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CAR-T Cell Therapy in Diffuse Large B Cell Lymphoma: Hype and Hope

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Abstract

Patients with non-Hodgkin lymphomas (NHLs) resistant to standard therapies have a dismal prognosis. The outcome is even poorer in patients relapsing after autologous stem cell transplantation. Most of these patients do not qualify for an allogeneic hematopoietic cell transplantation (HCT) due to refractory disease, lack of a suitable allogeneic donor, higher age, or cumulative toxicity of previous chemotherapy. Despite patients undergoing allogeneic HCT normally profit from a graft-versus-lymphoma effect, overall survival in patients with NHL after HCT remains short. Therefore, novel treatment modalities are urgently needed. Chimeric antigen receptor (CAR)-T cells, a new class of cellular immunotherapy involving ex vivo genetic modification of T cells to incorporate an engineered CAR have been used in clinical trials. In the majority of studies, B cell malignancies treated with CD19 targeting CAR-T cells have been analyzed.

Recently, results from 2 CD19 directed CAR-T cell trials with an increased follow-up of patients led to Food and Drug Administration and European Medicines Agency approval of tisagenlecleucel and axicabtagene ciloleucel. Common adverse events (AEs) include cytokine release syndrome and neurological toxicity, which may require admission to an intensive care unit, B cell aplasia and hemophagocytic lymphohistiocytosis. These AEs are manageable when treated by an appropriately trained team following established algorithm. In this review, we summarize the results of 3 large phase II CD19 CAR-T cell trials and focus on AEs. We also provide a perspective of ongoing activity in this field with the intend to improve the potency of this emerging novel therapy.

Introduction

Patients with diffuse large B cell lymphoma (DLBCL) usually achieve an overall response rate (ORR) of 60% after anthracycline and rituximab containing first-line chemotherapy and a long-term event-free survival of 50% is observed.[1,2] However, 30% to 40% patients eventually relapse and 10% are primary refractory. Despite intensive salvage chemotherapy including monoclonal antibodies (eg, rituximab or ofatumumab) and autologous stem cell transplantation (ASCT), outcome in these patients is poor, resulting in an ORR of 27% to 63% with long-term survival in up to 48%.[3–5] Only 50% of relapsed patients can proceed to ASCT, mostly due to insufficient response to salvage chemotherapy or stem cell collection failure.[4]

Allogeneic hematopoietic stem cell transplantation (HCT) transplantation is usually considered in the salvage setting, as an alternative to ASCT or after failure of autologous transplantation. However, due to refractory disease, lack of a suitable allogeneic donor, higher age, or cumulative toxicity of previous chemotherapy most of these patients do not qualify for allogeneic HCT. In an European society for blood and marrow transplantation registry of 101 DLBCL patients who underwent allo HCT after reduced (64%) or myeloablative conditioning (36%), the 3-year nonrelapse mortality overall survival (OS) was 28% and 54%, respectively.[6] An analysis of the Italian Group for Blood and Marrow Transplantation (GITMO) database reported 165 patients who relapsed after ASCT and further underwent allo HCT leading to an ORR of 49% including complete response (CR) in 72 of 165 patients (44%), and partial response (PR) in 9 of 165 (5%) patients.[7]

Recently, the SEAL study, including 7507 patients from 13 multicenter randomized controlled trials of active treatment in previously untreated DLBCL revealed that progression-free survival (PFS) at 24 months (PFS24) significantly correlated with OS treated with first-line anthracycline-based immuno-

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chemotherapy for DLBCL.[8] Moreover, patients remaining in remission at 2 years had excellent outcomes. In a subsequent study, OS stratified by PFS24 in a total of 5853 patients enrolled in the SEAL trial was analyzed. OS from PFS24 was defined as time from identified PFS24 status until death due to any cause. A total of 1423 assessable patients failed to achieve PFS24 and had a median OS of 7.2 months (95% confidence interval [CI] 6.8–8.1) after progression, resulting in a 5-year OS after progression of 19%. For those 3678 patients who achieved PFS24, the observed OS at 3, 5, and 7 years after achieving PFS24 was 93.1%, 87.6%, and 80.0%, respectively.[9] Use of this knowledge may expedite therapeutic development with the intent of bringing novel therapies to this patient population years before OS results are mature.[8,9]

Chimeric antigen receptor T cells

T cells can be genetically engineered ex vivo to express a chimeric antigen receptor (CAR) in addition to their natural T cell receptor (TCR). Unlike TCRs, CARs allow highly specific targeting of antigen in an major histocompatibility complex-independent fashion, counteracting cancer immune evasion mechanisms.[10]

A CAR is commonly composed of a specificity-conferring extracellular antibody single chain variable fragment (scFv), a hinge region transmembrane domain, one or more intracellular costimulatory domains (eg, CD28 or 4-1BB (CD137)), and a TCR signaling domain (CD3 ζ). CAR design has evolved over years to enhance efficacy and safety in particular immunologic settings.[10]

Recently, several generations of CARs can be distinguished. However, currently available cell products are second-generation CAR-T cells. The generations differ essentially by costimulating signaling domains (CD28, 4-1BB) being responsible for T cell activation and expansion.[11] The use of 4-1BB (CD137) as a costimulatory domain leads to an increased expansion of memory T cells and improved survival of CAR-T cells.[12]

Approved CAR-T cells in hematological disease

Encouraging data with CAR-T cells against the CD19 integral membrane glycoprotein, which is expressed on premature and mature B cells as well as on the majority of B cell malignancies, has recently led to Food and Drug Administration (FDA) approval of axicabtagene ciloleucel (KTE-C19) (Yescarta, Kite Pharma, Gilead) in October 2017 for the treatment of adult patients with refractory/relapsed (r/r) DLBCL and primary mediastinal B cell lymphoma (PMBCL) after 2 or more systemic lines of therapy. A second CAR-T cell product also targeting CD19, tisagenlecleucel (Kymriah, Novartis) for the treatment of pediatric and young adult patients up to 25 years of age with B cell acute lymphoblastic leukemia (ALL) that is refractory, in relapse post-transplant or in second or later relapse, and for adult patients with r/r DLBCL after 2 or more systemic lines of therapy has been approved by the FDA in May 2018. Both products achieved marketing authorization by the European Medicines Agency (EMA) in August 2018.

CAR-T cells in non-Hodgkin lymphoma

As conventional salvage chemotherapy often fails in relapsed patients, new strategies to overcome refractoriness and improve outcome are strongly warranted. With the introduction of CAR-T cells targeting CD19 positive lymphoid malignancies, encouraging response rates have been observed in heavily pretreated patients including patients with relapse after ASCT. To date, 3 international trials provide strong evidence of activity of different CAR-T cell products in adult patients with relapsed or refractory non-Hodgkin lymphoma (NHL).

Axicabtagene ciloleucel (KTE-C19) was the first CD19-directed CAR-T therapy (including a CD28 costimulatory domain) to demonstrate activity in DLBCL. Its efficacy was analyzed in the multicenter, phase II trial ZUMA-1 (NCT02348216) which was conducted in 77 patients with refractory DLBCL (Cohort 1) and 24 patients with PMBCL or transformed follicular NHL (Cohort 2) (Table 1).[13,14] After lymphodepleting chemotherapy with fludarabine 30 mg/m^2 per day and cyclophosphamide 500 mg/m^2 per day (FC 30/500) for 3 days, a total of 101 (91%) patients received a target dose of 2.0×10^6 CAR-T cells/kg body weight. The median time from leukapheresis to delivery of the final axicabtagene ciloleucel product was 17 days. Patients had a median age of 58 years (range, 51–64), had ≥3 lines of previous chemotherapy in 69% (69/101) including ASCT in 21% (21/101). Specific toxicity was cytokine release syndrome (CRS) in 93% (94/101), with grade ≥3 in 13% (13/101), neurological events in 64% (65/101), with grade ≥3 in 28% (28/101), and febrile neutropenia (FN) in 13%, all of grade ≥3. Three patients died from adverse events (AEs) during treatment. Patients achieved an ORR of 82% (83/101) with a CR rate of 54% (55/101) (Table 1). After a median follow-up of 15.4 months, 42% of patients continued to have a response, with 40% of patients still in sustained CR. For those patients who achieved a PR at 1 month, about a third (11/35) had converted to CR during follow-up. The median duration of response was 11.1 months for all responders and has not yet been reached by patients with CR. The OS rate at 18 months was 52% (53/101).

Similar data were obtained with tisagenlecleucel, the second CD19-directed CAR-T therapy (with a 4-1BB costimulatory domain) to be FDA approved. Tisagenlecleucel was applied to r/r DLBCL patients in a single-arm, open-labeled, multicenter, global phase II trial (JULIET; NCT02445248). Updated results of 111 patients with DLCBL after a median follow-up of 14 month were recently presented (Table 1).[15,16] After lymphodepleting chemotherapy with fludarabine 25 mg/m^2 per day and cyclophosphamide 250 mg/m^2 per day (FC 25/250) for 3 days, or bendamustine 90 mg/m^2 per day for 2 days in 93% (103/111) of patients, a median single dose of 3.0×10^8 (range, $0.1–6.0 \times 10^8$) CAR-T cells were infused. The median time from enrollment to infusion was 54 days (90% of patients received infusions between 30 and 92 days after enrollment). Of 111 patients, 102 (92%) patients bridging therapy was given between leukocyte collection and CAR-T cell infusion. Patients had a median age of 56 years (range, 22–76), received a median number of 3 (range, 1–8) prior lines of therapy including ASCT in 49% (54/111), and 76% (84/111) had stage III/IV disease. Cell of origin (COO) revealed germinal center B cell in 57% (63/111), activated B cell type in 41% (45/111), and 27% (19/70) had double/triple hit lymphoma. Specific toxicity of CAR-T cell therapy was CRS in 58% (64/111), with grade ≥3 in 22% (24/111), neurological toxicity in 21% (23/111), with grade ≥3 in 12% (13/111), and FN in 21% (23/111), with grade ≥3 in 14% (16/111). There was no therapy-related death observed. Best ORR was 52% (58/111; 95% CI, 41–62) with 40% (44/111) CR and 12% (13/111) PR. Those patients who had obtained CR at 3 months were also more likely to remain in remission at 6 months. Median duration of response was not reached. For patients achieving a CR, 12-month relapse-free survival rate was 79% with an OS of 95%. The OS

Table 1

Study Details

Name of Trial	JULIET	ZUMA-1	TRANSCEND001 "FULL"	TRANSCEND001 "CORE"
CAR-T product	CTL019 Tisagenlecleucel	KTE-C19 Axicabtagene ciloleucel	JCAR017 Lisocabtagene maraleucel	JCAR017 Lisocabtagene maraleucel
Patient characteristics				
Disease entity	DLBCL	DLBCL, tFL, PMBCL	DLBCL, tFL, PMBCL	DLBL, tFL
Enrolled/infused, n	165/111	111/101	134/102	n.r./73
Prior lines of CHT (n), median, range	3 (1–8)	3 (1–7)	3 (1–8)	3 (2–8)
Pre-ASCT, %	49	21	37	38
Bridging therapy	Allowed	Not allowed	Allowed	Allowed
Lymphodepleting CHT, mg/m^2 per day	Flu 25/Cy 250 day 1–3	Flu 30/Cy 500 day 1–3	Flu 30/Cy 300 day 1–3	Flu 30/Cy 300 day 1–3
Toxicity				
CRS, all/grade ≥3, %	58/22	93/13	38/1	37/1
Neurotoxicity, all/grade ≥3, %	21/12	64/28	23/13	25/15
Neutropenia ± fever, all/grade ≥3, %	21/14	35/31	n.r.	n.r.
Tocilizumab, %	15	43	17	n.r.
Response				
ORR, %	52	82	75	80
CR, %	40	54	55	59
3 months ORR, %	59	82	51	59
12 months PFS, %	65	73	n.r.	n.r.
12 months OS, %	49	51–65	n.r.	n.r.

ASCT = autologous stem cell transplantation, CAR-T = chimeric antigen receptor T cell, CHT = chemotherapy, CR = complete response, CRS = cytokine release syndrome, Cy = Cyclophosphamide, DLBCL = diffuse large B cell lymphoma, Flu = Fludarabine, n.r. = not reported, ORR = overall response rate, OS = overall survival, PFS = progression-free survival, PMBCL = primary mediastinal B cell lymphoma, tFL = transformed follicular lymphoma.

probability at 12-month for all infused patients was 49%. In 54% (13/24) of patients with PR, a conversion in CR during follow-up was observed, including 2 patients 9 to 12 months after initial response assessment. Interestingly there was no significant difference in ORR across different prognostic subgroups including prior ASCT, double/triple hit, or COO.[15,16]

A third CD19-directed CAR-T cell product, not yet approved, is currently investigated in the JCAR017-TRANSCEND trial study (CTN02631044) using lisocabtagene maraleucel (incorporating a 4-1BB costimulatory domain). It differentiates itself from the above-described products by being manufactured with a defined composition of CD4 and CD8 cells in a precise 1:1 ratio. For this study, patients with r/r DLBCL, high-grade B cell lymphoma (double/triple hit), PMBCL, follicular lymphoma 3B (FL3B), or mantle cell lymphoma were eligible and multiple dose levels (DLs) and administration schedules were evaluated. In the dose-finding cohorts, different DL of CAR-T cells were used: At DL1, 5×10^7 CAR-T cells (DL1), a single dose administered on day 1, or day 1 and day 14 were infused, whereas at DL2, a single dose of 1×10^8 CAR-T cells was given. DL2 was chosen for further pivotal DLBCL cohort.[17] Additionally, data were summarized in 2 datasets: the nonpivotal FULL dataset including all 102 patients in the DLBCL cohort (DLBCL NOS [not otherwise specified; de novo or transformed FL], PMBCL, FL3B) and the CORE dataset including 73 patients meeting inclusion criteria for the pivotal cohort with DLBCL NOS (de novo or transformed FL) and high-grade B cell lymphoma (double/triple hit). The latter cohort is described in more detail. Patients had a median of 3 prior therapies (range, 2–8) including ASCT in 38% (28/73). Lymphodepleting chemotherapy consisted of fludarabine 30 mg/m^2 per day and cyclophosphamide 300 mg/m^2 per day (FC 30/300) on 3 consecutive days.

Specific toxicity for both DLs was CRS in 37% (27/73), with grade ≥3 in 1% (1/73), neurological toxicity in 25% (18/73), with grade ≥3 in 15% (11/73), FN was not reported. Two patients died, one of septic shock unrelated to CAR-T cells, occurring in the setting of disease progression and one with diffuse alveolar damage while neutropenic. Best ORR in all patients (FULL), at both DLs, was 75% (95% CI, 65–83/102) with 55% of patients in CR (95% CI, 45–65/102) and for CORE, ORR was 80% (95% CI, 68–88/73) with 59% in CR (95% CI, 47–70/73), respectively (Table 1). A higher rate of durable response at DL2 was observed in the CORE population, with 6-month ORR and CR of 49% and 46% versus 42% and 33% at DL1.[17]

Assessment and management of AEs in CAR-T cell therapy

CAR-T cell therapy is associated with significant acute toxicities, which can be severe or even fatal.[18] Related to targeting malignant and normal B cells via CD19 depletion the following side effects can be observed: CRS ranging from low-grade symptoms to life-threatening multiorgan failure, rarely evolving into hemophagocytic lymphohistiocytosis (HLH)/macrophage-activation syndrome (MAS), CAR-T cell-related encephalopathy syndrome (CRES), cytopenia, prolonged B cell aplasia, infection, and hypo-gammaglobulinemia.

Cytokine release syndrome

One of the most important AEs of CAR-T cell therapy is the CRS, which is a nonantigen-specific inflammatory response often in the context of in vivo CAR-T cell expansion. The onset of CRS is commonly seen between 1 and 12 days after infusion. Clinical presentation may vary and typically may include unspecific symptoms like fever, hemodynamic instability, respiratory failure, and organ dysfunction. Besides expansion and activation of T cells, effector/pro-inflammatory cytokine release, for example, interleukin-6 (IL-6), INF-γ, TNF-α, IL-8, IL-12, IL-15, activation of macrophages, and endothelial factors (vWF,

ang-2) contribute to the pathomechanism.[19,20] Elevation of IL-6 levels has been found to correlate with timing of peak toxicity in CAR-T cell-treated patients and is thought to be a key mediator in this process.[21,22] Poor performance status (ECOG performance status 2), high tumor burden leading to rapid CAR-T cell expansion, or elevated pro-inflammatory parameters (eg, increased C-reactive protein or IL-6 levels) before CAR-T infusion are associated with higher risk of specific toxicity.[23,24] Other reasons mimicking a CRS, for example, infections, should be ruled out. However, distinction between CRS or infectious complication after lymphodepletion is ambiguous and therapy will comprise of broad-spectrum antibiotics and best supportive care. Some mild forms of CRS with temperature ≥38°C, treated only symptomatically with antipyretics, can be observed in almost all patients which CRS. In case of grade ≥2 CRS (including systolic blood pressure ≤ 90 mm Hg and/or FiO_2 ≥ 40%), interventions with IV fluids ± vasopressors and noninvasive oxygen supply through breathing mask, resuscitator or respirator might become necessary.

Scoring systems for CRS differ between studies and hinder to compare trials with respect to CRS and treatment of CRS. In the JULIET trial, the UPenn score was used, whereas the ZUMA-1 and TRANSCEND 001 utilized the scoring system published by Lee et al (Table 2).[25,26] The particular scoring systems result in different recommendations of CRS-management. According to the UPenn score, vasopressors are recommended for grade 3 CRS, in the Lee scoring system for grade 2, respectively. In patients with moderate to severe CRS not responding to supportive therapy, tocilizumab an IL-6 receptor antibody, is recommended. Tocilizumab inhibits direct binding of IL-6 or IL-6/soluble IL-6 receptor complex to cell membranes. The inhibition of the IL-6 signaling pathway hampers the inflammatory response and reduces or eliminates the symptoms of CRS. In contrast to corticosteroids no data exist, showing that tocilizumab declines T cell expansion.[14,27] Tocilizumab can be given at a dose of 8 mg/kg IV (max. 800 mg), every 8 hours with a maximum of 3 doses within 24 hours and a total of 4 doses. In case of CRS, grade ≥3 or patients not responding to tocilizumab within 24 hours, corticosteroids (methylprednisolone 1 g/kg or dexamethasone 10 mg twice daily) should be considered. In order not to interfere with in vivo T cell expansion the use of corticosteroids is generally not recommended as first-line therapy, albeit there is no clear evidence that corticosteroids will have a negative impact on PFS.[14] However, as long as an impact on T cell expansion cannot completely ruled out, corticosteroids should be considered as second-line therapy for severe CRS.

CAR-T cell-related encephalopathy syndrome

CRES is another major complication of CAR-T cell therapy, which can occur with or independently of CRS, mostly within 28 days after CAR-T cell infusion. The pathophysiology is less clearly defined and the pathomechanisms not yet fully understood. As CAR-T cells can cross the blood-brain barrier (BBB), endothelial cell activation might play a role in development of CRES and cerebral edema.[28] Clinical symptoms of CRES might vary and can range from diminished attention, confusion, word-finding difficulties, disorientation, aphasia, somnolence, seizures, or cerebral edema. Neurotoxicity occurs in 20% to 60% of patients, with grade ≥3 observed in 10% to 30%. Due to the fact that CRES is mostly reversible, only rare fatal cases have been observed.[18] CRES is associated with high CAR-T cell doses and high blood CAR-T cell peak. As tocilizumab is not expected to

Table 2

CRS Grading Scales[26,25,41]

Trial	UPenn Scale[41]	CTCAE v4.0[42]	Lee Scale[25]
Grade 1	Mild reaction: treated with supportive care such as antipyretics, antiemetics	Mild reaction: infusion interruption not indicated; intervention not indicated	Symptoms are not life-threatening and require symptomatic treatment only, eg, fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Moderate reaction: signs of organ dysfunction related to CRS and not attributable to any other condition. Hospitalization for management of CRS-related symptoms, including fevers associated with neutropenia, need for IV therapies (not including fluid resuscitation for hypotension)	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hours	Symptoms require and respond to moderate intervention. Oxygen requirement <40% or hypotension responsive to fluids or low-dose pressors or grade 2 organ toxicity
Grade 3	More severe reaction: hospitalization required for management of symptoms related to organ dysfunction, including grade 4 LFTs or grade 3 creatinine related to CRS and not attributable to any other conditions; this excludes management of fever or myalgias; includes hypotension treated with intravenous fluids (defined as multiple fluid boluses for blood pressure support) or low-dose vasopressors, coagulopathy requiring fresh frozen plasma or cryoprecipitate or fibrinogen concentrate, and hypoxia requiring supplemental oxygen (nasal cannula oxygen, high-flow oxygen, CPAP, or BiPAP). Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS	Prolonged reaction (eg, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (eg, renal impairment, pulmonary infiltrates)	Symptoms require and respond to aggressive intervention. Oxygen requirement ≥40% or hypotension requiring high-dose or multiple pressors or grade 3 organ toxicity or grade 4
Grade 4	Life-threatening complications such as hypotension requiring high-dose vasopressors, hypoxia requiring mechanical ventilation	Life-threatening consequences; pressor or ventilator support indicated	Life-threatening symptoms. Requirements for ventilator support or grade 4 oxygen toxicity (excluding transaminitis)

BiPAP = bilevel positive airway pressure, CPAP = continuous positive airway pressure therapy, CRS = cytokine release syndrome, CTCAE = Common Terminology Criteria for Adverse Events, IV = intravenous, LFT = liver function test, NSAID = nonsteroidal anti-inflammatory drug.

cross the BBB and could theoretically increase the amount of circulating IL-6 in the brain, dexamethasone 10 mg IV every 6 hours or methylprednisolone 1 mg/kg IV every 12 hours is considered as rescue therapy of CRES in patients without signs of CRS. If CRS and CRES are observed at the same time, administration of anti-IL-6 receptor antibody is preferred first-line therapy.

Consideration of neurology consults, brain imaging (computed tomography scan or magnetic resonance imaging) to rule out cerebral edema, lumbar puncture to rule out infection, or malignant infiltration should be performed at presentation of grade 2 symptoms and best supportive care as seizure prophylaxis or treatment with anticonvulsive drugs shall be initiated as soon as possible. A vigilant observation and close monitoring using a neurological assessment score is strongly recommended.[18] Assessment scores have to be available for adults and children. However, also scoring systems for CRES vary, for example, neurotoxicity is not incorporated in the UPenn score.[26]

B cell aplasia

As CAR-T therapies targeting CD19 cannot discriminate between malignant and normal B cells, profound B cell aplasia as on-target, off-tumor toxicity occurs. This often-prolonged AE exposes patients to infections even late after CAR-T infusion. Patients may require intermittent infusion of pooled immunoglobulin as auxiliary treatment of or prophylaxis from infectious complications.[21]

Hemophagocytic lymphohistiocytosis/ macrophage-activation syndrome

HLH/MAS is reported to occur very rare after CAR-T therapy.[13,18] However, patients with HLH/MAS or CRS may have similar clinical manifestations including high fever, multiorgan dysfunction, or central nervous system disturbances. Diagnosis of CAR-T cell related HLH/MAS is proposed by the following features: high ferritin levels of $>10{,}000$ ng/mL during the CRS phase, grade ≥ 3 organ toxicities involving liver, kidney, or lung, or hemophagocytosis in the bone marrow or other organs according.[18] Anti-IL-6 therapy and corticosteroids are recommended. In patients not responding within 48 hours, systemic etoposide or intrathecal cytarabine to treat neurotoxicity are recommended.

Comparing axicabtagene ciloleucel, lisocabtagene maraleucel, and tisagenlecleucel trials

Clinical data of the 3 large phase II trials ZUMA-1, JULIET, and TRANSCEND001 provide strong evidence of activity of CAR-T cell products in r/r DLBCL. Observed ORR range from 52% to 82% with a CR rate of 40% to 59%, a 12-month PFS and OS ranging from 54% to 73% and 49% to 74%, respectively. However, comparison of data is difficult as important issues, for example, inclusion criteria, bridging therapy, dosing of lymphodepleting chemotherapy, construction of CAR-T cell products, toxicity scoring, number of patients, and kind of pretreatment as percentage of ASCT (21–49%) before CAR-T cell infusion, vary between studies. For instance, bridging therapy was allowed in JULIET and TRANSCEND001 but not in the ZUMA-1 trial, most likely due to the different timespan between leukapheresis and product administration, which was only a median of 17 days in the ZUMA-1 trial. In addition, the ZUMA trial aimed to eliminate an additive effect of chemotherapy. Despite lymphodepleting chemotherapy was different across the studies (ZUMA-1: FC30/500, JULIET: FC 25/250, and TRANSCEND001: FC30/300), there is no clear evidence for increased toxicity with higher doses. Another factor might be attributed to cell dose, ranging from approximately 2×10^6 (ZUMA-1), 3.1×10^8, to DL1 5×10^7 or DL2 1×10^8 (TRANSCEND001).[16] With respect to AE management, different scoring systems (UPenn in JULIET, Lee score in ZUMA-1 and TRANSCEND001) were used prompting different management of CRS. Anti-IL-6 therapy tocilizumab was given in 12% to 43% of patients in the respective trials, according to different study recommendations (Table 1). Lisocabtagene maraleucel distinguishes itself from axicabtagene ciloleucel and tisagenlecleucel by demonstrating lower rates of severe toxicity by similar response rates.[21] All 3 products use the anti-CD19-antibody single chain fragment, whereas the costimulatory domain is CD28 in axicabtagene ciloleucel and 4-1BB in the other 2 products. Preclinical studies revealed an increased short-term expansion for CD28 containing CAR-T ("sprinter") but a longer persistence of 4-1BB containing CAR products ("marathon runner").[13,17]

FDA and EMA approved CAR-T cell products

To date, 2 CAR-T cell products are approved in United States and Europe for patients with r/r, B-ALL, DLBCL, and PMBCL. To minimize the risks associated with the treatment of CAR-T cells, the manufacturers have to ensure that hospitals and their associated centers that dispense these innovative advanced therapy medicinal products are specially qualified in accordance with an agreed control distribution program.

Besides the recommendations described in the EMA assessment report and package insert, development of intern strategies for patient selection, monitoring, detection, and adequate treatment of side effects is urgently required. Though clear guidelines are lacking, administration of CAR-T therapy in patients with late stage disease, high tumor burden, and higher ECOG status (eg, ECOG $\geq$ 2) should be avoided to prevent specific toxicity. Furthermore, an appropriate trained clinical staff and a well-established collaboration with other specialties as ICU, neurology, pharmacy, and apheresis unit is mandatory.[29]

Perspective

CAR-T cell therapies have shown promising results even in chemo-refractory lymphoma patients and provide a new option for patients who have no adequate treatment alternatives.[14–18,30] Roughly 50% to 70% of patients will be alive after 12 months of treatment. Patients showing CR at 3 months have an 80% to 90% probability of remaining in response, and up to 40% of patients with a PR can improve to a CR. However, not all patients respond to CAR-T cells. Tumor escape mechanisms as loss of epitope (eg, CD19 negative relapses) have been observed in ALL and lymphoma and more recently, specific mutations in patients with ALL relapsing after CAR-T cells were described.[31–33] In addition, it has been shown that upregulation of programmed death-1 (PD-1) within the tumor microenvironment inhibits CAR-T cell function. These findings suggest that PD-1/PD-ligand-1 (PD-L1) blockade may have a beneficial influence on the efficacy of CAR-T cells.[34]

For those patients not achieving a CR (eg, remaining in PR or progressive disease), the live expectancy is <1 year. Therefore,

effective strategies for improving the potency of CAR-T cells by combination with other agents targeting the immune system are currently under investigation. The phase Ib PORTIA study (NCT03630159) evaluates the efficacy of tisagenlecleucel in combination with pembrolizumab (anti-PD-1) in r/r DLBCL and the phase I to II ZUMA-6 study (NCT02926833) evaluates axicabtagene ciloleucel in combination with atezolizumab (anti-PD-L1) in refractory DLBCL.

Targeting other epitopes other CD19 might also improve outcome after CAR-T cell application. As an example, patients with ALL who relapse after CD19-targeted CAR-T cell associated by antigen loss or mutation, can successfully be treated with CAR-T cells targeting CD22.[35] Studies evaluating the safety and efficacy of T cells transduced with bivalent lentiviral vector (CD19/CD22. BB.z) expressing CD19/CD22 are currently conducted in patients with selected r/r B cell malignancies (NCT03289455, NCT03233854, NCT03448393).

Further development of second-generation CARs with 1 costimulatory domain resulted in third-generation CARs who harbor 2 costimulatory domains and fourth-generation CAR-T secreting immunomodulatory molecules (TRUCKS: T cells redirected for universal cytokine killing), mostly pro-inflammatory cytokines.[36,37] First clinical studies using TRUCKS are already ongoing (NCT02498912).

In addition, the role of CAR-T cell therapies as substitute for autologous—or even allogeneic—transplantation by enabling this treatment for patients in first relapse or with primary refractory disease should be explored. The results of the SEAL study supporting that PFS could serve as surrogate end point for OS and that this end point may expedite therapeutic development with the intent of bringing novel therapies to this patient population earlier.[8] A couple of randomized phase III studies have recently been activated, comparing CD19 CAR-T cells with standard of care second-line therapy (ASCT) in patients with r/r DLBCL (NCT03391466, NCT03570892, NCT03575351).

Limitation of autologous CAR-T therapies are failure to manufacture CAR-T products in certain patients (eg, insufficient T cell collection and/or transduction) and the timespan between leukapheresis and availability of the engineered product in which patients' clinical situation can deteriorate. An exceptional approach is the development of allogeneic CAR-T cells, which could be available as an "off-the-shelf" product in the future. Small cohorts of patients receiving donor derived CD19-CAR T cells for relapsed hematologic malignancies following allogeneic HCT have been published. In one study where patients did not receive prior lymphodepleting therapy, only 3 of 10 patients responded. None developed graft-versus-host-disease (GVHD).[38] More recently, preliminary results of a phase I study of allogeneic CD19-directed CAR-T cells (UCART19) in 6 pediatric patients with r/r B-ALL has been reported. In this trial patients received genetically modified CAR-T cell products manufactured from healthy donor cells, in which *TRAC* and *CD52* genes have been disrupted to allow administration in non-HLA matched patients. Besides CRS in all patients (1 hade grade 3), 1 patient experienced acute GVHD grade I of the skin. Five of six patients responded, but 2 of them relapsed 3 months after CAR T therapy.[39] Further studies are needed to evaluate the safety and efficacy of this approach. Allogeneic CAR-T products may help to reduce specific costs and enable quick access for patients in need, as disease progression can be observed in the waiting for autologous cell products. Specific gene editing may help to prevent a GVHD induced by allogeneic CAR-T products

We recently published practical considerations for the implementation of CAR-T cell therapies in Europe which remains an actual topic as 2 approved products have already entered the clinic.[40] However, additional trials testing other targets, more than 1 target, combinations with other drugs, and earlier application of CAR-T cells are currently performed. CAR-T cells have demonstrated significant clinical benefit in all studies published so far. Although AEs such as CRS, neurological toxicity, and B cell aplasia are common, the majority of events are manageable when treated by an appropriately trained multidisciplinary team.

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References

1. Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98. 5 Trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood.* 2010;116:2040–2045.
2. Pfreundschuh M, Kuhnt E, Trumper L, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. *Lancet Oncol.* 2011;12:1013–1022.
3. van Imhoff GW, McMillan A, Matasar MJ, et al. Ofatumumab versus rituximab salvage chemoimmunotherapy in relapsed or refractory diffuse large B-cell lymphoma: the ORCHARRD study. *J Clin Oncol.* 2017;35:544–551.
4. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol.* 2010;28:4184–4190.
5. Crump M, Neelapu SS, Farooq U, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood.* 2017;130:1800–1808.
6. van Kampen RJ, Canals C, Schouten HC, et al. Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European Group for Blood and Marrow Transplantation Registry. *J Clin Oncol.* 2011;29:1342–1348.
7. Rigacci L, Puccini B, Dodero A, et al. Allogeneic hematopoietic stem cell transplantation in patients with diffuse large B cell lymphoma relapsed after autologous stem cell transplantation: a GITMO study. *Ann Hematol.* 2012;91:931–939.
8. Shi Q, Schmitz N, Ou FS, et al. Progression-free survival as a surrogate end point for overall survival in first-line diffuse large B-cell lymphoma: an individual patient-level analysis of multiple randomized trials (SEAL). *J Clin Oncol.* 2018;36:2593–2602.
9. Maurer MJ, Habermann TM, Shi Q, et al. Progression-free survival at 24 months (PFS24) and subsequent outcome for patients with diffuse large B-cell lymphoma (DLBCL) enrolled on randomized clinical trials. *Ann Oncol.* 2018;29:1822–1827.
10. Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat Rev Cancer.* 2016; 16:566–581.
11. Wohlfarth P, Worel N, Hopfinger G. Chimeric antigen receptor T cell therapy—a hematological success story. *Memo.* 2018;11:116–121.
12. Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. *Nat Rev Clin Oncol.* 2018;15:31–46.
13. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med.* 2017;377:2531–2544.
14. Locke FL, Ghobadi A, Jacobson CA, et al. Durability of response in ZUMA-1, the pivotal phase 2 study of axicabtagene ciloleucel (Axi-Cel) in patients (Pts) with refractory large B-cell lymphoma. *J Clin Oncol.* 2017;36 (suppl):abstr3003.

15. Borchmann P, Tam CS, Jäger U, et al. An updated analysis of JULIET, a global pivotal phase 2 trial of tisagenlecleucel in adult patients with relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL) [Abstract S799]. 23rd EHA Congress, Stockholm; 2018.
16. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med.* 2019;380:45–56.
17. Abramson JS, Gordon LI, Palomba ML, et al. Updated safety and long term clinical outcomes in TRANSCEND NHL 001, pivotal trial of lisocabtagene maraleucel (JCAR017) in R/R aggressive NHL. *J Clin Oncol.* 2018;36:abstr7505.
18. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. *Nat Rev Clin Oncol.* 2018;15:47–62.
19. Obstfeld AE, Frey NV, Mansfield K, et al. Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. *Blood.* 2017;130:2569–2572.
20. Yang Y, Kohler ME, Chien CD, et al. TCR engagement negatively affects CD8 but not CD4 CAR T cell expansion and leukemic clearance. *Sci Transl Med.* 2017;9:417.
21. Havard R, Stephens DM. Anti-CD19 chimeric antigen receptor T cell therapies: harnessing the power of the immune system to fight diffuse large B cell lymphoma. *Curr Hematol Malig Rep.* 2018;13:534–542.
22. Maude SL, Barrett D, Teachey DT, et al. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J.* 2014;20:119–122.
23. Kochenderfer JN, Dudley ME, Feldman SA, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood.* 2012;119:2709–2720.
24. Siddiqi T, Li AJS, Brown D, et al. Patient characteristics and pre-infusion biomarkers of inflammation correlate with clinical outcomes after treatment with the defined composition, CD19-targeted CAR T cell product, JCAR017. *Blood.* 2017;130:193.
25. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood.* 2014;124:188–195.
26. Porter D, Frey N, Wood PA, et al. Grading of cytokine release syndrome associated with the CAR T cell therapy tisagenlecleucel. *J Hematol Oncol.* 2018;11:35.
27. June CH, O'Connor RS, Kawalekar OU, et al. CAR T cell immunotherapy for human cancer. *Science (New York, NY).* 2018;359:1361–1365.
28. Gust J, Hanafi HKA, Li LA, et al. Endothelial activation and blood–brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discov.* 2017;7:1–16.
29. Yakoub-Agha I. Clinical units to set up chimeric antigen receptor T-cell therapy (CAR T-cells): based on the recommendations of the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC). *Curr Res Transl Med.* 2018;66:57–58.
30. Chow VA, Shadman M, Gopal AK. Translating anti-CD19 CAR T-cell therapy into clinical practice for relapsed/refractory diffuse large B-cell lymphoma. *Blood.* 2018;132:777–781.
31. Brudno JN, Shi V, Stroncek D, et al. T cells expressing a novel fully-human anti-CD19 chimeric antigen receptor induce remissions of advanced lymphoma in a first-in-humans clinical trial. *Blood.* 2016;128:999.
32. Gardner R, Wu D, Cherian S, et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. *Blood.* 2016;127:2406–2410.
33. Orlando EJ, Han X, Tribouley C, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat Med.* 2018;24:1504–1506.
34. Cherkassky L, Morello A, Villena-Vargas J, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest.* 2016;126:3130–3144.
35. Fry TJ, Shah NN, Orentas RJ, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat Med.* 2018;24:20–28.
36. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther.* 2015;15:1145–1154.
37. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol Rev.* 2014;257:83–90.
38. Kochenderfer JN, Dudley ME, Carpenter RO, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood.* 2013;122:4129–4139.
39. Qasim W, Adams CO, Inglott S, et al. Phase I study of UCART19, an allogeneic anti-CD19 CAR T-cell product, in high risk pediatric patients with CD19+ relapsed/refractory (R/R) B-cell ALL: preliminary results of PALL study. *EHA Learning Center.* 2018.
40. Buechner J, Kersten MJ, Fuchs M, et al. Chimeric antigen receptor-T cell therapy: practical considerations for implementation in Europe. *HemaSphere.* 2018;2:e18.
41. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med.* 2015;7:303ra139.
42. US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE).V4.03; 2010. Available from: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed September 21, 2017.