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REVIEW

Potentials, challenges and future of chimeric antigen receptor T-cell therapy in non-Hodgkin lymphomas

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ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma subtype. Disease progression or relapse following frontline chemoimmunotherapy, largely in the form of standard R-CHOP, occurs in 30–40% patients. Relapsed/refractory (R/R) DLBCL represents a major unmet medical need. In particular, patients with primary refractory disease or those whose lymphoma relapses after autologous stem cell transplantation have historically had poor outcomes.

Material and methods: Chimeric antigen receptor T-cell (CART) therapy is a promising novel treatment with curative potential in this setting. CART is based on *ex vivo* genetic modification of autologous T-cells to express chimeric receptors targeting antigens highly expressed in tumors such as CD19 in DLBCL. After lymphocyte-depleting therapy, patients are infused with CARTs that expand *in vivo* and target CD19-positive lymphoma cells.

Results: In initial phase I–II trials, investigators have demonstrated complete responses in 40–50% of patients with R/R DLBCL, resulting in durable remission approaching 3 years of follow-up in most of these patients without further treatment. The logistics of delivery are complex as cell products require timely long-distance transfer between hospitals and production facilities. The unique toxicity profile of CARTs, including the risk of fatal immunological and neurologic events, also requires specific hospital wide management approaches and education. The substantial direct and indirect costs of CART will limit access even in countries with well resourced health care systems.

Conclusions: While only two products are commercially available at present, further approvals in coming years appear likely. Future directions include CARTs with reactivity to tumor antigens other than CD19 and products targeting multiple tumor antigens to overcome resistance. The availability of CART has altered the current treatment algorithm for R/R DLBCL, and indications will likely expand to earlier lines of therapy and other hematologic malignancies.

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Background

Diffuse large B-cell lymphoma (DLBCL), the most common subtype of non-Hodgkin lymphoma in the Western World, is an aggressive disease typically presenting with rapidly growing nodal or extranodal masses. R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) has been the established treatment of DLBCL for two decades, as 60–70% of patients are cured with this regimen [1]. Large-scale attempts to improve upon R-CHOP have been unsuccessful, with several negative studies showing no clear advantage of variations of R-CHOP, novel antibodies, more intensive approaches (dose-adjusted EPOCH-R, consolidative autologous stem cell transplantation (ASCT)) as well as difficulties in translating advances in the molecular genetic understanding of DLBCL into more diverse therapies [2–6].

The dismal outcomes for the 30–40% of DLBCL patients with relapsed/refractory (R/R) DLBCL constitute a significant unmet medical need. Primary refractory patients and those with early relapse are unlikely to achieve durable remissions to subsequent chemoimmunotherapy-based therapies with 2-year overall survival (OS) $\leq$20% [7,8]. Chimeric antigen receptor T-cell (CART) therapies may achieve impressive, sustained responses in this population. This review provides an introduction to the basic mechanisms, efficacy, toxicity and cost-effectiveness of CART therapies in R/R DLBCL, as well as future directions.

Chimeric antigen receptor T-cells – construct and mode of action

CARTs are genetically modified autologous T-cells that utilize the specificity of an antibody-binding domain to harness the

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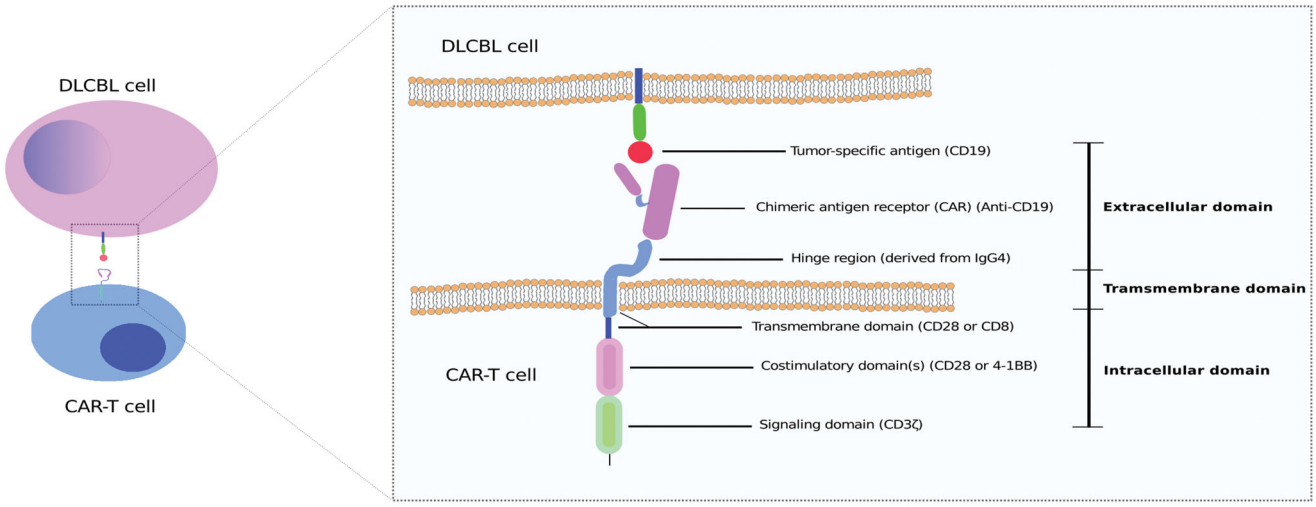

Figure 1. Overview of CART construct, showing the genetically modified T-cell expressing the chimeric antigen receptor directed against CD19 expressed on the surface of a tumor cell.

cytotoxic functions of T lymphocytes (Figure 1). Unlike normal T-cells, CARTs function independently of the T-cell receptor (TCR)/major histocompatibility complex (MHC) interaction and are therefore able to overcome some of the common strategies tumors utilize for immunologic escape such as loss of MHC expression [9].

Two decades ago, Eshhar and coworkers constructed a T-cell chimeric immune receptor with an antibody-like specificity able to transmit the signal for T-cell activation and trigger its effector function [10]. Such constructs have become the backbone of current CART products. A chimeric antigen receptor (CAR) consists of three domains including an extracellular, transmembrane and an intracellular domain (Figure 1). The extracellular component consists of an antigen recognizing (and binding) portion. This antigen-directed domain can be designed to target any particular antigen, but only CD19 targeting CARTs are commercially available for lymphoma at present. The transmembrane domain is an alpha helix spanning the cellular membrane and linking to the intracellular T-cell signaling domains which is composed of one or more signaling domains, including CD3ζ that contains three immunoreceptor tyrosine-based activation motifs (ITAMs). Phosphorylation of these ITAM domains allows binding of zeta associated proteins essential for the T-cell signaling cascade [11].

First generation CARTs, containing only CD3ζ as a signaling domain, failed to induce significant clinical responses and CART expansion [12]. Second generation CARTs, which are now commercially available, utilize a dual signal provided by the inclusion of a co-stimulatory molecule such as CD28 or 4-IBB (Figure 1). Among other things, this promotes IL-2 production, needed for expansion and persistence of infused CARTs [13]. Third generation CARTs utilize multiple co-stimulatory domains to further increase the cytokine production, and fourth generation CARTs more effectively eliminate antigen-negative tumor cells through co-activation of the innate immune system [14–16].

CART production requires multiple steps (Figure 2). The first step involves leukapheresis to collect patients' peripheral blood lymphocytes. T-cell enriched products then undergo activation and genetic modification to insert the CAR transgene, typically via lentiviral transduction. The resulting CARTs are then expanded and cryopreserved before shipped back to the administering institution. Prior to thawing and reinfusion, lymphodepleting chemotherapy is administered to the patient to prevent host immune response against the product. The CART product is then infused, with ensuing rapid CART expansion.

Commercially available CART products for relapsed/refractory DLBCL

At the time of writing two CART products have received regulatory approval by the European Medicines Agency (EMA) and the American Food and Drug Administration (FDA). The first FDA approved CART therapy for R/R DLBCL was axicabtagene ciloleucel (axicel, approved 2017) followed by tisagenlecleucel (approved 2018). Both approvals were based on single-arm phase 2 trials showing significantly favorable outcomes of R/R DLBCL compared to historical outcomes (Table 1).

ZUMA-1 was a multicenter phase 1/2 trial of axicel in R/R DLBCL (cohort 1) or R/R primary mediastinal B-cell lymphoma (PMBCL) and transformed follicular lymphoma (tFL) (cohort 2). Patients were refractory to the latest treatment line or had disease relapse within 12 months of ASCT. Patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide prior to CART infusion [19]. Out of 119 enrolled patients, 108 received CART infusion with a median time from leukapheresis to infusion of CART of 17 days [19,20]. CART manufacturing failed in only one patient. The efficacy population consisted of infused patients in the phase 2 part of the study ($n = 101$) and the primary endpoint of overall response rate (ORR) was observed in 83% patients, which included 58% achieving a complete response (CR) [19]. Responses were durable with median duration of CRs not reached at a median follow-up of 27 months [20]. The 2-year OS for CART treated patients was 51% and the 2-

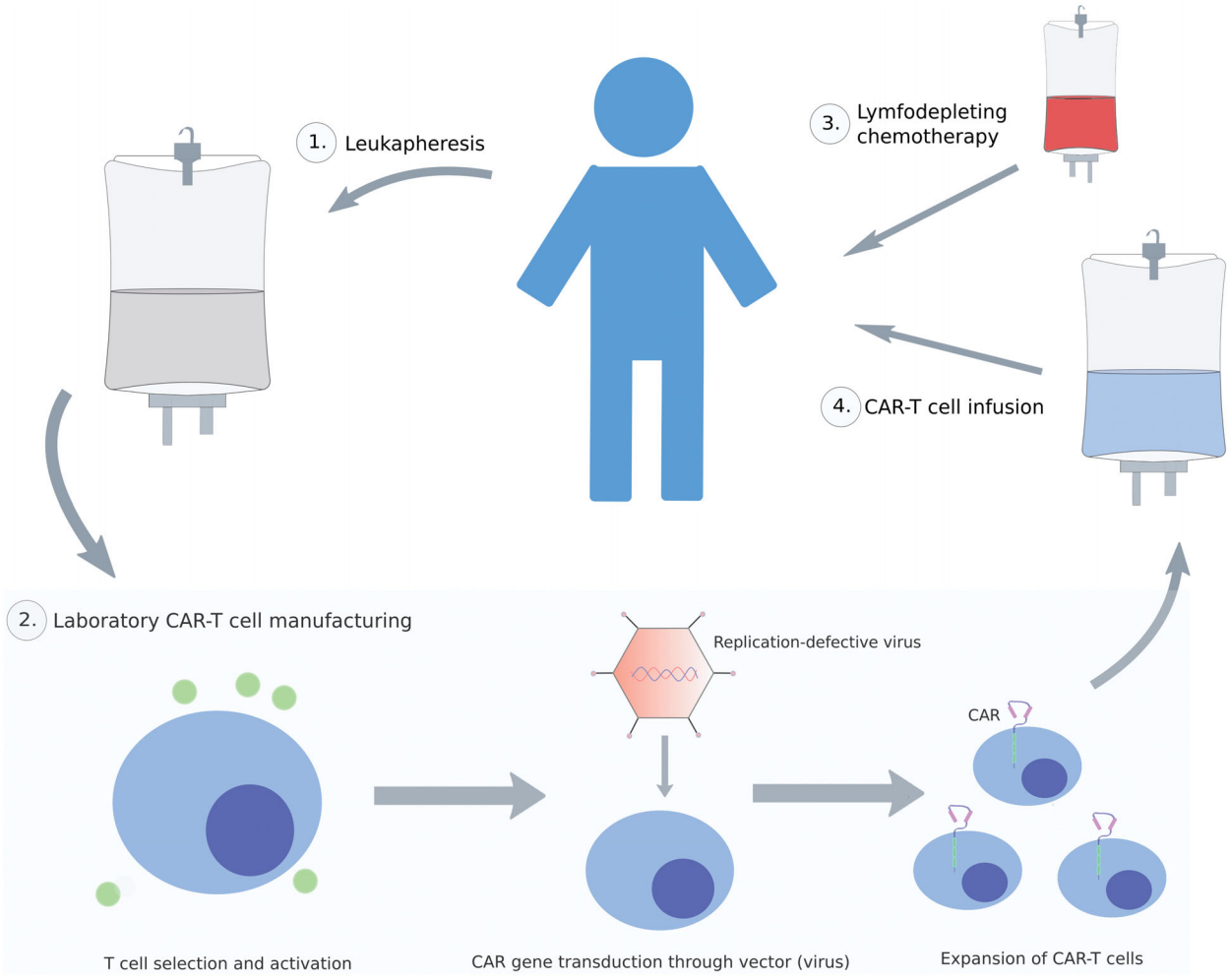

Figure 2. Overview of the CART manufacturing and treatment cycle. (1) Patient leukapheresis. (2) The T-cells are selected and activated *in vitro*, the CAR gene is transduced through a vector (here shown as a virus-vector for example) and the product of CART-cells is purified and prepared for the treatment. (3) A lymphodepleting course of chemotherapy is given prior to the CAR-T cell infusion to allow expansion of CART cells *in vivo* (4).

Table 1. Patient characteristics and efficacy data from the registrational trials of Tisacel/Axicel and ongoing trial of Lisocel.

	CAR-T registration trials		Trials	Real-world data for R/R DLBCL	
Product/study	Tisacel JULIET [17,18]	Axicel ZUMA-1 [19,20]	Lisocel TRANSCEND [21]	SCHOLAR-1 [8]	Ekstroem Smedby et al. [22]
Patient characteristics					
Patients enrolled, *n*	165	119	342	636	713
Patients infused, *n*	111	108	268	NA	NA
Median age, years (range)	56 (22–76)	58 (23–76)	63 (18–86)	55 (19–81)	71 (18–95)
Median follow up, months	19.3	27.1	10.8	ND	ND
DLBCL subtype	DLBCL, tFL	DLBCL, tFL, PMBCL	DLBCL, tFL, tIL, PMBCL, HGBCL, FL3B	DLBCL, tFL, PMBCL	DLBCL
Prior therapy, %					
≥3 prior lines of therapy	51	64	26%[a]	49%[b]	ND
Primary refractory disease	5	3	ND	28%	ND
Refractory to last therapy	55	77	67%	78%	ND
Prior or relapse after ASCT	49	21	34%	22%	ND
Efficacy					
Efficacy population, *n*	93	101	255	523	713
Response (ORR/CRR), %	52/40	83/58	73/53	25/7	ND
PFS (6/12/18 months), %	66/64/64	49/44/ND	ND	ND	ND
OS (6/12/18 months), %	ND/49/43	78/59/51	ND/ND ND	ND/ND/28/20[c]	26%, 2y
Median OS, months	11.1	NR	19.9	6.3	ND
Median PFS, months	NR	5.9	6.8	ND	ND
mDOR, months	NR	11.1	13.3	ND	ND

OS: overall survival; ORR: objective response rates; CRR: complete remission rates; PFS: progression-free survival; mDOR: median duration of response; ASCT: autologous stem-cell transplantation; DLBCL: diffuse large B-cell lymphoma; tFL: follicular lymphoma; tIL: transformed indolent lymphoma; PMBCL: primary mediastinal large B-cell lymphoma; HGBCL: high grade B-cell lymphoma; FL3B: follicular lymphoma grade 3b; NR: not reached; ND: no data.
Observational data from the SCHOLAR-1 and the Swedish Lymphoma Register on R/R DLBCL for comparison.
[a]26% had ≥4 prior lines of treatment, median of 3 (range, 1–8).
[b]49% had ≥2 prior lines of therapy.
[c]28% 12 months OS, 20% 24 months OS for 603 patients.

Table 2. Efficacy and safety data from studies of CART therapies in a real-world setting, JULIET and ZUMA-1 studies for comparison.

	CAR-T registration trials		Real-world CAR-T				
Product/study	Tisacel JULIET [17,18]	Axicel ZUMA-1 [19,20]	Axicel Pasquini et al. [24]	Tisacel Chong et al. [25]	Axicel Riedell et al. [26]	Tisacel Riedell et al. [26]	Tisacel/Axicel Kuhnl et al. [27]
Patient characteristics							
Patients treated, *n*	114	108	295	13	163	79	91[b]
Median age, years (range)	56 (22–76)	58 (23–76)	61 (19–81)	68 (42–75)	58 (18–85)	67 (36–88)	57 (18–75)
Prior therapy, %							
≥3 prior lines of therapy	51	64	ND	ND	ND	ND	43
Prior therapy, median (range)	ND	ND	ND	3 (2-5)	3 (2-11)	4 (2-9)	ND
Refractory to last therapy	55	77	66	ND	ND	ND	72
Prior or relapse after ASCT	49	21	34	ND	29	23	16/4[c]
Efficacy							
Response (ORR/CRR), %	52/40	83/58	70/52	64/55	72/43	59/44	36/20
Safety							
CRS all grades/≥3, %	58/15	88/11	83/11	23/0	ND/13	ND/1	ND/11
Neurotoxicity all grades/≥3, %	21/12	64/32	ND/61	0/0	ND/41	ND/3	ND/13
Tocilizumab/steroid use, %	15/11	43/26	70/56	ND	62/57	13/7	65/29
AEs Grade ≥3/5, %	89/ND	98/8[a]	ND/>1	ND	ND/3	ND/ND	ND[d]

ORR: objective response rates; CRR: complete remission rates; autologous stem-cell transplantation; AEs: adverse events; ND: no data.
[a]Nine patients (8%) had AE grade 5 including 5 who died of progressive disease.
[b]62 and 29 were infused with Axicel and Tisacel, respectively.
[c]16% had prior auto-SCT and 4% prior allo-SCT.
[d]Treatment-related mortality at 2%.

year progression-free survival (PFS) for patients responding 3 months after infusion was >70% [20].

JULIET was the pivotal trial of tisagenlecleucel in R/R DLBCL. This study included patients with R/R DLBCL after at least two lines of therapy and who were ineligible for, or had disease progression after ASCT [17]. Lymphodepleting chemotherapy was more flexible with options such as fludarabine and cyclophosphamide, bendamustine, or none if the absolute lymphocyte count was ≤1000/mm^3. The median time from enrollment until CART infusion was 54 days, but in contrast to ZUMA-1, bridging therapy was allowed and used in 92% of patients. In total, 238 patients were screened, 165 enrolled and 111 ultimately received CART infusion. Based on 93 patients in the main cohort with follow-up >3 months, the primary endpoint, ORR by PET/CT, was achieved in 52% of the patients (CR 40%) [17]. The median duration of response was not reached and 79% of patients with CR were projected to remain relapse-free at 12 months. The 12-month OS in the efficacy population was 49% [17].

The SCHOLAR-1 study of 636 historical R/R DLBCL patients pooled from observational studies and clinical trials provided the benchmarking for the ZUMA-1 trial [8]. In a cohort of R/R DLBCL defined by progressive or stable disease as best response at any point during chemotherapy or relapse ≤12 months from ASCT (resembling the ZUMA-1 population), the ORR was 26% with CR 7% and the median OS was only 6.3 months [8]. In this light, CART therapies represent a major improvement in terms of response rates as well as survival outcomes. However, the actual improvement associated with CART therapy cannot necessarily be quantified from comparisons to historical data. Although populations may appear similar based on R/R DLBCL definitions, patient-related factors such as performance status and comorbidities impact their ability to tolerate salvage therapy, and disease characteristics such as the degree of resistance to chemoimmunotherapy is a continuum rather than a binary factor. For example, enrollment in the CART trials required the expectation that the R/R DLBCL could be controlled during the screening and CART manufacturing periods. Furthermore, primary efficacy analyses were limited to patients dosed with CART, introducing immortal bias compared to unadjusted historical data.

Head-to-head comparisons of the two commercially available CART therapies do not exist, and differences in R/R DLBCL definitions and the use of bridging therapy preclude informative cross-trial comparisons. However, such data are relevant as the products have unique features in regards to manufacturing process (viral transfection technology) and CART co-stimulatory domains (CD28 for axicel and 4-1BB for tisagenlecleucel) [17,19]. For example, the use of 4-1BB as co-stimulatory domain is associated with longer persistence, whereas CD28 leads to more rapid expansion [23].

Real-world data on CART therapy

Early real-world studies of CART therapy have reported mixed results, with some observing similar efficacy as in the above clinical trials and others not (Table 2). A large series of 300 R/R DLBCL patients from the US Lymphoma CART Consortium, which includes 17 US academic centers, reported the need for bridging therapy in 53% patients treated with axicel (this was not allowed in ZUMA-1), which was associated with a higher risk of lymphoma-related deaths. Only 23/300 patients did not receive CART infusion after leukapheresis, mostly due to progressive lymphoma while waiting for product turnaround [28].

In a report from the US Center for International Blood and Marrow Transplant Research (CIBMTR) registry, 295 patients treated with axicel and with at least 3 months of follow-up, the 70% ORR and 52% CR rates were also comparable to clinical trial observations. Even though 101/295 patients were over the age of 65 years and up to the age of 81 years, only two patients died from acute toxicity, suggesting CART therapy is safe and effective in patients typically not considered eligible for intensive salvage therapies [24]. This is consistent with a single institution experience in which 24 patients

≥65 years of age with R/R DLBCL, many with significant comorbidities, had similar outcomes to younger patients [29].

However, the experience with CART therapy in the National Health Service (NHS), United Kingdom, has been more disappointing. Patients were assessed for eligibility to CART therapy by a national CART clinical panel, with eligibility criteria broadly following those of the ZUMA-1 and JULIET trials. Out of 125 patients approved for CART, 91 were infused (62 axicel, 29 tisagenlecleucel) after a median time of 63 days. A total of 80 patients were evaluable for response, CR rate was 20% for axicel and 17% for tisagenlecleucel. Median EFS was only 3 months [27].

Interestingly, CART therapies have also shown efficacy in patients with R/R DLBCL secondarily involving the CNS in real-world studies. With intensive salvage therapies, this group of patients have a 2-year OS of only 20% [30], but four of eight patients with CNS involvement had clinically relevant responses to tisagenlecleucel [31].

CART toxicities

Most acute CART toxicities are caused by immune reactions shortly after infusion. The cytokine release syndrome (CRS) and the immune effector cell-associated neurotoxicity syndrome (ICANS) constitute the two best characterized acute immunological toxicities. CRS typically manifests as mild flu-like symptoms with fever, myalgia, rigors, fatigue and loss of appetite, but multiorgan dysfunction with circulatory collapse and fulminant hemophagocytic lymphohistiocytosis (HLH) can occur in the most severe cases. Close observation and early treatment with tocilizumab, an anti-IL-6-receptor antibody, with or without steroids can effectively manage life-threatening CRS complications without comprising treatment efficacy [32–34]. In the most severe CRS cases, dual therapy with corticosteroids and tocilizumab is effective, although a possible lymphotoxic effect of corticosteroids (on the CART cells) has been a concern which requires further investigation [33].

ICANS is the second most common acute toxicity and features symptoms such as aphasia and confusion. In the more severe cases, coma, seizures, motor weakness and cerebral edema can potentially lead to death [33,35]. ICANS is reversible with supportive care alone in mild cases and corticosteroids in severe cases. The pathophysiology behind ICANS is not fully understood, but breakdown of the blood–brain endothelial barrier with entry of inflammatory cytokines and CART cells into the CNS is a plausible cause [36].

While only two products are commercially available at present, an increase in the number of available CART therapies is expected in the foreseeable future. Comparisons of safety profiles of different products will be essential to establish key differences and therefore harmonization of CRS and ICAN reporting is critical. Recently, the American Society for Transplantation and Cellular Therapy (ASTCT) has proposed a Consensus Grading System for CRS and ICANS [35]. This grading system is anticipated to become standard for reporting in both clinical trials and routine care (Table 3).

Myelosuppression in the form of grade 3 and 4 cytopenias has also been reported with both axicel and tisagenlecleucel in approximately 30% patients [17,19]. These cytopenias typically resolve but may require supportive care with growth factors and/or transfusions for variable periods of time [37]. On-target, off-tumor effects of CART therapy result in eradication of normal CD19 positive B-lymphocytes and may lead to hypogammaglobulinemia with a need for parenteral immunoglobulin replacement therapy [20]. However, B-cell recovery occurs in many patients over time and without loss of disease control [20].

Knowledge about late toxicities to CART therapy is limited at this stage, but ongoing pharmacovigilance is necessary as CART cells may persist for 10 years or longer after infusion [38]. This requires longer observation for toxicities than typically mandated for conventional therapies. Late potential risks could include effects on the immune system (e.g., autoimmune events), pregnancy outcomes and secondary malignancies from vector-mediated insertional mutagenesis [39–41]. The network of population-based Nordic healthcare databases may provide additional safety and efficacy data on these products [42]. In particular, the opportunity for very long follow-up periods with little loss to follow-up will be informative.

Costs, cost-effectiveness and access outside clinical trials

CARTs are among the most expensive cancer interventions today. The manufacturing cost of the CART product itself is high, currently $3,73,000 USD for both axicel and tisagenlecleucel for R/R DLBCL although the cost of the latter is $475,000 USD for R/R B-cell ALL. The cost of both products in the United Kingdom remains confidential, and the cost in other developed countries remains variable, yet comparable. For example, the cost of tisagenlecleucel in Japan is $306,000 USD [43,44]. Other direct costs, which often add up to a significantly greater sum than the CART product itself, include necessary procedures around the CART administration such as leukapheresis, bridging and lymphodepleting chemotherapy, admission for CART infusion, and supportive care including IL-6 inhibitors for adverse events [45–47]. This means the total cost of a single CART treatment may well exceed $1,000,000 USD.

Cost-effectiveness analyses comparing the two currently approved CARTs against other treatments for R/R DLBCL, in particular salvage chemotherapy and ASCT, have generated variable estimates often exceeding $150,000 USD/quality-adjusted life year (QALY). The current literature suggests cost-effectiveness could be as low as $100,000 USD/QALY or as high as >$1,000,000 USD/QALY [45–47], depending on the assumptions of direct and indirect costs, as well as the estimated long-term outcomes. Therefore, CART will only become evidently cost-effective when all direct costs related to the CART product as well as the indirect costs are reduced through cheaper, safer and more active CART constructs.

The two commercially available CART products were introduced into the market under the existing system of approval and reimbursement used for non-cellular therapies. Therefore, public and private payers are likely to restrict

Table 3. American Society for Transplantation and Cellular Therapy (ASTCT) grading for cytokine release syndrome (CRS) and IEC-associated neurotoxicity syndrome (ICANS) [35].

Severity	Grade 1	Grade 2	Grade 3	Grade 4
CRS parameter				
Fever[a]	≥38 °C	≥38 °C +	≥38 °C +	≥38 °C +
Hypotension	None	Not requiring vasopressors and/or	Requiring a vasopressor with or without vasopressin and/or	Requiring multiple vasopressors (excluding vasopressin) and/or
Hypoxia[b]	None	Requiring low-flow O_2-therapy	Requiring high-flow O_2-therapy	Requiring positive pressure O_2-therapy
ICANS parameter[c]				
ICE score[d]	7–9	3–6	0–2	0 (unable to perform ICE)
Depressed level of consciousness[e]	None	None	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	None	None	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min) or repetitive clinical or electrical seizures without return to baseline in between
Motor findings	None	None	None	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/cerebral edema	None	None	Focal/local edema on neuroimaging	Diffuse cerebral edema on neuroimaging[f]

ICP: intracranial pressure.
[a]Fever not attributable to any other cause. If antipyretic or anti-CRS treatment is started (tocilizumab or steroids), CRS grading is subsequent driven by hypotension and/or hypoxia.
[b]Low flow O_2-therapy is defined as oxygen delivery ≤6 L/min by nasal cannula or blow-by. High-flow O_2-therapy is defined as oxygen delivery ≥6 L/min by nasal cannula, facemask, nonrebreather mask or venturi mask. Positive pressure O_2-therapy includes CPAP, BiPAP, intubation and mechanical ventilation.
[c]ICANS grade is determined by the most severe event not attributable to any other cause.
[d]Immune effector cell-associated encephalopathy (ICE) score [35].
[e]Depressed level of consciousness should be attributable to no other cause (e.g., sedatives).
[f]Decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad. Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading.

coverage of this extremely expensive therapy outside of clinical trials to patients who meet the strict regulatory indications provided in the approval labels. For example, Lin et al. estimate that providing CART to all patients with R/R DLBCL in the United States who meet FDA-approved indications would increase health care costs by ∼$10 billion over a 5-year time frame [45]. Consequently, off-label use of CART will likely remain uncommon in the foreseeable future. If the indications were to expand further, a growing proportion of payers will not be able to keep up with the high costs of CART.

CART failures and future improvements to CART therapy

Despite the impressive response rates seen with CD19 CART therapy in NHL, approximately 50–60% of patients will either not respond or experienced disease relapse. Relapse after CART therapy can be broadly divided into CD19 negative and CD19 positive disease relapse.

CD19 negative relapse occurs in approximately one-third of cases after CART therapy and can occur in the presence of ongoing CART persistence. Loss of target (CD19) is a well-characterized mechanism for resistance and develops through selection of tumor cells with CD19 exon splice variants or gene mutations that leads to loss/altered CD19 expression [48]. In order to overcome loss of CD19, other antigens may be targeted alone or in combination with CD19, such as CD20, CD22 or BAFF-R. As a single target, two small single center phase I trials of CD20 CARTs have demonstrated ORR 80–83% with CR 17–50% [49,50]. *In vitro* and murine *in vivo* studies with one of these constructs demonstrates that CD20 CARTs retain efficacy *in vivo* at clinically significant levels of rituximab, suggesting that recent rituximab exposure should not significantly interfere with CD20 CART activity [51]. CARTs targeting another B-cell marker, CD22, have demonstrated efficacy in B-cell acute lymphoblastic leukemia (ALL) and this target is now to be explored in NHL (ClinicalTrials.gov Identifiers: NCT04088890; NCT02315612) [52]. Single antigen targeting, however, potentially allows for tumor escape by clonal selection pressure; therefore, a logical step from these studies is to combine targets to make dual-antigen targeting CARTs. Given the success with CD19, the majority of these dual targeting strategies incorporate CD19 plus either CD20 or CD22. And example of this is AUTO3, a CART product designed to target CD19 and CD22 and which has shown promising response rates in combination with pembrolizumab (and PD1-receptor blocking antibody that increase T-cell activity) in early trials [53]. These dual antigen studies are ongoing, but despite the theoretical advantages of this approach it is unclear whether or not upfront combinatorial targeting is truly superior to single antigen approaches.

CD19 positive DLBCL relapse after CART therapy can generally be attributed to tumor/host factors or inadequacy of the infused CART product [54]. Tumor factors leading to CART treatment failures can be large tumor volumes or expression of T-cell inhibitory ligands such as PD-L1 in tumor microenvironment. The host cytokine profile may also be an important determinant of CART outcomes. For example, intensive lymphocyte depletion leading to high levels of MCP-1 seem to increase *in vivo* CART expansion and

correlate with better response [55]. Potency of CARTs have also been shown to be determined by the clonality of infused T-cells, with a high degree of polyclonality being associated with stronger better efficacy [56]. The T-cells used for CART construction have often been exposed to several lines of chemotherapy, potentially leading to exhaustion and impairment of their effector function. High expression of PD-1 on CARTs makes them sensitive to inhibitory signaling in the tumor microenvironment. To prevent CD19 positive relapse, variations could be made to the intracellular signaling molecules on the CART construct to either improve persistence or prevent exhaustion of the CARTs.

Clinical perspectives on future CART use in other indications

Given the promising response rates in high risk R/R DLBCL, there is strong interest in moving CART therapy forward in the treatment paradigm of DLBCL. One clinically relevant question is whether CART therapy could replace high dose chemotherapy and ASCT in R/R DLBCL. ZUMA-7 (NCT03391466) and BELINDA (NCT03570892) are phase 3 studies in which patients with aggressive B-cell lymphoma and either refractory disease or relapse following primary therapy are randomized to receive anti-CD19 CART therapy or high dose cytotoxic therapy and ASCT [57,58]. As salvage chemotherapy and ASCT can cure up to 50% of patients, the rates of cure with CAR-T therapy would need to be significantly more to justify the current costs of this therapy. A risk adapted approach for those at high risk of treatment failure with chemotherapy is another strategy to effectively utilize these therapies and the subgroup analysis of the ongoing phase 3 studies will be essential for developing these protocols.

Although most trials have enrolled patients with aggressive B-cell lymphomas, activity has also been observed in patients with low grade lymphomas. Tisagenlecleucel was tested in 14 patients with follicular lymphoma of which >50% were refractory to both an alkylating agent and rituximab. Among these patients, the PFS was 70% at a median follow up of 28.6 months and median OS was not reached [59]. Hirayama et al. also showed promising preliminary activity of CD19 direct CART therapy in 8 patients FL with CR 88%; all remain in remission at a median follow up of 24 months [60]. The ongoing ELARA study (NCT03568461), a phase 2, single-arm, multicenter, open label trial in which patients with follicular lymphoma and ≥2 prior lines of therapy receive liso-cel, with a primary endpoint of CR rate will define the role of CART in FL. Anti-CD19 CARTs also have considerable activity in mantle cell lymphoma (MCL). Wang et al. recently presented the results of ZUMA-2, a phase II study in which 74 patients with R/R MCL (median of 4 prior lines treatment, all patients had prior BTK inhibitor) received KTE-X19. The investigator-assessed ORR according to intention to treat was 86% (CR 57%) with 12-month PFS rate 61%. Though non-randomized, these data are encouraging for this high risk population and may result in FDA approval for this indication [61].

Conclusions

CART therapy is the first treatment modality to provide substantially improved outcomes for R/R DLBCL patients with the highest unmet need, and two products have already received marketing authorization based on phase II trials. Initial reports of CART treatments performed in the real-world setting confirm the high response rates reported in clinical trials in some series, but others report significantly poorer outcomes. Nonetheless, more than half of patients receiving CD19 directed CART treatment will likely experience relapse/progression. CART-associated immunological toxicities have required modest adjustments to the current healthcare system infrastructure to ensure their optimal management. The next generation of CART therapies will involve highly active, yet safer products, ideally with faster turnaround times and at more reasonable costs. Expectations of expansion of indications to earlier lines of therapy as well as other lymphomas will add to the complexity of the sustainability question. Allogeneic CARTs and productions at local hospitals may increase access while lowering production costs.

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