype. Within the ABC-DLBCL group, lymphomas with *NOTCH1* mutations or co-occurring *MYD88* and *CD79B* mutations have a poor prognosis.[7]

Clinical studies using CAR-T in DLBCL. Several studies have explored the safety and efficacy of CAR-T in diffuse large B-cell lymphomas (DLBCL). These patients may be cured with first-line therapy; however, up to 30% to 40% of them may become refractory or relapse.

Clinical studies with Axi-Cel. The ZUMA-1 and ZUMA-7 trials evaluated the safety and efficacy of Axi-Cel in different clinical settings of patients with LBCL. The ZUMA-7 study is a phase III trial in which patients with early relapsed or refractory DLBCL were randomized to receive either Axi-Cel (180 patients) or standard care (179 patients) that consisted of salvage chemotherapy followed by high-dose chemotherapy with autologous stem cell transplantation (ASCT) in second-line therapy.[8] At the latest median follow-up explored (47.2 months), the following results were observed: 82 deaths in the Axi-Cel group and 95 in the standard-care group; median overall survival (mOS) not reached in the Axi-Cel group and 31.1 months in the standard-care group; median progression-free survival (mPPFS) was 14.7 months in the Axi-Cel group and 3.7 months in the standard-care group.[8-9] A subgroup analysis of the ZUMA-7 study limited to patients 65 years of age or older showed a higher rate of ORR and CRR in the Axi-Cel group compared to the standard care group (ORR 88% vs 52%, respectively; CRR 75% vs 33%, respectively).[10] In particular, no grade 5 cytokine release syndrome or neurologic events occurred in this group of patients who are more fragile and at risk for complications.[10]

The ZUMA-7 trial was preceded by the ZUMA-1 trial exploring the efficacy of Axi-Cel in third-line therapy. ZUMA-1 was a single-arm phase I/II study enrolling LBCL patients with refractory or relapsed disease after autologous stem cell transplantation; the patients received a target dose of $2x10^6$ CAR-T cells per Kg of body weight after conditioning chemotherapy with fludarabine and cyclophosphamide.[11] One hundred one patients were enrolled in this study, and after a median follow-up of 63.1 months, the following results were observed: 83% of ORR, with 58% of CRR; mOS was 25.8 months, with a disease-specific survival at 5 years of 51%.[11-12] These results suggested a curative potential of Axi-Cel in a subset of LBCL patients.[11-12] A comparison of 2-year outcomes with CAR-T cells of patients enrolled in the ZUMA-1 trial showed better results observed with CAR-T cell therapy compared to salvage chemotherapy in a comparable group of patients (ORR 83% vs. 34% and CRR 54% vs. 20%, respectively).[13] The 2-year survival rate was 54% with Axi-Cel and 20% with salvage therapy.[13]

The analysis of the clinical results observed in a real-world setting of 275 relapsed-refractory DLBCL patients receiving Axi-Cel confirmed the results observed in the ZUMA-1 study, with ORR 82% and CRR 64%. At a median follow-up of 12.9 months, the PFS was 8.3 months.[14]

The 58% achieved a complete response rate following treatment with Axi-Cel in LBCL patients participating to the ZUMA-1 study offered the opportunity to explore the existence of tumor-related and tumor-associated parameters differentially expressed in responding and non-responding patients. Thus, many studies have shown the existence of several clinical, biochemical, and biological parameters that either negatively or positively affect the response to Axi-Cel.

A high tumor burden, measured through evaluation of baseline metabolic tumor volume (MTV) on ^{18}F fluorodeoxyglucose positron emission tomography, was associated with decreased efficacy of Axi-Cel in LBCL patients.[15] A second study based on the analysis of patients enrolled in the ZUMA-1 study subdivided patients into three subgroups (responders, non-responders, and relapsed). Low baseline tumor burden, high CAR-T cells/tumor burden ratio, low systemic inflammation, and high product CD8 and $CCR7^+$, $CD45RA^+$ T cells were associated with better tumor response.[16] A third study showed that resistance to Axi-Cel is related to immune dysregulation that is frequently observed in LBCL and leads to insufficient *in vivo* Axi-Cel expansion consisting of high blood levels of monocytic myeloid-derived suppressor cells (M-MDSCs) and tumor interferon signaling, giving rise also to expression of immune checkpoint ligands.[17] Finally, a fourth study provided evidence that tumor immune contexture is a major determinant of Axi-Cel efficacy. In particular, clinical response and overall survival were associated with immunological parameters that can be identified using Immunoscore (tumor-infiltrating T cell density) and Immunosign 21 (immune-related gene expression profile).[18] Furthermore, circulating CAR-T cell levels were associated with post-treatment T cell exhaustion in the tumor microenvironment.[18]

Clinical studies with Liso-Cel. Several studies have evaluated Liso-Cel in the treatment of relapsed or refractory LBCLs. The phase I TRASCEND study evaluated 269 LBCL patients with relapsed/refractory disease who received at least two previous lines of therapy and were treated with Liso-Cel using three different dose levels ($50x10^6$ or $100x10^6$ or $150x10^6$ CAR-T cells).[19] The first results of this study showed high response rates (ORR 73%, CRR 53%), with a low incidence of grade 3 or worse cytokine release syndrome and neurological events.[19]

The TRANSFORM phase III trial randomized 184 LBCL relapsed/refractory patients, candidates for autologous SCT, to treatment with either standard-of-care therapy or Liso-Cel ($100x10^6$ CAR-T cells).[20] With a median follow-up of 6.2 months, the EFS was 10.1

months in the Liso-Cel group compared to 2.3 months in the standard-of-care group.[20] An analysis of the results observed in this trial after a follow-up of 17.5 months showed: a CRR of 74% in the Liso-Cel arm compared to 43% in the SOC arm; a PFS not reached in the Liso-Cel group compared to 6.2 months in the SOC group; a mOS not reached in the Liso-Cel arm compared to 29.9 months in the SOC arm.[21] The safety profile of treatment with Liso-Cel was favorable, with grade 3 cytokine release syndrome and neurological events occurring in 1 and 4% of patients, respectively.[21]

An analysis of the parameters related to the quality of life (QoL) showed that the Liso-Cel arm showed a higher improvement in QoL parameters and a lower deterioration than the SOC arm.[22]

Olson and coworkers explored tumor biology and microenvironment from lymph node biopsies of DLBCL patients undergoing treatment with Liso-Cel. The authors compared gene expression profiles between responding and non-responding patients.[23] Tumor microenvironment and tumor-associated macrophage stromal gene signatures had been previously associated with adverse outcomes to standard chemoimmunotherapy treatment in DLBCL.[24] Their study was carried out on 78 patients with DLBCL included in the TRASCEND NHL 001 trial and showed that pre-treatment biopsies from patients achieving a complete response showed higher expression levels of T-cell and stroma-associated genes and lower expression of cell-cycle-related genes in the responding patients, post-treatment biopsies had higher levels of CAR-T-cell densities and CAR gene expression, general immune infiltration, and immune activation.[23]

Clinical studies with Tisa-Cel. Other studies have explored the safety and efficacy of Tisa-Cel in adult relapsed/refractory DLBCL patients. The phase II multicentre JULIET study initially explored the safety and the efficacy of Tisa-Cel in a group of 93 patients with relapsed/refractory DLBCL, showing 52% of ORR, with 40% of CR and 12% of PR; 22% of patients displayed a cytokine release syndrome and 12% neurologic events.[25] Analysis of long-term outcomes in the JULIET trial extended to 115 patients treated with Tisa-Cel showed the following results: an ORR and a CRR in 53% and 39% of cases, respectively; mPFS and mOS were 2.9 months and 11.1 months, respectively; among 34 patients with CR at 6 months, only 3 relapsed within 12 months. Post-hoc analysis showed that PFS and OS were not reached among patients reaching a CR after 6 months.[26]

A comparison of the results of the JULIET trial with historical results (CORAL study) observed in a similar patient group treated with standard chemotherapy supported the superiority of CAR-T-based treatment compared to chemotherapy (mOS of 12.48 months compared to 4.40 months, respectively).[27]

The BELINDA phase III clinical trial randomized 322 patients with aggressive B-cell lymphomas (about 70% of the patients had DLBCLs) to treatment with Tisa-Cel or with standard of care (salvage chemotherapy and autologous HSCT). The ORR and EFS were similar in the two groups of patients.[28] Tisa-Cel was not superior to standard salvage therapy in this trial.

In a retrospective study, 418 relapsed/refractory DLBCL patients, included in the French DESCART-T registry, were treated with CAR-T cell therapy either using Axi-Cel or Tisa-Cel. Treatment results were compared after 1:1 propensity score matching. With a median follow-up of 11.7 months, the 1-year PFS was 46.6% for Axi-Cel and 33.2% for Tisa-Cel, 1-year OS was 63.5% for Axi-Cel and 48.8% for Tisa-Cel, the ORR was 80% for Axi-Cel compared to 66% for Tisa-Cel and the CRR was 60% for Axi-Cel compared to 42% for Tisa-Cel.[29] However, immune effector cell-associated neurotoxicity syndrome (ICANS) and cytokine release syndrome (CRS) were more frequent in the Axi-Cel than in the Tisa-Cel group.[29] In conclusion, this retrospective study supports a higher efficacy and higher Axi-Cel toxicity than Tisa-Cel.[29]

The analysis of the safety profile of DLBCL patients treated with Tisa-Cel-based CAR-T in the context of the JULIET trial showed the occurrence of manageable long-term (LT) adverse events: 14% of responding patients displayed LT cytopenias lasting 90 days; patients treated with rituximab displayed hypogammaglobulinemia that in some patients was exacerbated by CAR-T treatment; few responding patients had LT infections (severe or opportunistic infections).[30]

The phase Ib PORTIA study explored the safety and the efficacy of Tisa-Cel in association with the anti-PD-1 inhibitor pembrolizumab in relapsed/refractory DLBCL patients; the patients enrolled in this study were subdivided into three cohorts: 4 patients were treated with pembrolizumab on day 15, 4 on day 8 and 4 patients on day -1, for CAR-T cell infusion.[31] The best response rates were observed in patients treated with pembrolizumab before Tisa-Cel, but definitive conclusions cannot be drawn given the limited number of patients studied; the drug association displayed a manageable safety profile; pembrolizumab did not stimulate the cellular expansion of Tisa-Cel but delayed peak expansion in the day -1 cohort.[31]

Comparative analysis of the results obtained in phase III studies on CAR-T cell therapy in the second-line. Three prospective randomized phase III clinical trials, ZUMA-7 (Axi-Cel), TRANSFORM (Liso-Cel), and BELINDA (Tiso-Cel), have compared CAR-T cell therapy to standard of care (high-dose chemotherapy with auto-HSCT) in DLBCL patients with early relapsed/refractory

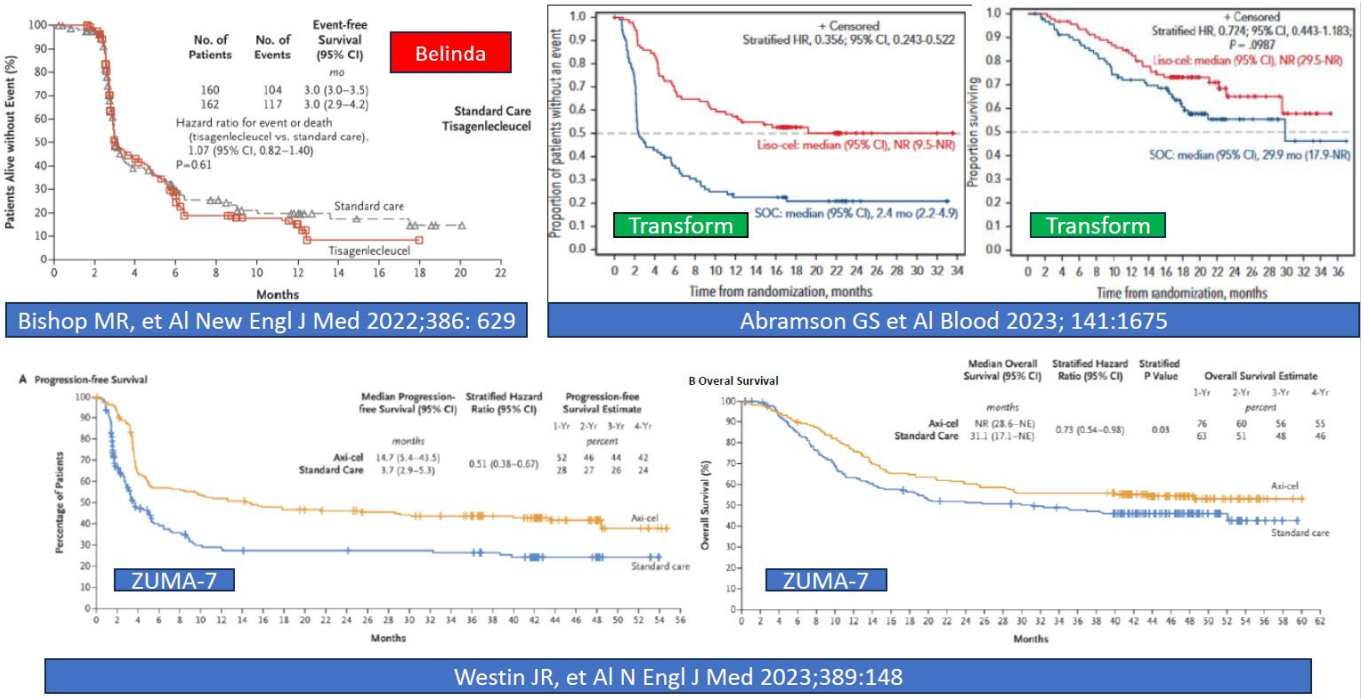

Figure 2. Patients DCBL Disease-free Survival and Overall Survival of the three randomized trials of 19-CAR T-cell vs. Standard Care: BELINDA, Engl J Med 2022, 386(7): 629-639; ZUMA-7, N Engl J Med 2023, 2023 Jul 13;389(2):148-157; TRANSFORM, Blood 2023; 141(14): 1675-1684. No differences in the Belinda trial; significant differences in the two other trials, mostly evident for Disease free survival.

disease (<12 months after chemoimmunotherapy). The ZUMA-7 study reported an improved event-free survival in the Axi-Cel arm compared to the SOC arm (8.3 months vs 2.0 months).[8-9] In the TRANSFORM study, median EFS was longer in the Liso-Cel arm than in the SOC arm (not reached vs 2.4 months).[19-20] In contrast, in the BELINDA study, no difference in median EFS was observed between Lisa-Cel and SOC arm (3.0 vs 3.0 months).[28] Based on these data in 2022, the FDA and EMA approved Axi-Cel and Lisa-Cel for DLBCL patients with refractory disease or relapsed within 12 months of first-line treatment. In the BELINDA study, the Tisa-Cel arm included a higher proportion of patients with intermediate or higher IPI scores and double-hit lymphoma compared to the SOC arm (65.4% vs 57.5%, respectively).[28]

CAR-T cell therapy in first-line treatment of DLBCL. In addition to the studies carried out in second and third-line refractory/relapsed DLBCL patients, a recent study explored the safety and the efficacy of Axi-Cel in first-line therapy in a population of high-risk DLBCL patients.[32] This study enrolled 40 patients: 22 with DLBCL, 16 with double- or triple-hit lymphomas, and 2 with high-grade B-cell lymphoma (HGBL) not otherwise specified.[32] Double-hit or triple-hit lymphomas correspond to a subtype of DLBCLs with *MYC* rearrangement concurrent with a rearrangement in *BCL2*, *BCL6*, or both. They are associated with poor outcome after standard chemotherapy treatment.[7] All treated patients received two cycles of one previous systemic therapy, most commonly R-CHOP or DA-EPOCH-R; 40% had PD, suggesting a primary chemorefractory disease.[32] The following results were observed: a CRR of 78%, with a median time to CR of 30 days; an ORR of 89%, with a median time to objective response of 29 days; the estimated rates for DoR, PFS, EFS, and OS at 12 months were 81%, 75%, 73%, and 91%, respectively.[32] Grade 3 cytokine release syndrome and grade 3 neurologic events were observed in 8% and 18% of patients, respectively.[32]

Given the results observed in the ZUMA-12 study, the ZUMA-23 trial was proposed as a phase III randomized controlled trial involving the evaluation of Axi-Cel as a first-line regimen in comparison with standard of care in about 300 LBCL adult patients with a high-risk disease, defined as International Prognostic Index 4-5.[33]

CAR-T with double targeting. Failure to achieve sustained responses is observed in about 60-70% of relapsed/refractory LBCL patients treated with CD19-directed CAR-T cells. This result is due to different mechanisms: (i) CD19-negative relapse due to antigen downregulation or loss of CAR-T selection pressure; (ii) impaired CAR-T cell expansion and T-cell exhaustion; (iii) overexpression of programmed cell death protein 1 (PD-1) and/or high expression of PD-L1. One of the possible strategies to bypass these resistance mechanisms consists of dual antigen targeting, such as dual targeting of CD19 and CD22 with bicistronic CAR-T cells. Thus, AUTO3, a dual-targeting, humanized, second-generation autologous CD19/CD22 CAR-T product, was developed using a bicistronic vector

encoding CD19 CAR and CD22 CAR within a single construct.[34] In the phase I ALEXANDER trial AUTO3 plus PD-1 blockade with pembrolizumab was evaluated as third-line therapy in 52 adult patients with relapsed/refractory LBCL.[34] AUTO3 administration was well tolerated with only rare events of drug-related toxicity.[34] The ORR was 66%, with 54% of CR; the mDoR was 8.3 months; for patients with CR, the mDoR was not reached; mPFS was 3.3 months and the mOS was 13.8 months.[34]

The results of this study indicated that dual-targeting CAR-T and pembrolizumab as third-line therapy was able to induce a significant therapeutic response in 55% of relapsed/refractory LBCL patients and future studies will attempt to develop a new generation of AUTO3, endowed with a higher capacity of *in-vivo* expansion.[34]

Other studies have explored another strategy based on the combined targeting of CD19 and CD20 on the surface of B-lymphoid cells. In this context, Tong et al. reported the development of optimized tandem CD19/CD20 CAR-engineered T cells (TanCAR7 T cells) that target these two antigens simultaneously or separately.[35] TanCAR7 T cells were shown to possess a marked antitumor activity *in vitro;* furthermore, early clinical evidence demonstrated an acceptable safety profile and clinical efficacy: 28 patients with relapsed/refractory NHLs, including 16 patients with DLBCL, were treated with TanCAR7 T cells.[35] 75% of patients with DLBCL had a response, with an mPFS not reached, and 75% of patients showed no disease progression at 12 months after infusion.[35] Concerning the safety profile, 14% of patients displayed a grade 3 CRS and no cases of grade 3 T-cell-related encephalopathy syndrome were observed.[35]

A more recent study reported the results obtained with this strategy in 87 NHL patients (66% with DLBCL).[36] Among DLBCL patients, 78% of objective responses were observed, with a CRR of 71%, a median DoR not reached 74% of patients remaining in remission 12 months after having a response, a median PFS of 23.5 months, and 59% of patients showing no disease progression at 12 months after infusion.[36] The mOS was not reached, and the probability of survival was 88% at 6 months and 75% at 12 months.[36]

The DALY II multicenter trial evaluated the safety and efficacy of bispecific targeted CD20/CD19 therapy with ZAMTO-CEL in 22 patients with relapsed/refractory DLBCL (68% with high-risk disease with IPI ≥3 and 13% exposed to previous CAR-T cell therapy).[37] At 3 months post-treatment, the ORR was 52% with 38% CR and 14% PR.[37]

Role of bridging therapy during CAR-T cell therapy. Bridging therapy (BT) is the anticancer therapy administered in patients during CAR-T cell manufacturing. It is a tool to stabilize or debulk disease between leukapheresis and CAR-T cell administration.[38] However, there is no clear indication for administering or not BT, and the use of BT is guided by physician and patient preferences. A recent study explored BT modality and response in 375 patients undergoing treatment with either Axi-Cel or Lisa-Cel; most patients received BT as chemotherapy or radiotherapy.[39] This analysis showed that complete or partial response to BT conferred a 42% reduction in disease progression and death after CD19 CAR-T therapy.[39] The best responses to BT were observed in patients treated with polatuzumab-containing chemotherapy regimens.[39]

The decision to perform bridging therapy should be individualized at the level of the single patient, taking into account several factors, such as tumor burden, number and types of previous lines of therapy, and the expected timing for CAR-T cell infusion compared to apheresis.[38]

Hubblings and coworkers have evaluated the role of bridging therapy based on radiotherapy in a group of 33 DLBCL patients undergoing CD19 CAR-T cell therapy.[40] Bridging radiotherapy induced a significant reduction of the diameter of lymphoma lesions, MTV, SUV (standard uptake value), and LDH levels, all predictors of poor outcomes post-CAR-T therapy outcomes.[40] Therefore, bridging radiotherapy may help to convert poor-risk LBCL patients into patients with better risk.[40]

Clonal Hematopoiesis and CAR-T Cell Therapy. A recent study explored the potential risk caused by clonal hematopoiesis (CH) in DLBCL patients undergoing treatment with anti-CD19 CAR-T cells. CH is a condition of clonal expansion of hematopoietic stem/progenitor cells bearing somatic gene mutations.[41] CH is associated with an increased risk of hematological malignancies, cytopenias, and nonhematological conditions such as atherosclerosis and cardiovascular and cerebrovascular disease.[41]

Sinai et al. explored 114 LBCL patients undergoing treatment with CAR-T cells (105 with Axi-Cel and 9 with Tisa-Cel) for CH detected in 36.8% of cases. The two most frequently mutated genes were *PPMN1D* (19/114) and *TP53* (13/114).[42] The incidence of therapy-related neurotoxicity was higher in CH-positive than in CH-negative patients (45.2% vs 25.0%, respectively). Higher neurological toxicities were preferentially associated with *DNMT3A, TET2,* and *ASXL1* genes.[41] A higher incidence of grade ≥3 cytokine release syndrome was observed in the CH-positive than in CH-negative patients (17.7% vs 4.2%, respectively).[42] Finally, the 24-month cumulative incidence of therapy-related myeloid neoplasms after CAR-T cell therapy was higher in CH-positive than in CH-negative patients (19% vs 4.2%, respectively).[42]

Other studies have reported myeloid malignancies

development in LBCL patients undergoing anti-CD19 CAR-T cell treatments.[43-44] The precise mechanism behind the increased risk of tMN has to be elucidated and remains subject to speculation. It remains unclear whether the particular immune dysregulation in patients after CAR-T plays an important role or whether the occurrence of tMN is simply the consequence of genetic damage induced by the precedent lines of therapy in these mostly heavily pretreated patients.

Autologous Stem Cell Transplantation vs CAR-T Cell Treatment for DLBCL Patients in Partial Remission. Randomized clinical trials have evaluated CAR-T treatment's safety and efficacy in a subset of DLBCL patients with early treatment failure. These patients were randomized to salvage therapy followed by auto-HSCT consolidation in responding patients or directly to CAR-T treatment without attempting salvage therapy. However, the efficacy of auto-HSCT and CAR-T treatment was not comparatively evaluated in a population of DLBCL patients achieving a partial response to initial standard therapy. DLBCL patients achieving only partial response after chemoimmunotherapy show durable remissions after autologous HSCT with a 5-year PFS of 41% and an OS of 51%-63%.[45]

In a retrospective analysis carried out in a group of patients with DLBCL patients in partial response post-salvage therapy, auto HSCT (266 patients) and CAR-T cell therapy with Axi-Cel (145 patients) gave 2-year PFS of 52% vs 42% and OS of 69% vs 47%, respectively.[46] Therefore, this study showed a slightly longer PFS and OS in DLBCL patients in partial response after salvage therapy treated with auto-HSCT compared to CAR-T cell therapy.[46]

At variance with the findings of this study, Akhtar et al. performed a retrospective analysis on 125 older (≥65 years) DLBCL patients in partial response after salvage therapy undergoing treatment with either auto-HSCT or CAR-T infusion: no statistically significant differences between auto-HSCT and CAR-T groups in 1-year OS (68% vs 72%, respectively) and 1-year PFS (56% vs 59%, respectively) were observed.[47] Furthermore, patients in the CAR-T group showed a trend to lower non-relapse mortality compared to those in the auto-HSCT group.[47] According to these observations, the authors suggest that in older patients with refractory/relapsed DLBCL patients achieving a partial response to salvage chemotherapy, CAR-T treatment resulted in outcomes comparable to auto-HSCT.[47]

Allogeneic HSCT and CAR-T Therapy after Auto-HSCT Failure in DLBCL. A retrospective noncomparative registry study analyzed outcomes in 584 patients with DLBCL undergoing a reduced intensity allo-HSCT or CAR-T therapy with Axi-Cel after a prior auto-HSCT failure. The 1-year relapse, non-relapse mortality, overall survival, and progression-free survival after auto-HSCT failure were: for CAR-T treatment, 39.5%, 4.8%, 73.4%, and 55.7%, respectively; for the allo-HSCT cohort, 26.2%, 20.0%, 65.6%, and 53.8%, respectively.[48] Therefore, both CAR-T cell treatment and allo-HSCT can provide durable remissions in a subset of DLBCL patients relapsing after auto-HSCT.[48]

Therapy of DLBCL after CAR-T Failure. Over 60% of LBCL patients ultimately progress or relapse following CD19-CAR-T cell therapy. The treatment of patients relapsing after CAR-T cell therapy failure is extremely challenging and largely undefined.

Tomas et al. explored 182 LBCL patients experiencing disease recurrence or progression after CAR-T therapy; 74% received anticancer treatment post-CAR-T failure, with a mOS of 8 months.[49] Most of these patients were treated with standard chemotherapy, polatuzumab-based therapies, or lenalidomide-based therapies: no CRs were observed in patients treated with

Table 1. Main CD19-CAR-T cell therapies. Four CAR products are currently available commercially and three of them were approved for the treatment of DLBCL patients.

CART Product	Antigen-binding domain	Hinge region	Transmembrane region	Co-stimulatory domain	T cell activation domain	Therapeutic indication
Tisagenleucel	Anti-CD19	CD8a	CD8a	4-1BB	CD3ζ	R/R B-ALL Relapsed LBCL (after second-line) Relapsed FL (after second-line)
Axicabtagene ciloleucel	Anti-CD19	CD28	CD28	CD28	CD3ζ	Relapsed LBCL (after fist-line) Relapsed LBCL (after second-line) Relapsed FL (after second-line)
Brexucabtagene autoleucel	Anti-CD19	CD28	CD28	CD28	CD3ζ	R/R B-ALL R/R MCL
Lisocabtagene maraleucel	Anti-CD19	IgG4	CD28	4-1BB	CD3ζ	Relapsed LBCL (after first-line) Relapsed LBCL (after second-line)

conventional chemotherapy, while ≥30% CRs were observed among patients treated with polatuzumab- or lenalidomide-based therapies.[49] Factors associated with poor overall survival among patients treated post-CAR-T failure were represented by pre-CAR-T bulky disease, lack of response to CAR-T therapy, age >65 years, and elevated LDH at post-CAR-T treatment: the presence of ≥2 of these factors was associated with lower OS compared to ≤1.[49]

Another study retrospectively evaluated 83 patients with LBCL receiving an allo-HSC after anti-CD19 CAR-T cell therapy failure.[49] The median number of lines of therapy between CAR-T infusion and allo-HSCT was 1; low-intensity conditioning was used in 77% of cases, and peripheral blood was the most common graft source; the most common donor types were matched unrelated donor (39%), followed by haploidentical (30%) and matched-related donor (26%).[50] One year OS, PFS, and GVHD were 59%, 45%, and 39%, respectively.[50] These findings concluded that allo-HSCT after CAR-T failure can provide durable remissions in a subset of patients.[50]

CAR-T Therapy in DLBCL: Prognostic Factors and Mechanisms of Relapse. Identifying a subgroup of DLBCL patients who benefit from anti-CD19 CAR-T cell therapy remains a key challenge. The clinical trials with Axi-Cel and Tisa-Cel failed to identify clinical covariates predictive of efficacy.

Clinical factors. Vercellino and coworkers have investigated the predictive factors for early progression after CAR-T cell therapy in 116 refractory/relapsed LBCL patients; 55 of these patients failed treatment, and 49% of these patients relapsed within the early months after CAR-T cell therapy and therefore are early progressors.[51] Risk factors identified for early progression at the time of diagnosis and at the time of treatment are represented by extranodal site involvement (≥2 sites) and lymphoma tumor burden as measured by total metabolic tumor volume (TMTV) assessment and LDH levels.[51]

As discussed above, the tumor burden is a major determinant of outcomes of DLBCL patients at the moment of CAR-T cell therapy with Axi-Cel.[14-15] In line with these observations, Nastoupil et al., in a retrospective analysis, showed an association between achieving CR at 12 months after Axi-Cel treatment and no need for bridging therapy.[13] Since the need for bridging therapy reflects either a higher tumor burden or a more rapidly progressive disease, it is evident why it emerges as a negative prognostic factor.

Hirayama and coworkers have explored some prognostic factors associated with durable responses in patients with aggressive NHL (mostly DLBCL and

Table 2. Main clinical trials carried out in DLBCL patients using CD19-CAR-T cells. Abbreviations: SOC (standard-of-care); ORR (Overall Response Rate); CRR (Complete Response Rate); NR (Not Reported); PFS (Progression-Free-Survival); OS (Overall Survival); EFS (Event-Free Survival).

Clinical trial	Patients	CAR product	Median follow-up	ORR (%) CRR (%)	PFS	OS
ZUMA-7 Phase-III randomized	246 DLBCL	Axi-Cel SOC: 120 patients Axi-Cel: 126 patients	47.2 months	ORR SOC: 50 Axi-Cel: 83 CRR SOC: 32 Axi-Cel: 65	SOC: 3.7 months Axi-Cel: 14.7 months	SOC: 31.1 months Axi-Cel: Not Reached
ZUMA-1 Phase I/II single-arm	77 DLBCL	Axi-Cel	63.1 months	ORR: 81 CRR. 58	5.9 months	11 months
ZUMA-12 Phase I	22 DLBCL 16 DH or TH-L 2 HGBL	Axi-Cel	15.2 months	ORR: 89 CRR: 78	75% at 12 months	81% at 12 months
TRANSCEND NHL-001 Phase I multicentre	270 LBCL	Liso-Cel	6.8 months	ORR: 73 CRR: 53	6.8 months	NR
TRANSFORM Phase III randomized	184 LBCL	Liso-Cel SOC: 92 patients Liso-Cel: 92 patients	17.5 months	SOC: 43 Liso-Cel: 74	SOC: 2.4 months Liso-Cel: not reached	EFS SOC: 2.3 months Liso-Cel: 10.3 months
JULIET Phase I	115 LBCL, HGBL, tFL	Tisa-Cel	40 months	ORR: 53 CRR: 39	2.9 months	11.1 months
BELINDA Phase III randomized	322 aggr. B-lymphoma (70% DLBCL)	Tisa-Cel SOC: 160 patients Tisa-Cel: 162 patients	18 months	ORR: 46 vs 45 CRR: 28 vs 27	3 months vs 3 months	At 18 months: SOC: 60% Tisa-Cel: 60%

HGBL-DH or TH). These patients received lymphodepletion with cyclophosphamide and fludarabine, followed by CAR-T cell infusion.[52] This analysis identified lower serum lactate dehydrogenase and a favorable cytokine profile (defined as serum day 0 monocyte chemoattractant protein-1 (MCP-1) and interleukin-7 (IL-7) above the median level) as serum biomarkers associated with a better PFS.[52]

CAR-T cell therapy is associated with two main early toxicities represented by cytokine release syndrome and neurotoxicity; the frequency and severity of these toxicities are partly associated with baseline disease and patient characteristics. Both the Cumulative Illness Rating Scale (CIRS) and the International Prognostic Index (IPI) are associated with outcomes in DLBCL patients after CAR-T cell therapy.[53-54] A recent study used the CIRS to define a prognostic score predictive of outcomes of CAR-T cell therapy in DLBCL patients.[55] Particularly, a CIRS ≥3 in the respiratory, upper gastrointestinal, hepatic, or renal system, defined as "severe 4", predicted shorter PFS and OS and a CRS of grade ≥3.[55] Therefore, a simplified CIRS-derived comorbidity index may predict adverse outcomes in DLBCL patients undergoing CAR-T cell therapy.

CD19 antigen escape is one of the mechanisms of relapse observed in some DLBCL patients relapsing after CAR-T cell therapy. In this context, Plaks and coworkers explored 20 DLBCL patients treated in the ZUMA-1 trial with Axi-Cel for CD19 expression at RNA and protein level and for CD19 gene mutational status.[56] 30% of these patients showed a relapse characterized by negative/low CD19 expression; the mechanism responsible for the generation of a CD19-negative relapse seems to be related to indirect treatment-related selection of tumor cells with low-very low CD19 protein expression in the context of removal of antigen-positive tumor cells rather than alternative splicing or CD19 mutation.[56]

Tumor-related genomics. Typical tumor-related features, such as double- or triple-hit translocations, activated B-cell-like, and cells of origin phenotype, are not informative of outcomes in LBCL patients undergoing CAR-T cell treatment. To identify tumor-related factors that could be associated with response to CAR-T cell therapy, Shouval et al. have characterized the mutational profile of 153 LBCL patients undergoing CD19-CAR-T cell therapy; 37% of these patients displayed *TP53* alterations (either mutations and/or copy number alterations): the 1-year OS of *TP53*-altered LBCL was 44% compared to 1-year OS of 76% among *TP53*-WT patients.[57] Transcriptomic studies showed that *TP53* alterations are associated with dysregulation of pathways associated with CAR-T-cell cytotoxicity and reduced CD8 T-cell tumor infiltration.[57]

Jain and coworkers have analyzed the genomic profile of 49 LBCL patients undergoing CAR-T cell therapy by whole-genome sequencing.[58] The analysis showed that the pre-treatment presence of complex structural variants, APOBEC mutational signatures, and genomic damage deriving from reactive oxygen species predict CAR-T resistance; furthermore, the recurrent 3p21.31 chromosomal deletion englobing the *RHOA* tumor suppressor gene was markedly enriched in patients with failure to CAR-T cell therapy.[58]

Zhou et al. have used low-pass whole genome sequencing of ct-DNA to explore copy number alterations (CNAs) in pre-treatment plasma samples of 122 LBCL patients before CAR-T cell therapy.[59] A high focal CNA score, denoting genomic instability, was the most significant pre-treatment CNA associated with inferior 3-month CRR, PFS, and OS.[59] Among the 34 unique focal CNAs observed in these patients, deletion at 10q23.2, determining the loss of the FAS death receptor, was most significantly associated with poor outcomes.[59]

Other studies have evaluated the residual tumor disease in DLBCL patients undergoing CAR-T cell therapy using noninvasive monitoring for treatment response and predicting disease relapse after therapy. Routine surveillance by tumor imaging for DLBCL patients achieving remission is of limited utility. In contrast, molecular disease evaluation by immunoglobulin high-throughput sequencing from peripheral blood provides a more sensitive strategy for surveillance. Molecular disease can be detected in peripheral blood cells and plasma; molecular disease detection often precedes PET/CT detection of relapse in patients initially achieving remission.[60]

Frank et al. have evaluated the role of monitoring circulating tumor DNA in detecting relapse following CAR-T cell therapy with Axi-Cel. 69 LCBL patients with a tumor clonotype were explored by analysis of ctDNA: high pre-treatment ctDNA concentrations were associated with progression after Axi-Cel infusion and development of CRS and immune effector cell-associated neurotoxicity syndrome; 70% of patients with durable response compared to 13% of progressing patients showed non-detectable ctDNA one week after Axi-Cel infusions. At day 28, patients with detectable ctDNA compared to those with undetectable ctDNA had a PFS of 3 months vs not reached and an OS of 19 months vs not reached; ctDNA was detected at or before radiographic relapse in 94% of patients, while 100% of durably responding patients had undetectable ctDNA; in patients with radiographic PR or stable disease, 10% of those with concurrently undetectable ctDNA relapsed and 92% of those with concurrently detectable ctDNA relapsed.[61]

A recent study by Sworder et al. provided fundamental information on the genomic mechanisms of resistance to CAR-T cell therapy observed in 138

relapsed/refractory LBCL patients undergoing treatment with Axi-Cel.[62] In this study, a peculiar methodology was developed for the simultaneous assessment of ctDNA, cell-free CAR19 (cfCAR19) retroviral fragments (for evaluation of CAR-T cell expansion and functional persistence *in vivo* after their infusion), and cell-free T cell receptor rearrangements (cfTCR) that enabled noninvasive profiling and integration of tumor dynamics and of T cell expansion and TCR diversification in CAR19 patients.[62] Baseline and dynamic ctDNA levels were prognostic for outcome: patients experiencing disease progression had significantly higher pre-treatment ctDNA levels; at 4 weeks post-infusion, patients that achieved a ctDNA major molecular response showed significantly better outcomes.[62] The analysis of cfCAR19 showed similar levels between patients responding or not to CAR-T cell therapy, without any significant difference between these two groups.[62] However, cfTCR levels at 4 weeks after CAR19 infusion were higher in patients with durable response than in patients with disease progression.[62] The analysis of the mutational profile showed that mutations in several genes are significantly associated with inferior event-free survival, such as alterations of *TMEM30A, IRF8, PAX5, TP53,* and *DXT1* genes; other mutations appeared in patients relapsing after CAR-T therapy, such as multiple *CD19* alterations and *PPM1D* mutations.[62] Relapsing patients also displayed gene amplifications of *PD-L1* or *PD-L2*. These somatic mutations affect CAR-T cell therapy at various levels, including CAR-T cell expansion, persistence, and tumor microenvironment. Resistant DLBCL tumors may display either abundant infiltrating CAR-T cells or low/absent CAR-T cells: tumors with high infiltration demonstrate different microenvironmental and inflammatory signatures compared to tumors with low CAR-T infiltration, thus suggesting different mechanisms of resistance.[62]

Gene expression studies. Several biological mechanisms contribute to the heterogeneity of DLBCL, such as cell-of-origin subtypes, genomic alterations, and differences in composition and activation of cellular elements present in the tumor microenvironment.

Gene expression profiling (GEP) studies have refined the molecular classification of DLBCL. GEP studies have characterized the consistent heterogeneity in the lymphoma microenvironment. In this context, Kotlov and coworkers have analyzed the publicly available gene expression profiles of 4655 DLBCL patients; using this approach, they have identified 25 functional gene expression signatures (F^{GES}) corresponding to subtypes of the microenvironment, non-cellular components of the tumor microenvironment, biological processes, and signaling pathways.[63] According to these F^{GES}, four types of lymphoma microenvironment were identified: a germinal center-like LME1 (15%), with the presence of F^{GES} of cell types present in germinal centers; a mesenchymal LME2 (33%), due to the presence of stromal and extracellular matrix pathways; an inflammatory LME3 (25%), due to the presence of F^{GES} associated with inflammatory cells and pathways; and a depleted LME4 (27%), due to the low presence of microenvironment-related F^{GES} and to the presence of proliferation-related F^{GES}.[63] The four LME categories of DLBCLs are associated with specific genomic alterations and distinct clinical outcomes: a better PFS and OS for LME1 and LME2 than for LME3 and LME4.[63]

Steen and coworkers have implemented a machine learning algorithm, termed Eco Typer, to integrate transcriptomic deconvolution and single-cell RNA sequencing to define states and ecosystems present in DLBCLs.[64] B-cell states were defined by COO subtypes GBC and ABC and subdivided into centrocytes, centroblasts, memory B cells, and plasmablasts. This approach identified five different cell states of malignant B cell differentiation associated with differences in prognosis.[64]

Several studies have shown that the heterogeneous characteristics of the TME in LBCL are associated with clinical responses to anti-CD19 CAR-T cell therapy. Scholler et al. have explored the dynamic changes in TME occurring in LBCL patients undergoing treatment with Axi-Cel in the context of the ZUMA-1 trial.[18] In this analysis the patients were subdivided into two groups, responders and non-responders; responders showed an early and rapid increase of cytotoxic T cell-related genes, such as CD8α, T cell growth factor genes such as IL-15, interferon-γ-regulated immune checkpoint encoding genes (CD274, CD276 and CTLA-4), myeloid-related genes and chemokines; in non-responders, no increase in immune-related genes was observed, except for pro-inflammatory chemokines such as CXCL10 and CXCL11.[18] Immunohistochemical studies in a few patients have confirmed these observations by gene expression analysis, showing higher T cell densities among responders reflecting pre-treatment T cell density.[18] The infiltration of TME with exhausted T cytotoxic lymphocytes observed in non-responding patients correlated with poor CAR-T cell expansion in blood. The pre-treatment quantification of tumor-infiltrating T cell density by Immunoscore and of a panel of immune genes by Immunosign 21 positively correlated with overall survival after CAR-T cell therapy.[18]

Batlevi and coworkers extensively characterized 49 DLBCL patients treated with CD19-CAR-T cell therapy using whole exome sequencing performed on tumor samples, defining the cell of origin, assessing double hit gene signatures and the lymphoma microenvironment, analyzing gene expression according to Kotlov et al.[63] In these patients, the overall response at 3 months was

77.6%, with 59.2% CR and 18.4% PR, PFS at 6 months was 49%; prognostic biomarkers to CAR-T therapy, such as LDH levels, MTV, and SUV were confirmed.[65] The major findings of this study were that: *PIK3CA* amplification was associated with improved PFS; increased *MHCII* expression, associated with centrocyte-like phenotype,[66] was higher in DLBCL patients with GCB phenotype responsive to CD19-CAR-T therapy; DLBCL patients with GCB phenotype and with higher *SMAD1* expression are usually responsive to CD19-CAR-T cell therapy; germinal-center-like and mesenchymal LME subtypes exhibited increased OS compared to those with inflamed and depleted LME subtypes.[65]

Haradhval et al. used single-cell RNA sequencing to explore cellular dynamics associated with response to CAR-T therapy for DLBCL using Axi-Cel or Tisa-Cel.[66] Axi-Cel and Tisa-Cel, as discussed above, differ for many characteristics related to differences in CAR design (4-1BB vs. CD28 costimulatory domain, CD8 vs. CD28 transmembrane domain for Tisa-Cel vs Axi-Cel, respectively), in vectors used for their delivery, and in manufacturing processes (fresh vs frozen apheresis products, activation by antibody-coated beads vs soluble antibody and cytokines). This study showed that Tisa-Cel responses were associated with the expansion of central-memory CD8 cell populations, while Axi-Cel responders displayed more heterogeneous cell populations.[67] Despite these differences in cell types associated with response, both Axi-Cel and Tisa-Cel CAR-T cells displayed at day seven after infusion a remarkable increase in the expression of genes related to cellular proliferation and activation.[67] In Axi-Cel non-responders, a population of regulatory T-cells with CAR transcripts in the infusion product was expanded *in vivo* and could exert an immunosuppressive activity.[67]

Gene expression studies have also contributed to understanding the consistent heterogeneity of CAR-T products obtained from different DLBCL patients. Deng and coworkers analyzed the cellular and molecular features of CAR-T infusion cell products prepared using Axi-Cel to identify transcriptomic (by single-cell RNA sequencing) features associated with efficacy and toxicity in 24 LBCL patients. 50% of these patients had progressive disease, 4% a partial response and 38% a complete response.[68] Patients achieving a complete response at 3 months had 3-fold higher frequencies of CD8 T cells expressing memory signatures compared to patients with partial responses or progressive disease.[68] Molecular responses at day 8 post-infusion were significantly associated with the clinical response signature of CD8 T-cell exhaustion associated with a poor molecular response.[67] Finally, a rare cell population with monocytic features was associated with ICANS occurrence.[68]

Hematologic toxicity. Hematologic toxicity is frequently observed in DLBCL patients undergoing CAR-T cell therapy. In 258 patients receiving CD19-CAR-T cell therapy, profound neutropenia was observed in 72% of cases and prolonged neutropenia in 64% of patients; in these patients, predictive biomarkers of hematologic toxicity were baseline cytopenia (thrombocytopenia) and inflammatory state (hyperferritinemia).[69] According to these observations, a predictive model for hematologic toxicity (CAR-HEMATOTOX) was generated based on markers associated with hematopoietic reserve, such as platelet count, absolute neutrophil count, and hemoglobin level, and baseline inflammation markers, such as C-reactive protein and ferritin.[68] A high CAR-HEMATOTOX score predicted a longer neutropenia duration and a higher incidence of thrombocytopenia and anemia.[69]

Infectious complications represent the key determinant of non-relapse mortality after CAR-T cells. They are favored not only by neutropenia but also by the immune disturbance caused by the T-CAR cells. The temporal distribution of these risk factors shapes different infection patterns early versus late post-CAR-T-cell infusion. Furthermore, due to the expression of their targets on B lineage cells at different stages of differentiation, CD19 and B-cell maturation antigen (BCMA), CAR-T cells induce distinct immune deficits that could require different prevention strategies. Infection incidence is the highest during the first-month post-infusion and decreases afterward. However, infections remain relatively common even a year after infusion. Bacterial infections predominate early after CD19, while an equal distribution between bacterial and viral causes is seen after BCMA CAR-T-cell therapy, and fungal infections are universally rare. Cytomegalovirus (CMV) and other herpesviruses are increasingly reported.[70]

Toxicity associated with the immune effector response. CAR T cells can result in significant toxicities directly associated with the induction of powerful immune effector responses. Cytokine release syndrome (CRS), neurotoxicity,[71-73] or more rarely cardiotoxicity[74] represent the most frequent manifestations of this toxicity, which is in relationship with the immunological effects of CAR T cells.[71-73] Toxicities may be related both to the activation of T cells with the release of high levels of cytokines and the interaction between CAR and CAR-target antigens expressed on non-malignant cells. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are well-known inflammatory side effects of CAR T-cell therapy.[71–73] CRS typically presents with constitutional symptoms such as fever, myalgia, and arthralgia or constitutional symptoms such as rigors, fatigue, malaise, and anorexia. However, rapid progression to

hemodynamic instability, respiratory failure, organ dysfunction, shock, and hemophagocytic lymphohistiocytosis has also been reported.[71,72] Depending on the product, CRS typically occurs within 1–2 weeks of CART-cell infusion but can occur as early as a few hours post-infusion.[71,72] CRS has a reported incidence between 37% and 93% across different studies. Factors associated with CRS include the product type, tumor burden, disease indication, elevation in baseline inflammatory markers (i.e., ferritin, C-reactive protein), and concomitant infection. In cases where CRS develops early and is higher grade, severe ICANS is more likely. This occurrence may be partly associated with a high dose of CART cells or usually robust and rapid CART-cell expansion. Notably, ICANS can also infrequently develop in the absence of CRS. ICANS presents as dysgraphia, word-finding difficulties, headache, tremor, confusion, somnolence, expressive aphasia, seizure, and coma. Rarely, death from cerebral edema has been reported (1%–2% estimated incidence).[71-73] Rubin et al.[73] reported that among 100 treated cases, the most commonly occurring neurological symptoms were encephalopathy (57%), headache (42%), tremor (38%), aphasia (35%) and focal weakness (11%). Focal neurological deficits were frequently observed after chimeric antigen receptor T-cell therapy and are associated with regional EEG abnormalities, FDG-PET hypometabolism, and elevated velocities on transcranial Doppler ultrasound. In contrast, structural imaging was typically normal, ICANS may co-occur with CRS or immediately following CRS. Neurologic signs and symptoms typically begin 3–6 days after CART-cell infusion and peak around day 7 or 8, with complete symptom resolution by days 14–21. The rate and grade of ICANS toxicity varies greatly among CAR T-cell products. ICANS occurs in 20%– 70 % of patients treated with CD19 CART cells.[71–73] Inflammatory cytokines released by macrophages, specifically IL-6 and IL-1, have been widely identified as critical components in the pathogenesis of CRS and ICANS, respectively.[71–72] Elevated serum levels of IL-6 are one of the most correlated findings with CRS Activated CART cells release IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) to induce tumor cell cytolysis.86 However, these cytokines also activate macrophages, which release IL-6 and TNF-α.[71,72]

Cardiotoxicity hits about 10% of patients and manifests as cardiomyopathy, heart failure, arrhythmias, and myocardial infarction. Patients undergoing T-cell therapies should be screened for cardiovascular conditions that may not be able to withstand the hemodynamic perturbations imposed by CRS.[74]

Brammer JE et al.[75] report 102 CAR-T-treated patients; of them, 90 were identified as treated with single-agent therapy, of which 88.9% developed toxicity (80 CRS, 41 neurotoxicity, and 17 cardiotoxicity), including 28.9% with high-grade (≥3) events. The most common manifestations were hypotension at 96.6% and fever at 94.8%. Among patients with cardiac events, there was a non-significant trend toward a higher prevalence of concurrent or preceding high-grade (≥3) CRS. 50.0% required tocilizumab or corticosteroids. The median time to toxicity was 3 days; high-grade CRS development was associated with cardiac and neurotoxicity. In multivariable regression, accounting for disease severity and traditional predictors of disease response, moderate (maximum grade 2) CRS development was associated with higher complete response at 1 year (HR: 2.34; p=0.07), and longer PFS (HR: 0.41; p=0.02, in landmark analysis), and OS (HR: 0.43; p=0.03). Among those with CRS, relative blood pressure (HR: 2.25; p=0.004), respectively, was also associated with improved PFS. No difference in disease outcomes or maximum toxicity grade (CRS, neurotoxicity, or cardiotoxicity) was observed based on the presence or absence of early CRS-directed therapies. Therefore, moderate toxicity predicts a good outcome. Nonhematological toxicity also depends on tumor burden; patients with DLBCL without residual lymphoma at the time of CD19 CAR T-cell therapy show low toxicity and excellent outcomes.[76]

Anti-inflammatory therapy, specifically targeting IL6, has become the cornerstone of CRS management.[77,78] Tocilizumab, a humanized IgG1k anti-IL-6R antibody, binds to both soluble and membrane-bound IL-6R, blocking the downstream signal transduction pathways implicated in CRS. It is currently the only anti-IL6 therapy approved by the FDA for treating severe or life-threatening CAR T cell–induced CRS.[77,78] While it is approved for severe or life-threatening CRS, current guidelines and product information recommend initiating tocilizumab for treating grade ≥ 2 or grade 1 CRS in patients at high risk of early and severe CRS or those whose symptoms persist greater than 24 h. For severe (grade ≥ 3) or refractory CRS, the addition of steroids is recommended.[78] A recent analysis[77] of the ZUMA-1 study of axicabtagene-ciloleucel (axi-cel) shows prophylactic corticosteroids and earlier corticosteroid and/or tocilizumab intervention resulted in no grade 3 or higher CRS, a low rate of grade 3 or higher NEs and high response rates in this study population. 95% and 80% objective and complete response rates, respectively, for patients who received prophylactic steroids (dexamethasone 10 mg on day 0 (pre-infusion), day 1 and 2) or early addition of steroids to tocilizumab for CRS[77] Although tocilizumab and steroids are first-line interventions for prevention and treatment of CRS and ICANS, with high response, data for outlining the treatment of refractory CRS and/or ICANS, are lacking. However, there is an emerging use of anakinra and an improvement of mitigation strategies and supportive care

measures to ameliorate outcomes of patients who develop these refractory toxicities.[79]

Conclusions. The studies carried out in the last ten years have clearly supported and defined a role for CD19-CAR-T cells in the therapy of DLBCL patients with refractory/relapsed disease. This therapeutic role was established for patients with refractory disease and early relapse. For DLBCL patients with partial response after salvage therapy, CD19-CAR-T cells also have shown consistent therapeutic activity, but additional studies are required to compare their efficacy to auto-HSCT carefully. Similarly, CD19-CAR-T cells have shown efficacy in treating high-risk DLBCL patients in first-line, but additional studies are required to assess their efficacy compared to standard treatments. At present, there are no data suggesting which of the four CAR products commercially available for patients with B cell lymphomas: Axicabtagene ciloleucel (Axi-Cel), Brexacubtagene autoleucel (Brexa-Cel), Lisocabtagene macrolevel (Liso-Cel) and Tisagenlecleucel (Tisa-Cel), could be the best in term of efficacy and side effects. The results of the contemporary publication in the NEJM of two randomized trials employing one the Tisa-Cel,[28] the other the Axi-Cel,[8] could induce to think a superior efficacy of Axi-Cel; however, the criteria for enrollment of patients are different, so no comparison is possible.[80,81] Another problem is the cost of this therapy. Utilization of CAR T cell therapy is very expensive, but papers comparing the Cost-Effectiveness ratio of the different products are rare.[82] However, the best standard salvage care requires fewer resources in comparison with CAR-T.[83]

Although the efficacy of CD19-CAR-T cell therapy in refractory/relapsed DLBCL patients was well documented, only about 40% of relapsed/refractory DLBCL patients are responding to this treatment, and the remaining are refractory or rapidly relapse. Several strategies seem to be required to improve the outcomes of these patients: (i) decrease tumor burden using novel bridging therapies that include chemoimmunotherapy or radiation therapy prior to CAR-T cell therapy; (ii) use CAR-T cells engineered with double targeting activity, such as CD19/CD20 or CD19/CD22; (iii) optimize CAR-T cell expansion and persistence by increasing the number of infusions or the dosing of infused cells; (iv) modify the therapy in patients who do not show an adequate clearing of lymphoma cells following CAR-T cell infusion; (v) define alternative treatments in DLBCL patients displaying genomic alterations predicting resistance to CAR-T cell therapy.

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