



Immunotherapy

CD19 chimeric antigen receptor-T cells in B-cell leukemia and lymphoma: current status and perspectives

Mohamad Mohty¹ · Jordan Gautier² · Florent Malard¹ · Mahmoud Aljurf³ · Ali Bazarbachi⁴ · Christian Chabannon⁵ · Mohamed A. Kharfan-Dabaja⁶ · Bipin N. Savani⁷ · He Huang⁸ · Saad Kenderian⁹ · Arnon Nagler¹⁰ · Miguel-Angel Perales^{11,12}

Received: 10 June 2019 / Revised: 8 August 2019 / Accepted: 14 August 2019 / Published online: 5 November 2019
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Abstract

The approval of tisagenlecleucel and axicabtagene ciloleucel represents a breakthrough in the field of immune and cellular therapy for hematologic malignancies. These anti-CD19 chimeric antigen receptor-T cells (CAR) proved to be highly effective in the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) and specific histologic subtypes of B-cell non-Hodgkin lymphomas. This expert review aims to summarize the current available research evidence in this field, with a special focus on the different challenges faced by treating physicians, and we also provide future perspectives.

Introduction

The development of chimeric antigen receptor (CAR) T-cell therapy for hematologic malignancies was a breakthrough technological advancement which generated a lot of enthusiasm and hope for many patients, with as yet incurable disease (Fig. 1). This review aims to provide an updated overview of CAR T-cells clinical results and a

comprehensive discussion on their use in therapeutic strategies for the two groups of malignancies that are included in FDA and EMA marketing approvals, acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), as well as chronic lymphocytic leukemia (CLL) for which there is no approval yet. Issues of CAR T-cell toxicity will also be covered. Regulatory requirements, manufacturing processes, and biology of CAR T-cells,

✉ Mohamad Mohty
mohamad.mohty@inserm.fr

¹ Service d'Hématologie Clinique et Thérapie Cellulaire, Hôpital Saint-Antoine, Sorbonne Université, INSERM UMRs 938, Paris, France

² Clinical Research Division, Integrated Immunotherapy Research Center, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

³ Oncology Center, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

⁴ Department of Internal Medicine/ American University of Beirut, Beirut, Lebanon

⁵ Institut Paoli-Calmettes, Inserm CBT-1409 & Aix-Marseille Université, Marseille, France

⁶ Blood and Marrow Transplantation Program, Division of Hematology-Oncology, Mayo Clinic, Jacksonville, FL, USA

⁷ Hematology and Stem Cell Transplantation Section, Division of Hematology/Oncology, Department of Medicine, Vanderbilt

University Medical Center and Veterans Affairs Medical Center, Nashville, TN, USA

⁸ Bone Marrow Transplantation Center, the First Affiliated Hospital, School of Medicine, Zhejiang University, Institute of Hematology, Zhejiang Province Engineering Laboratory for Stem Cell and Immunity Therapy, Hangzhou 310058, China

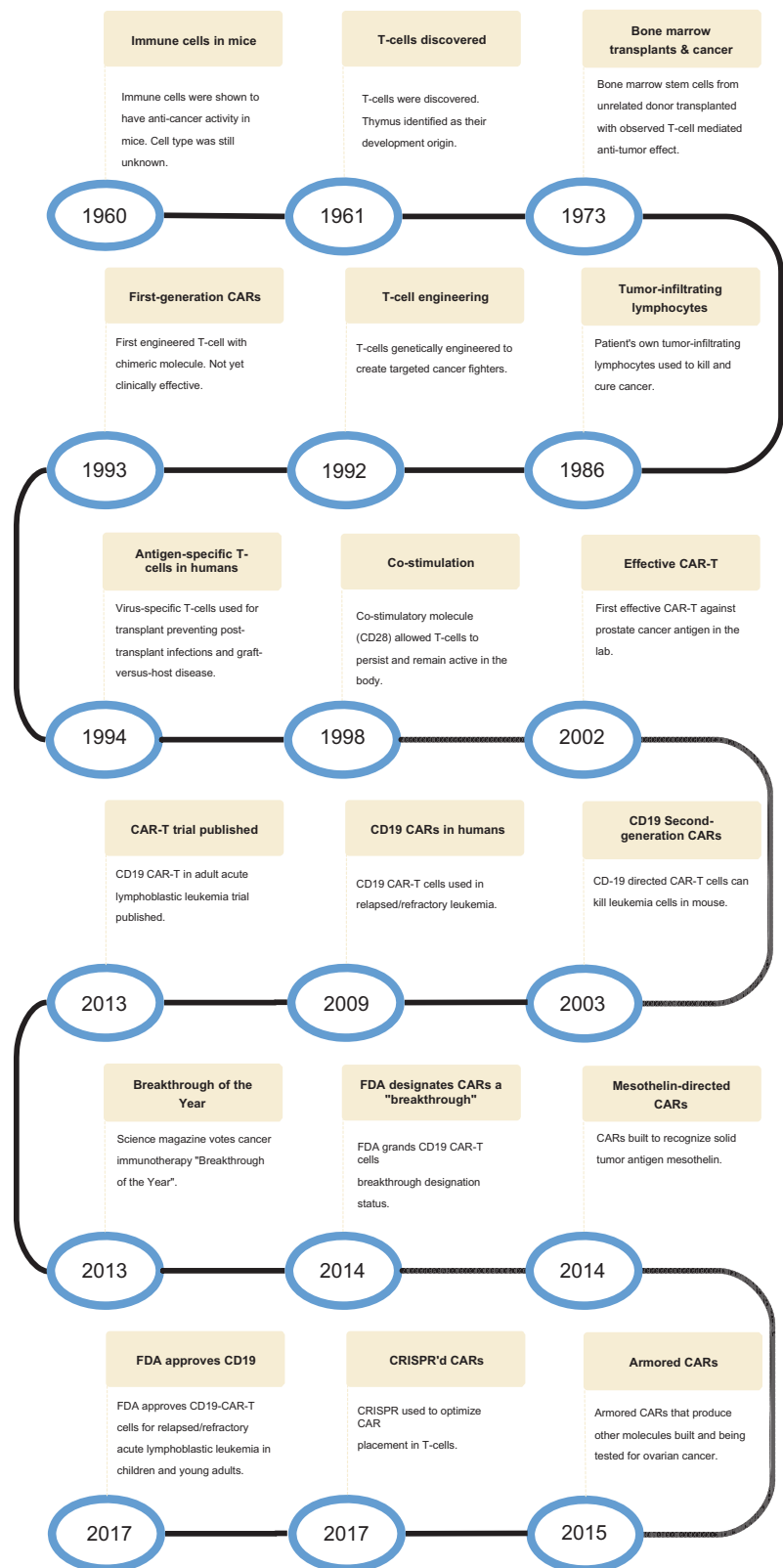
⁹ Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, USA

¹⁰ The Chaim Sheba Medical Center, Tel-Hashomer, Affiliated with the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

¹¹ Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

¹² Department of Medicine, Weill Cornell Medical College, New York, NY, USA

Fig. 1 CAR T-cell development



including immune reconstitution and mechanisms of relapse have been recently reviewed elsewhere and will not be covered here [1–3].

There are major structural differences between the T cell antigen receptor (TCR), and the CAR. TCRs usually recognize the antigen as peptides bound to major

histocompatibility complex (MHC) molecules, thus requiring the dual specificity of the tumor antigen and the MHC molecule [4]. In contrast, the antigen recognition domain of a CAR is a single-chain variable fragment (scFv) derived from an antibody which is then integrated into the TCR machinery. This means that CARs recognize antigens on the surface of cells in an HLA-independent fashion. For example, the CD19 antigen, commonly present on B cells, will be recognized in everyone by the same CAR. In contrast with the TCR, there is both antigen and MHC restriction, so that if for example, one develops a TCR against the human tumor antigen NY-ESO-1 in the context of HLA-A201, (currently being tested in pediatric sarcoma), this TCR will only work in patients who are HLA-A201 positive. This difference explains the faster clinical development and wider applicability of CAR T-cell therapy compared with the TCR approach, both in terms of scientific development and clinical trial progress as the potential benefits will be experienced by a larger population.

Effective T cell activation and proliferation requires two signals. Signal 1 is provided by T cells recognizing an antigen, while signal 2 is often delivered by costimulatory molecules. There are several costimulatory molecules including CD28 and 4-1BB which are currently being used clinically as well as a series of potential costimulatory molecules being tested in preclinical studies as well as in clinical trials [4]. CAR T-cell design has evolved from first-generation (T cell signal only) to those second-generation CAR T-cells used in the clinic today, which recognize not only the antigen but also include a costimulatory molecule signal, either CD28 or 4-1BB. Third-generation CAR T-cell products are engineered to contain more than one costimulatory molecule such as CD28 plus 4-1BB, or CD28 plus OX40, and are currently being tested in clinical trials [5]. While fourth-generation products are on their way combining for example a second-generation CAR with signaling domains from cytokine receptors or inducible expression of inflammatory cytokines, such as interleukin-12 (IL-12) or IL-18. Finally, CD19-specific CARs are the only ones approved for commercial use at the present time. CD19 is expressed on the surface of most B-cell malignancies and expression is restricted to B cells and possibly follicular dendritic cells. It is not expressed on pluripotent bone marrow stem cells. CD19 has the advantage of being broadly expressed on the surface of all B cells, with the possible exception of end-stage and fully differentiated plasma cells that express low or undetectable levels. Thus CD19 is a biomarker for B lymphocyte development that can be utilized as a target across a wide range of B cell malignancies from ALL, to NHL and CLL [6]. In contrast, CD20 which has a narrow spectrum of expression currently the target of rituximab and other CD20 directed antibodies

is variably expressed in ALL and might not be the target of choice.

Pertaining to the structure of CAR T-cells, there are some common elements and others that diverge between the products being tested at different centres. The common CARs used clinically or in preclinical development all have a scFv which is a chimeric protein that recognizes the antigen. While, the currently used scFv are derived either from murine monoclonal antibodies, new CD19-targeted CAR T-cells are engineered with humanized or fully human scFv to decrease immunogenicity [7]. The most commonly used costimulatory molecule is CD28 or 4-1BB. CD28-based CARs have been utilized at MSKCC, the National Cancer Institute, and the Baylor College of Medicine. 4-1BB-based CARs have been utilized at the Children's Hospital of Philadelphia /University of Pennsylvania (CHOP/UPenn) and the Fred Hutchinson Cancer Research Center (FHCRC)/Seattle Children's Hospital (FHCRC) [8].

In the last few years there has been dramatic development and approval of these engineered T cell products and in the USA, there is now approval for three different indications: the first was for tisagenlecleucel, in August 2017, for patients with relapsed/refractory B cell ALL in patients up to 25 years of age. Subsequently, tisagenlecleucel and axicabtagene ciloleucel were both approved for adult patients with relapsed or refractory (R/R) large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and transformed DLBCL arising from follicular lymphoma (TFL). Axicabtagene ciloleucel is also approved for treatment of R/R primary mediastinal large B-cell lymphoma (PMBCL). Simultaneously, academic anti-CD19 CAR T-cell program is under development in some centers [9].

CD19 CAR T-cells in acute lymphoblastic leukemia (ALL)

Data from both the British (MRC UKALL2/ ECOG2993) and French (LALA-94) showed that patients with ALL who relapse have a very poor prognosis and low long-term survival, particularly older patients [10, 11]. The UK study of 609 patients reported a 5-year overall survival (OS) of 7% [10] and in the French study including 421 patients, the 2- and 5-year OS were 11% and 8%, respectively [11]. Results in pediatric ALL are much better, however 15% of patients will not respond to current upfront protocols and R/R disease remains associated with an extremely poor prognosis [12]. Accordingly, this represents an area of unmet need. The main CAR T-cell therapy products for ALL that have been investigated in clinical trials today incorporate the CD28-CD3 ζ (MSKCC and NCI trials) and

Table 1 Main results of CD19 CAR T-cells in ALL

Signaling domain	Vector	Patients	CR Rate	CRS	Neurologic toxicity	References
CD28-CD3 ζ	Gamma-retrovirus	<i>N</i> = 53 (ALL) Adults	83%	26% severe	42% Gr 3–4	MSK Park et al. [14]
CD28-CD3 ζ	Gamma-retrovirus	<i>N</i> = 21 (ALL) Peds and AYA	67% (ITT)	76% (28% severe)	29%	NCI Lee et al. [13]
4-1BB-CD3 ζ	Lentiviral	<i>N</i> = 30 (ALL) 25Peds, five adults	90%	100% (27% Severe)	43%	UPenn/CHOP Maude et al. [21]
4-1BB-CD3 ζ	Lentiviral	<i>N</i> = 30 (ALL) Adults	93%	83%	50% severe	Seattle Turtle et al. [53]

CR complete remission, CRS cytokine release syndrome, ALL acute lymphoblastic leukemia, Peds pediatrics, AYA adolescents and young adults

4-1BB-CD3 ζ , (CHOP/UPenn and FHCRC trials) domains, transduced with a gamma-retrovirus or lentiviral vector, respectively. Complete remission (CR) rates range from 67% in the 2015 NCI trial [13] to over 80 and 90% in the more recent MSKCC and FHCRC trials [14, 15] (Table 1). CAR T brought significant CR rates in patients with relatively aggressive and relapsed/refractory disease that have not been observed previously with any other conventional therapies. However, CAR T-cell therapy is not without toxicity, namely cytokine release syndrome (CRS) and neurotoxicity. These side effects can also be seen with other treatments such as bispecific antibodies [16], but seem to be particularly frequent in patients receiving CAR T-cells. There is variation in the incidence rates and severity of CRS and neurotoxicity across the trials which could be due in part to the choice of either CD28 or 4-1BB costimulatory molecules but also to the different grading systems that were used across different studies [14, 17–19].

From a commercial standpoint, the CTL019 product (tisagenlecleucel, KYMRIAH[®], Novartis) is a CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of predominantly pediatric patients and young adults aged up to 25 years with B-cell precursor ALL that is refractory or in second or later relapse. It is administered 2–14 days after receiving a standard lymphodepletion regimen which combines fludarabine and cyclophosphamide [20]. The impressive results from the ELIANA study [21] (NCT02435849) led to the approval of tisagenlecleucel by the United States Food and Drug Administration in August 2017. This was a single-arm, open-label, international multicenter, Phase 2 trial of 92 enrolled patients with relapsed/refractory B-cell ALL. Of the 75 evaluable patients, 61% received one prior stem cell transplant procedure and the median number of previous lines of treatment was 3 (range, 1–8). The primary endpoint was overall remission rate (ORR), defined as best overall response of CR or CR with incomplete blood count recovery (CRi) within 3 months. With a median follow-up of 13.1 months, the CR/CRi was 81% (61 of 75; 95%

confidence interval [CI], 71–89) and the median duration of response (DOR) was not reached at the time of publication by the 61 patients who responded. This study confirmed the efficacy of a single infusion of CTL019, without additional therapy.

Currently, there are no approved CAR T-cell products for adult patients with ALL older than 25 years of age, although there are promising results from several ongoing trials. Kite-Gilead are conducting two Phase 1/2 trials, namely ZUMA-3 (NCT02614066) [22] and ZUMA-4 (NCT02625480) [23] with adult and pediatric/adolescent patients, respectively, receiving axicabtagene ciloleucel (Yescarta). Although numbers are small (<40 patients), so far similar high CR rates of 72 and 100% in the ZUMA-3 and ZUMA-4 trials, respectively are already evident [22, 23]. In another Phase 1/2 trial of 53 adult patients using a CD19-targeted CAR T-cell product of defined composition (1:1 ratio of CD4⁺:CD8⁺CAR T-cells, JCAR014, NCT01865617, Juno Therapeutics/Celgene Corporation), 85% of patients achieved CR [19]. These results raise hopes for future approval of CAR T-cell therapy for adult ALL patients.

A recently published phase 1 study from the MSKCC [14], reported on the first 53 adults patients treated with CD19-28z CAR (NCT01044069). The patients underwent leukapheresis, and then received either fludarabine and cyclophosphamide or cyclophosphamide only, for lymphodepletion. After the first 20 patients had been treated with 3×10^6 CAR T-cells/kg, two different dose levels were used, patients with detectable minimal residual disease (MRD) or those with MRD-negative status received the same dose of 3×10^6 CAR T-cells/kg, while patients with a high disease burden received a lower dose of 1×10^6 CAR T-cells/kg. The rationale for this approach was that lower disease burden would provide less stimulus to expand the cells. There is also an association between toxicity and higher disease burden, which further supports using fewer cells in this setting [14]. Of the 53 patients, 44 (83; 95% CI, 70–92) had a CR and most were MRD-negative. A total of

48 patients had sufficient bone marrow samples for the assessment of MRD, of whom 32 (67; 95% CI, 52–80) were in MRD-negative CR. Subgroup analysis showed that regardless of disease burden, pre-CAR hematopoietic stem cell transplantation (HSCT), number of prior therapies, conditioning regimen and age group, the CR rates were similar with no significant differences between the subgroups. Although the CR rates are quite high, patients do relapse after CAR T-cell therapy. At a median follow-up of 29 months (range, 1–65), the median event-free survival (EFS) was 6.1 months (95% CI, 5.0–11.5), and the median OS was 12.9 months (95% CI, 8.7–23.4). Patients with a low disease burden (<5% bone marrow blasts) before treatment had markedly enhanced remission duration and survival, with a median EFS of 10.6 months (95% CI, 5.9—not reached) and a median OS of 20.1 months (95% CI, 8.7—not reached). Patients with a higher burden of disease ($\geq 5\%$ bone marrow blasts or extramedullary disease) had a greater incidence of CRS ($p = 0.004$) and neurotoxic events ($p = 0.002$) and shorter long-term survival than did patients with a low disease burden [14]. Patients who had a MRD-negative CR had a significantly better EFS ($p < 0.001$) and OS ($p < 0.001$) compared with those who had a MRD-positive CR or no response. From a cost-effectiveness point of view, it is still uncertain whether CAR T-cell therapy should be considered as a definitive treatment or as a bridge to allogeneic HSCT (allo-HSCT) consolidation following CR after CAR T therapy. In the latter study, survival was found to be independent of allo-HSCT. There was no significant difference in EFS or OS between patients who proceeded to allo-HSCT after CAR T-cells and those who did not [14]. It should be noted that these patients were not randomized, some were not suitable for allo-HSCT, some elected not to proceed to allo-HSCT, and for some patients no donor could be identified. But these results do suggest that some patients could be treated with CAR T-cells alone, and, in our opinion, this is an area that deserves further investigation to determine whether consolidation with a transplant is necessary in a particular subgroup of patients [24, 25]. Currently, a phase 2 study is being carried out by the Childrens' Oncology Group in the USA, looking into consolidation with CAR T-cell therapy after first-line therapy in MRD-positive patients (NCT03876769) [26].

CD19 CAR T-cells in non-Hodgkin lymphoma (NHL)

DLBCL is the commonest subtype of NHL and constitutes about 30–40% of adult NHLs. Although 5-year survival rates in the first-line setting range from 60 to 70, up to 50% of patients become refractory to or relapse after treatment. Patients with RR-DLBCL have a poor prognosis and if left

untreated, have a life expectancy of 3–4 months. The SCHOLAR-1 trial [27], an international, multicohort NHL research study retrospectively evaluated outcomes in 636 patients with refractory DLBCL which, for this study, was defined as progressive disease or stable disease as best response at any point during chemotherapy (greater than four cycles of first-line or two cycles of later-line therapy) or relapsed at ≤ 12 months from autologous HSCT (auto-HSCT). Pooled data from two phase 3 clinical trials and two observational cohorts were included in that analysis. Overall the SCHOLAR-1 trial confirmed the very poor prognosis of RR-DLBCL with 20% of patients alive at 2 years and outcomes consistently poor across patient subgroups and study cohorts. This is another area of unmet need and these survival rates of around 20% form the basis of approval, in the USA and in Europe, for CAR T-cells therapy for DLBCL [28]. A recently published review [29] showcases the clinical efficacy and unique toxicities of individually developed CAR T-cell products for the treatment of lymphomas and their evolution from the laboratory bench to commercialization.

The commercial product axicabtagene ciloleucel (YES-CARTA[®], Kite-Gilead) [30] is an autologous CD19-directed genetically modified T-cell immunotherapy indicated for the treatment of adult patients with RR-DLBCL, including DLBCL not otherwise specified, PMBCL, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma (FL). This product was developed at the NCI and the label, derived from the ZUMA-1 trial (NCT02348216), is very similar to that of tisagenlecleucel which is also indicated for all these B-cell lymphoma variants, except PMBCL for which axicabtagene ciloleucel is the only approved CAR T product. The ZUMA-1, phase 1/2 trial response rates given in the axicabtagene ciloleucel label for objective response rate and CR rate are 72% and 51%, respectively ($n = 73$). The median DOR was 9.2 months and the median follow-up was 7.9 months. It is important to note that these results are captured at a 1-month time point, which is quite early as most clinicians who perform autologous transplants for lymphoma typically assess disease at 3-month imaging PET/CAT scans and would not perform a scan this early unless clinically indicated. As absence of early CR in patients treated with CAR T is associated with inferior PFS and OS, early staging (by day 60) is a helpful prognostic marker that helps identify the patients destined to do poorly. The primary analysis of the ZUMA-1 phase 2 trial, and an updated analysis with 1 year of follow-up, and which led to approval, was published in December 2017 [31]. This trial looked at 77 patients with DLBCL and a group of 24 patients with PMBCL or TFL. The CR rate was 82% and rates for DLBCL and PMBCL were 82% and 83%, respectively. There were no significant differences in response across key covariates including age, disease stage,

Table 2 Main results of CD19 CAR T-cells in NHL

	KTE-C19	CTL019	JCAR017
Drug name	Axicabtagene ciloleucel	Tisagenlecleucel-T	Lisocabtagene maraleucel
Clinical trial	ZUMA-1 NCT02348216	JULIET NCT02445248	TRANSCEND NHL 001 NCT02631044
Phase	Phase 1/2	Phase 2a	Phase 1
Dose level	2×10^6 cells/kg	5×10^8 cells	5×10^7 cells
Conditioning chemotherapy	Low dose Cy/Flu \times 3 days	Variety; based on clinical features and past therapies	Low dose Cy/Flu \times 3 days
Evaluable patients (N)	DLBCL ($N = 77$) TFL/PMBCL ($N = 24$)	DLBCL ($N = 51$)	DLBCL ($N = 40$) Transformed DLBCL ($N = 14$) FL grade 3B ($n = 1$)
Response rates	ORR = 82% CR = 54%	ORR = 59% CR = 43%	ORR = 74% CR = 52%
CRS	Overall: NA Severe: 11%	Overall: 58% Severe: 22%	Overall: 35% Severe: 1%
Neurotoxicity	Overall: NA Severe: 32%	Overall: 21% Severe: 12%	Overall: 19% Severe: 12%

Cy cyclophosphamide, *Flu* fludarabine, *DLBCL* diffuse large B cell lymphoma, *TFL* transformed follicular lymphoma, *PMBCL* primary mediastinal B cell lymphoma, *FL* follicular lymphoma, *ORR* overall response rate, *CR* complete remission, *CRS* cytokine release syndrome, *NA* not available

extra-nodal disease, treatment history, International Prognostic Index (IPI) score at enrollment, presence or absence of bulky disease, and cell-of-origin subtype. Many of these patients developed CRS or neurotoxicity or both, and a big concern at the start was whether tocilizumab or steroid (glucocorticoid) would affect the efficacy of CAR T-cells, as tocilizumab blocks IL-6 and steroids are immunosuppressive. However, the response rates did not appear to be influenced by use of these agents, thus they do not appear to reduce CAR T-cell efficacy, at least in the ZUMA-1 trial.

As with pediatric ALL, there is a drop off in PFS and then stability at around 6 months. The recently published long-term follow-up [32] confirmed a median duration of PFS of 5.8 months (95% CI, 3.3–15.0) with PFS rates of 49% (95% CI, 39–58) at 6 months, 44% (95% CI, 34–53) at 12 months, and 41% (95% CI, 31–50) at 15 months. The median OS was not yet reached (95% CI, 12.8 months—could not be estimated) with OS rates of 78% (95% CI, 69–85) at 6 months, 59% (95% CI, 49–68) at 12 months, and 51% (95% CI, 40–60) at 24 months. A total of 56% of patients remained alive at the time of the data cutoff. Two patients who had a response underwent allo-HSCT.

According to its label, tisagenlecleucel is also indicated for the treatment of adult patients with RR-large B-cell lymphoma after two or more lines of systemic therapy including DLBCL not otherwise specified, high grade B-cell lymphoma and DLBCL arising from FL. As noted above, these indications are the same as for axicabtagene ciloleucel except for PMBCL, but the lymphodepletion regimen is different. One can either use fludarabine and cyclophosphamide or other options, such as bendamustine.

Data from the cohort which led to approval of tisagenlecleucel were recently reported by Schuster et al. [33]. Survival in DLBCL patients after CD19 CAR T therapy is similar to that seen in the ZUMA-1 trial [32] with a median OS of 12 months (95% CI, 7 months—not yet reached) [33]. In FL patients however, the results are most impressive with the median OS not reached and 93% remained alive at the median follow-up of 28.6 months [34].

In summary, there are currently two approved CAR T-cells products for the treatment of NHL: axicabtagene ciloleucel (KTE-C19, ZUMA-1 [32]) and tisagenlecleucel-T (CTL019, JULIET [33]) while a third product lisocabtagene maraleucel (JCAR017) is currently under investigation in the phase 1 TRANSCEND NHL 001 trial (NCT02631044) [35] (Table 2). The ORR rates for these three products are 82%, 59%, and 74%, and the CR rates are 54%, 43%, and 52%, respectively. Although these are not phase 3 randomized trials, all these products seem to be efficacious and have similar toxicity profiles with respect to CRS, while severe neurotoxicity seems to be less frequent with tisagenlecleucel-T and lisocabtagene maraleucel. There are also differences in time of onset of some of the toxicities which may be due to the difference in the costimulatory molecules, CD28 and 4-1BB. For example, the median time to onset of CRS is 1 day earlier with axicabtagene ciloleucel (which uses CD28), being 48 h versus 72 h for tisagenlecleucel (which uses 4-1BB). The logistics also are different, with axicabtagene ciloleucel and lisocabtagene maraleucel being manufactured at a central manufacturing organization (CMO) from the freshly collected cells that are immediately shipped from the collection facility performing apheresis,

while tisagenlecleucel is manufactured at a CMO from cryopreserved autologous blood mononuclear cells that are frozen onsite immediately after apheresis, by a hospital or blood bank operated processing facility. The different organizations, together with persistent restrictions in manufacturing capacities in view of the worldwide demand, explain while several Health Technology Assessment (HTA) agencies differently differently, the medical value of tisagenlecleucel and axicabtagene ciloleucel, taking into account different failure rates for the manufacturing process.

In NHL, several questions can be asked in relation to the lymphodepleting regimen prior to CAR T-cell therapy, one being the best choice of lymphodepletion. This question cannot be easily answered. A study of 14 patients with DLBCL used several conditioning regimens and the authors' conclusion was that the choice of lymphodepletion did not make any difference [34]. In contrast, Hirayama et al. analyzed the impact of clinical and treatment characteristics, serum biomarker and CAR T-cell manufacturing and pharmacokinetic data on PFS after CAR T-cell therapy in 65 NHL patients and found that lymphodepletion impacted PFS [36]. They found that lower pre-lymphodepletion serum lactate dehydrogenase (LDH) level and a favorable cytokine profile, defined as serum day 0 monocyte chemoattractant protein-1 (MCP-1) and peak interleukin-7 (IL-7) concentrations above the median, were associated with better PFS. Furthermore, MCP-1 and IL-7 concentrations increased after lymphodepletion, and higher intensity of cyclophosphamide (Cy) and fludarabine (Flu) lymphodepletion was associated with higher probability of a favorable cytokine profile. Overall PFS was superior in patients who received high-intensity lymphodepletion and achieved a favorable cytokine profile compared with those who received the same intensity of lymphodepletion without achieving a favorable cytokine profile, suggesting that the biological effects of the lymphodepleting regimen are likely more important than the intensity of lymphodepletion to improve CAR T-cell efficacy [37].

For approved products however, the product label regimens are usually prescribed.

Another question is whether auto-HSCT could be used instead of lymphodepletion in preparation for CAR T-cell infusion. This has been investigated in a study by Sauter et al. (NCT01840566) [38]. Patients with poor-risk R/R NHL ($n = 15$) had an auto-HSCT performed and then were immediately infused with CAR T-cells on days +2 and +3 after HSCT. With a median follow-up of 31 months, the 2-year PFS rate was 30% (95% CI, 20–70) similar to what one would expect for auto-HSCT patients with the caveat that such patients would not normally have been expected to do quite so well. This combination of auto-HSCT and CAR T-cells was associated with toxicity. As a result, future trials

should be planned to address the dose of CAR T-cells as well as the timing in relation to the autologous transplant. This is a promising area of research and shows the potential for combining ASCT and CAR T-cell therapy.

Given the documented efficacy of CAR T-cell therapy in DLBCL patients who relapse after transplant, another area of active investigation of CAR T-cell therapy in NHL, is how it performs compared with the standard of care high-dose therapy and auto-HSCT (Table 3). This is being examined in the phase 3 ZUMA-7 trial (NCT03391466) [39]. Adult patients with relapsed or refractory disease after first-line chemoimmunotherapy are randomized to receive either CAR T-cells (axicabtagene ciloleucel) or standard of care therapy comprising platinum-based salvage chemotherapy regimen followed by HDT-HSCT. There are two other similar trials being run by Novartis (NCT 03570892) and Celgene (NCT03575351) with the difference being that in the Novartis trial patients in the CAR T-cell arm could optionally receive a chemotherapy salvage regimen similar to the HDT-HSCT arm. These large phase 3 trials will, in a few years provide data that might allow an expanded use of CAR T-cells at an earlier disease stage.

Studies are also being planned or are in early development looking at CAR T-cell therapy in high-risk patients such as those with double-hit lymphomas where it may be given as front-line therapy or to those with CNS lymphoma [40]. There is a small single-center study [30] that is combining a checkpoint inhibitor (pembrolizumab) with CAR T-cell therapy to augment the immune response in 12 DLBCL patients with immune exhaustion in relapse or progression after a previous CAR T-cell treatment [41]. Although further studies are needed, initial results have been positive with a best ORR of 25% (one CR, two PR, one stable disease, and eight progressive disease). Another ongoing study reports an ORR of 50% (five CR and one PR) in the 12 evaluable patients with R/R NHL treated with CAR T-cells and the anti-PDL1 monoclonal antibody darvolumab [42].

There is also a rationale to evaluate CD19 CAR T-cells in Hodgkin lymphoma (HL). HL is a complex disease driven by the Reed–Sternberg cell. In a pilot trial [43] of five relapsed or refractory classical HL, the researchers tested the hypothesis that by eradicating CD19+ B cells within the tumor microenvironment and the circulating CD19+ progenitor Hodgkin Reed–Sternberg cells from the blood using an anti-CD19-directed CAR-modified T-cells (CART19), this may indirectly affect HRS cells, which do not express CD19. There are also studies of CD30 CAR T-cells in HL, including studies at Baylor Medical Center (NCT02917083) [44], University of North Carolina (NCT02690545), and centers in Spain that are going to open CD30 CAR T studies in HL by the end of 2019, as CD30 may be a more relevant tumor antigen than CD19 in this context.

Table 3 Ongoing phase III trials of CD19 CAR T-cells in ALL and NHL

Trial	Patients	CAR T-cells	Comparative arm	Primary endpoint
NCT03628053 OBERON (Novartis)	N = 220 Adults R/R ALL	-Lymphodepleting chemotherapy -Tisagenlecleucel	-Optional bridging chemotherapy -Blinatumomab or inotuzumab	Overall survival
NCT03391466 ZUMA-7 trial (Gilead)	N = 350 Adults R/R NHL	-Lymphodepleting chemotherapy (Flu + Cy) -Axicabtagene ciloleucel	Platinum-based immunochemotherapy (R-ICE, R-DHAP, R-ESHAP, or R-GDP) + high-dose chemotherapy (i.e., BEAM) + autologous HSCT	Event-free survival
NCT 03570892 BELINDA (Novartis)	N = 318 Adults R/R NHL	-Optional platinum-based immunochemotherapy (i.e., R-ICE, R-GemOx, R-GDP, R-DHAP) -Lymphodepleting chemotherapy (Flu + Cy or bendamustine) -Tisagenlecleucel	Platinum-based immunochemotherapy (i.e., R-ICE, R-GemOx, R-GDP or R-DHAP) + High-dose chemotherapy (i.e., BEAM) + autologous HSCT.	Event-free survival
NCT03575351 TRANSFORM (Celgene)	N = 182 Adults R/R NHL	-Lymphodepleting chemotherapy (Flu + Cy) -JCAR017	Platinum-based immunochemotherapy (i.e., R-ICE, R-GDP or R-DHAP) + High-dose chemotherapy (i.e., BEAM) + autologous HSCT.	Event-free survival

Cy is for cyclophosphamide, *Flu* fludarabine, *NHL* non-Hodgkin lymphoma, *ALL* acute lymphoblastic leukemia, *R-ICE* rituximab, ifosfamide, carboplatin, etoposide, *R-DHAP* rituximab, dexamethasone, cytarabine, cisplatin, *R-ESHAP* rituximab, etoposide, cisplatin, methylprednisolone, cytarabine, methy/prednisolone, cytarabine, *R-GDP* rituximab, gemcitabine, dexamethasone, cisplatin, *BEAM* carmustine, cytarabine, etoposide, melphalan, *HSCT* hematopoietic stem cell transplantation

CD19 CAR T-cells in chronic lymphocytic lymphoma (CLL)

One of the first areas of development of CAR T-cell therapy was in CLL patients and just as these efforts were initiated, several drugs became available, such as ibrutinib [45], idelalisib [46], venetoclax (ABT-199) [47], duvelisib (IPI-145) [48], and obinutuzumab [49]. These treatments were easier to deliver than CAR T-cells and have had a noticeable impact on the use of allogeneic HSCT. In the US, the number of allo-HSCTs in CLL patients dropped after 2013 due to the availability of alternative treatments for CLL, in contrast to a continued increase in the number of patients with AML, MDS, and ALL [50]. However, CAR T-cell therapy for CLL patients is still an area of interest. In 2015, the Abramson Cancer Center (University of Pennsylvania) treated 14 patients with relapsed or refractory CLL with CAR T-cells and the results were reasonable with an ORR in 8 of the 14 included in the study (57%), four CR and four partial remissions [51]. The authors concluded that the responses were durable and that no patient in CR had relapsed. Eradication of the malignant clone was sustained without further therapy and responses were associated with high levels of CTL019 expansion and long-term persistence and B cell aplasia for years after infusion.

CAR T-cells can be manufactured for CLL patients who have failed prior standard purine analog-based chemioimmunotherapy. The group at MSKCC reported a median PFS of 13.6 months in a small cohort of eight patients [52], which resembles that found in the above mentioned study at the Abramson Cancer Center [51]. An objective response was observed in three of eight patients (38%), and two patients had a CR lasting more than 28 months. None developed CRS or neurotoxicity. An interesting area is that of combination therapies as well as the use of CAR T-cells in patients with CLL who are refractory to what we now consider to be standard therapy such as ibrutinib or venetoclax. In 2017, a study in Seattle [53] of 24 CLL patients, including 5 Richter's transformation, who were refractory to ibrutinib ($n = 19$) and venetoclax ($n = 6$ of whom five were refractory to ibrutinib as well) reported an ORR of 71% (17 of 24). The PFS was similar in patients with lymph node PR or CR by IWCLL criteria. Twenty-three of these patients had high-risk cytogenetics (complex karyotype and/or 17p deletion). Patients who were negative for malignant IGH sequences had better PFS compared with those with persistent malignant IGH sequences ($P = 0.0253$). Median OS was not reached in either group. The positive effect on outcome of marrow clearance demonstrated by IGH sequencing on outcome was also observed when the analysis was restricted to PFS in patients who responded (CR or PR) by IWCLL criteria ($P = 0.063$; median PFS for IGHseq-positive, 8.5 months;

median PFS for IGH-negative was not reached). The study concluded that CD19 CAR T-cells were highly effective in this group of high-risk patients with CLL after they have experienced treatment failure with ibrutinib therapy. Even with the new treatments, patients will eventually progress and although they may benefit from CAR T-cells, there is as yet, no consensus as to whether this should become routine practice, but it is a promising area of research. Of note, there was some concern because ibrutinib targets both Bruton tyrosine kinase and interleukin (IL)-2 inducible T-cell kinase (ITK); this may interfere with T-cell function, but it was shown that prolonged treatment with ibrutinib restored CLL patient T-cell functions and that concurrent ibrutinib treatment enhanced the efficacy of CAR T-cell engraftment [54]. There is now clinical data for 19 patients treated with a combination of ibrutinib and CTL119, the humanized CD19-directed CAR T-cell therapy [55]. Seventeen of eighteen patients who were evaluable for response had no evidence of disease in their bone marrow at 3 months, including 15 patients with negative MRD. At 12 months, 11 patients had evaluable marrows of which 10 (91%) were in morphologic CR including seven with negative MRD. Another group performed a phase 1–2 study evaluating the combination of ibrutinib, a Cy/Flu lymphodepleting regimen and JCAR14 CAR T-cells in 17 patients with R/R CLL [56]. Their outcomes were compared with a group of 19R/R CLL patients treated with Cy/Flu + JCAR14 without ibrutinib. The ORR (CR + PR) was higher in the ibrutinib cohort, being 88% versus 56% in the no ibrutinib cohort ($p = 0.06$). There was no difference in the incidence of neurotoxicity and grade ≥ 1 CRS between both groups, however the incidence of severe (\geq grade 3) CRS was significantly lower in the ibrutinib cohort, being 0%, versus 26% ($p = 0.05$). It may be possible in the future for this combination to be used as first-line therapy which would remove the need for chronic therapy.

Safety and toxicity management of CAR T-cell therapy

The main toxicities associated with the use of CAR T-cells, are CRS and neurologic toxicities. They require different management strategies. CRS is a multi-organ complex toxicity syndrome with neurological components and hematological as well as multi-organ complications [57]. Interleukin-6 receptor blockade with tocilizumab remains the mainstay pharmacologic therapy for CRS, though indications for administration vary among centers. It is important to emphasize that centers must document availability of two doses of tocilizumab prior to infusion of CAR T-cells. Corticosteroids should be reserved for neurologic toxicities and CRS not responsive to tocilizumab. Patients

can become quite sick and it is imperative to have a multi-disciplinary team approach to their medical management. Hematologists and oncologists must work closely with physicians in the intensive care unit (ICU) as well as with the neurology team. In many centers, patients have a neurological consultation and an MRI of the brain before admission which greatly reduces the threshold at which ICU and/or neurologists are called to see patients who develop complications. Procedures warranting a safe CAR T-cells administration have been recently reviewed elsewhere [1].

The critical aspect in management of these patients is early recognition of the signs of these complications and early and aggressive treatment. The labels that come with the approved drugs contain the instructions on how to manage patients but many centers have developed their own treatment algorithms based on these instructions. One of the challenges in patient management is what to do when different products are used at the same time with differing label instructions. What approach should be used when for example, a patient with lymphoma develops CRS on day +3 after axicabtagene ciloleucel versus tisagenlecleucel, the former being a CD28 product and the latter using 4-1BB. The timeline and severity of toxicity are likely different, warranting distinct therapeutic approaches. The situation is very complex and necessitates a treatment algorithm that has been developed in collaboration with the neurologists and the ICU team. Improving the management of CAR T-cell toxicity is one of the most important avenues for overall improvement in the field of CAR T-cell therapies; this will need improvements in the design of CAR T-cells themselves (such as incorporating a switch in the molecular construct, allowing to definitively or temporarily inhibit CAR T-cell activity *in vivo*) and improvements in hospital organization, including much-needed training of all categories of healthcare personnel and proper logistics (such as capacity in the ICU). Challenges regarding actual and future role of ICU in management of CAR T-cells toxicities have been recently reviewed [58] and a detailed toxicity of CAR T-cells is a subject beyond the scope of this paper, but some important references are provided [59]. The latter report presents a novel system to grade the severity of CRS (Lee criterion) in individual patients and a treatment algorithm for management of CRS based on severity. The goal of such an approach is to maximize the chance for therapeutic benefit from the immunotherapy while minimizing the risk for life threatening complications of CRS. In another work [60], the authors document the development of consensus guidelines on CAR T-cell-related toxicities, mostly with reference to axicabtagene ciloleucel, the anti-CD19 CAR construct containing CD28, in patients with aggressive forms of NHL.

In terms of neurotoxicity, which remains poorly understood, an important publication by Gust et al. [61] provides

a detailed clinical, radiologic, and pathologic characterization of neurotoxicity after CD19 CAR T-cells and identifies risk factors for neurotoxicity. The authors showed endothelial dysfunction and increased blood brain barrier permeability in neurotoxicity and found that patients with evidence of endothelial activation before lymphodepletion may be at increased risk of neurotoxicity. Another work by Santomasso et al. detailed the neurologic symptoms and blood, cerebrospinal fluid, and neuroimaging correlates of neurotoxicity associated with CD19 CAR T-cells and identified neurotoxicity risk factors [62]. Their findings implicate cellular components other than T-cells and suggest novel links between systemic inflammation and characteristic neurotoxicity symptoms.

On top of severe adverse events in the treated patients, comes the concern of the financial impact on the global healthcare systems. Approved CAR T-cells are among the most expensive medicinal products ever marketed. In addition to drug pricing, the costs of hospital reorganization to fully master their contribution to industry manufacturing of these highly personalized gene therapies, as well as additional patient costs are presently not fully evaluated. There is a need for innovative reimbursement modalities, if the situation is to remain sustainable for all interested parties.

Future developments of CD19 CAR T-cells

A number of recent papers provide guidance on how to set up a program for CAR T-cells [63]. One of the challenges is that centers administering CAR T-cells have developed and employed different CRS grading scales (e.g., Penn, Lee etc.), making it difficult to compare toxicity severity and outcomes, with and without interventions, between studies. As a result of this additional complexity, the American Society for Transplantation and Cellular Therapy (ASTCT), formerly known as American Society for Bone Marrow Transplantation (ASBMT) convened about 50 experts in June 2018 representing the large centers and the companies with the aim of developing consensus criteria and thus a single grading system for CAR T-cell toxicity, both for CRS and neurotoxicity, regardless of the product used [64]. This system will be freely available in the ASBMT mobile application. A joint publication by ASBMT and EBMT covers FAQs in relation to the practical aspects of management of patients after CAR T-cell therapy [65].

The area of engineered CAR T-cells is a very exciting one. There is now approval for treatment of NHL and ALL. The future for NHL is expanding the use to earlier stages. There are ongoing studies in ALL looking to expand the label including the use in adult patients and there will probably be approval of additional products for ALL, for example, consolidation with CAR T-cells after first-line

therapy in MRD-positive patients [26]. An indication for CAR T-cells is also expected either in CLL patients who have failed the new therapies such as venetoclax/ibrutinib and others or in combination with some of these therapies. Regarding multiple myeloma (MM), it is noteworthy that one patient was reported to be successfully treated with autologous CD19 CAR T-Cells, despite the low level of expression of CD19 on terminally differentiated plasma cells [66]. Nevertheless, most of the work in the field of MM and CAR T-cells is being focussed on CAR T-cells engineered to target the B-cell maturation antigen (BCMA), and so it has not been covered here [67]. Approval of one of the BCMA CAR T-cell products for MM may be forthcoming in late 2019/early 2020.

Although the CAR T-cell era is still in its infancy, it is probably the most important development in cancer therapy, especially for hematological malignancies. It is currently leading immunotherapy-based approaches with extremely encouraging clinical results, leading to very fast approval of the first two products to now benefit this desperate group of patients with advanced disease.

Author contributions All authors contributed substantially to the conception, writing, critical review and final approval of the manuscript.

Compliance with ethical standards

Conflict of interest MAP reports honoraria from Abbvie, Bellicum, Bristol-Myers Squibb, Incyte, Merck, Novartis, Nektar Therapeutics, and Takeda. He serves on DSMBs for Servier and Medigene, and the scientific advisory boards of MolMed and NexImmune. He has received research support for clinical trials from Incyte, Kite (Gilead) and Miltenyi. Christian Chabanon reports honoraria from Kite/Gilead and Novartis. The remaining authors declare that they have no conflict of interest.

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