Chimeric antigen receptor T-cells: a new therapeutic option for relapsed/refractory B-cell malignancies and beyond

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Chimeric antigen receptor (CAR) T-cell therapy is a new cancer immunotherapy targeting specific cell surface antigens. This type of adoptive cell immunotherapy has been a breakthrough in the treatment of aggressive B-cell lymphoma and B-cell precursor acute lymphoblastic leukaemia (ALL) and is currently also being studied in other cancer types, including multiple myeloma and chronic lymphocytic leukaemia. This review will discuss the recent clinical developments and future perspectives of CAR T-cell therapy, with a focus on the clinical trials that led to the FDA and EMA approval of tisagenlecleucel (Kymriah®, Novartis) and axicabtagene ciloleucel (Yescarta®, Gilead) for the treatment of childhood/adult relapsed/refractory (r/r) B-cell precursor ALL and aggressive B-cell non-Hodgkin lymphoma.

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INTRODUCTION

For decades, the treatment of haematological malignancies was dominated by systemic chemotherapy, radiation therapy and stem cell transplantation. More recently, new insights in the genetic and molecular basis of these malignancies paved the way for the development of targeted therapies, while the increased understanding of the interplay between the patients' immune system and cancer cells led to the development of several innovative immunotherapies. One of these immunology-based treatment strategies that recently generated much excitement is chimeric antigen receptor (CAR) T-cell therapy.[1] This type of adoptive cell therapy (ACT) already proved to be a real breakthrough in the treatment of aggressive B-cell lymphoma and B-cell precursor acute lymphoblastic leukaemia (ALL), and is currently also being evaluated in other haematological cancer types, including multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL).[1]

Exploiting the immune system to attack cancer cells is not a new concept. In fact, the development of allogeneic stem cell transplantation (alloSCT) has first highlighted the potential of T-cells to eliminate cancer cells. In this respect, *Kolb et al.* showed that donor lymphocyte infusions can induce long-lasting remissions in patients with relapsed chronic myeloid leukaemia (CML).[2] With ACT, immune cells are collected from a patient or a donor after which they are manipulated and/or expanded *ex vivo* and reinfused to the patients.[1] The success of ACT mainly depends on the presence of an adequate amount of effector cells in the patients, which in turn requires precursors with either natural anti-tumour recognition, or engineering of T-cells to provide this recognition.[1] Therefore, researchers have developed several strategies to improve the tumour recognition of adoptively stimulated cells. Genetic engineering of novel receptors (i.e. CARs) led to the development of molecules that can both recognise proteins present on the surface of

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tumour cells and provide T-cell activation, proliferation, and memory.[3] CAR constructs are hybrid molecules; the extracellular part is based on the structure of a monoclonal antibody and responsible for surface antigen recognition. This recognition occurs in a major histocompatibility complex (MHC)-independent manner. The intracellular part is based on the structure of the T-cell receptor (TCR) coupled with one or more co-stimulatory domains, allowing to transduce the antigen recognition into T-cell activation.[3]

CAR T-CELL DESIGN

In general, CARs consist of three major domains: an ectodomain, a transmembrane domain and an endodomain. The ectodomain or extracellular portion of the CAR typically consists of heavy and light chains derived from an antibody in single-chain variable fragment format, and a hinge region. It redirects the specificity of the receptor to recognise antigens on the cell surface independently of MHC molecules. CD19 has been most frequently chosen as target antigen for several reasons: its frequent and high-level expression in B-cell leukaemia and lymphoma, with a broader and higher expression relative to other potential targets like CD20 or CD22, and its restriction to the B-cell lineage in healthy tissue. The transmembrane domain of the CAR construct primarily plays a role in stabilizing the CAR, while the intracellular endodomain provides the necessary signals to activate the T cells after antigen recognition.[3]

The design of CARs considerably evolved over the years. First-generation CARs were designed similarly to the endogenous TCR complex. In these initial constructs, the intracellular component usually consisted of CD3ζ, which was linked to an extracellular antigen-recognition domain that allowed for direct, MHC-independent recognition of antigens on the tumour cell surface.[3] Importantly, these first-generation designs did not include co-stimulatory domains and, as such, did not provide a second signal for full T-cell activation. As a result, these first-generation CAR T-cells were more prone to apoptosis and had limited *in vivo* expansion potential, resulting in poor cytotoxicity.[3,4] The addition of co-stimulatory signalling domains (e.g. CD28, 4-1BB) in second-generation CARs resulted in improved T-cell activation, enhanced survival capabilities and a more effective expansion of the modified T-cells *in vivo*.[5] These second-generation receptors form the basis of the currently approved CAR T-cell therapies. It is now becoming increasingly clear that each type of co-stimulatory domain has specific roles in CAR signalling; for example, CD28-based CAR T-cells exhibit more potent effector cell functions but limited persistence, whereas 4-1BB tends to drive the CAR T-cells towards a central memory phenotype resulting in improved persistence.[6] Third-generation CAR T-cells combine the signalling potential of two co-stimulatory domains (e.g., both CD28 and 4-1BB). The antitumour activity of fourth-generation CARs, including T-cells redirected for universal cytokine-mediated killing (TRUCKs), is even further enhanced by additional genetic modifications, for example by the addition of transgenes for cytokine secretion (e.g., IL-12).[7-9]

CAR T-CELL MANUFACTURING AND ADMINISTRATION

Although allogeneic CAR T-cells have been used, the production of CAR T-cells typically starts with the collection of peripheral blood mononuclear cells (PBMCs) from the patient (autologous) using a large volume leukapheresis procedure. The cells are then transferred to a cell-processing centre where they are brought into culture to induce proliferation. Then, the cells are loaded with the CAR, usually by incubating them with CAR-encoding viral vectors, which enter the T-cells and introduce the CAR gene RNA. This CAR RNA is then reverse-transcribed into DNA, which recombines into the T-cell genome, resulting in permanent CAR gene incorporation. Both lentiviral, and to a lesser extent, gamma-retroviral vectors have been used for CAR gene transduction of primary T-cells.[10]

The modified T-cells are then transferred back to the centre for infusion, which typically happens as a single infusion. The median time from leukapheresis to CAR T-cell administration is 4-5 weeks and the entire process from referral to infusion typically takes 2 months.[11] Therefore, physicians often perform bridging chemotherapy to avoid rapid disease progression and to maintain the patient's general condition during the CAR T-cell production period. Lymphodepleting (LD) chemotherapy, such as fludarabine and cyclophosphamide, is often administered prior to the infusion of the CAR T-cells.[12] LD chemotherapy decreases the number of T-cells *in vivo*, including regulatory T-cells, and consequently upregulates cytokines such as IL-7 and IL-15.[10] These cytokines promote T-cell expansion, including CAR T-cells, and augment the antitumour activity of the CAR T-cell therapy.

EFFICACY OF CAR T-CELL THERAPY IN HAEMATOLOGICAL MALIGNANCIES

CAR T-cell therapy has emerged rapidly over the last few years, ultimately leading to the approval of the first two CAR T-cell medicines (tisagenlecleucel [Kymriah®, Novartis] and axicabtagene ciloleucel [Yescarta®, Gilead]) both by the US Food and Drug Administration (FDA) and later by the European Medicines Agency (EMA) for the treatment of relapsed/refractory (r/r) B-cell precursor acute lymphoblastic leukaemia (ALL) in children and young adults, and aggressive

TABLE 1. Patient characteristics in the three anti-CD19 CAR T-cell therapy multicentre trials in r/r B-cell non-Hodgkin lymphoma.

Characteristics	ZUMA-1 [17,18] (Axicabtagene ciloleucel)	JULIET [20] (Tisagenlecleucel)	TRANSCEND* [26] (Lisocabtagene maraleucel)
N enrolled (N infused)	111 (101)	165 (111)	134 (114 -> 102#)CORE cohort: 73
Median age (range), yrs	58 (23-76)	56 (22-76)	60 (20-82)
Age ≥ 65 years	24%	23%	33%
Lymphoma subtypes (N)	DLBCL (77), TFL (16), PMBCL (8)	DLBCL (88), TFL (21), other (2)	DLBCL (53), TFL (20)
Double-hit lymphoma	NR	27%	22%
≥ 3 lines of therapy	69%	52%	50%
Primary refractory	26%	NR	49%
Refractory to last therapy	64%	45%	67%
Prior ASCT	21%	49%	38%

*: data presented for CORE cohort only; #: 12 patients received nonconforming product; *ASCT: autologous stem cell transplantation; DLBCL: diffuse large B cell lymphoma; N: number; NR: not reported; PMBCL: primary mediastinal B cell lymphoma; TFL: transformed follicular lymphoma; yrs: years.*

B-cell non-Hodgkin lymphoma (NHL; more specifically diffuse large B-cell lymphoma [DLBCL] and primary mediastinal large B-cell lymphoma [PMBCL]). In addition to this, the potential of CAR T-cell therapy is also being explored in other haematological cancers, such as MM and CLL.

NON-HODGKIN LYMPHOMA

B-cell NHL is the most frequent haematological malignancy, with DLBCL being the most common subtype. Despite substantial refinements in chemo-immunotherapeutic treatment regimens for DLBCL, a substantial proportion of patients develops chemorefractory disease. Currently, approximately two-thirds of patients with newly diagnosed DLBCL are cured with first-line cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) therapy in combination with rituximab.[13] The standard of care second-line treatment for fit patients with relapsed/refractory DLBCL is salvage chemotherapy followed by autologous SCT (ASCT). Unfortunately, approximately half of the patients will remain refractory or experience a relapse after second-line treatment.[13] Relapsed/refractory DLBCL faces a grim prognosis; based on data from the SCHOLAR-1 study, a multicohort, retrospective study involving 636 patients with pooled data from two phase III studies (CORAL and LY.12) and two observational cohorts, the median overall survival (OS) for patients with relapsed/refractory DLBCL in only 6.3 months (95%CI: 5.9-7.0 months).[14] To overcome this chemo-refractoriness in DLBCL, several novel therapeutic strategies have been explored, including CAR T-cell therapy. Several early, single-centre studies demonstrated significant anti-lymphoma activity of CD19-directed CAR T-cell therapy in NHL patients and formed the basis for the design of three larger multicentre clinical trials.[15,16]

The phase II portion of the ZUMA-1 trial evaluated axicabtagene ciloleucel (axi-cel) in patients with refractory, high-grade B-cell lymphoma. In this study, no bridging therapy was allowed and the LD regimen consisted of cyclophosphamide and fludarabine. Patients in the trial were divided in two cohorts: cohort one included all DLBCL patients, while cohort two consisted of patients with PMBCL and transformed follicular lymphoma (TFL).[17] An overview of the patients' characteristics in this trial is depicted in *Table 1*. The primary endpoint in ZUMA-1 was overall response rate (ORR) in patients with more than 6 months

TABLE 2. CAR characteristics and main efficacy results of the three multicentre trials evaluating CD19 CAR T-cell therapy in r/r B cell non-Hodgkin lymphoma.

Characteristics	ZUMA-1 [17,18] (Axicabtagene ciloleucel)	JULIET [20] (Tisagenlecleucel)	TRANSCEND [26] (Lisocabtagene maraleucel)
Viral vector	Retrovirus	Lentivirus	Lentivirus
Co-stimulatory domain	CD28	4-1BB	4-1BB
Cell source	PBMCs (fresh)	PBMCs (cryopreserved)	CD4:CD8 (1:1)
CAR T dose	$2x10^6$ cells/kg	median $3x10^8$ CAR^+ cells	DL1: $5.0 x10^7$ cells DL2: $1.0 x10^8$ cells
LD regimen	Flu: 30mg/m² x3 days Cy: 500mg/m² x3 days	Flu: 25mg/m² x3 days Cy: 250mg/m² x3 days or B: 90mg/m² x2 days	Flu: 30mg/m² x3 days Cy: 300mg/m² x3 days
Median follow-up	27.1 mo	14.0 mo†	12.0 mo
N response-evaluable	101	93	73*
Best ORR (CR)	83% (58%)	52% (40%)	80% (59%)
Median DoR	11.1 mo (4.2 mo-n.e.)	Not reached (10.0 mo-n.e.)	Not reached (5.0 mo-n.e.)
Median PFS	5.9 mo (3.3-15.0 mo) 24-mo PFS rate for pts in CR at 3 mo: 72% (PR: 75%)	NR 12-mo PFS rate for pts in CR/PR at 3 mo: 83%	NR
Median OS	Not reached (12.8 mo-n.e.) Estimated 24-mo OS rate: 50.5%	12.0 mo (7.0 mo-n.e.)§ Estimated 12-mo OS rate: 49% (90% for pts in CR)	Not reached (10.7 mo-n.e.) Estimated 12-mo OS rate: 63% (89% for pts in CR)

†: from time of infusion to data cut-off; *: data presented for CORE cohort only; §: median OS reported for the infused population (N=111); *B: bendamustine; CR: complete response; PR: partial response; Cy: cyclophosphamide; DL: dose level; DoR: duration of response; Flu: fludarabine; LD: lymphodepletion; mo: months; N: number; n.e.: not estimable; NR: not reported; ORR: objective response rate; OS: overall survival; PBMCs: peripheral blood mononuclear cells; PFS: progression-free survival.*

follow-up after axi-cel infusion, as compared with historical control (SCHOLAR-114). In total, 111 patients were enrolled of whom 101 received axi-cel. More than two-thirds of the patients were refractory to at least three lines of therapy and 21% relapsed within 12 months after an ASCT. In the most recent report of this trial, with a median follow-up of 27.1 months, an ORR of 83% was demonstrated with a CR rate of 58%.[17] This represents an eightfold higher CR rate in comparison with SCHOLAR-1.[14,17,18] The median duration of response is still not reached for patients with a CR (95%CI: 12.9 months–not estimable), underscoring the durability of the responses to axi-cel.[17] A more detailed overview of the efficacy data in ZUMA-1 can be found in *Table 2*, with the Kaplan Meier curve for overall survival (OS) being depicted in *Figure 2*.[17]

The JULIET trial is a phase II multicentre global study in patients with refractory B-cell NHL utilizing the anti-CD19 CAR T-cell product tisagenlecleucel. Key eligibility criteria in JULIET included aggressive B-cell lymphoma (DLBCL or TFL), relapse after an ASCT, ineligibility for an ASCT, or refractory after two lines of therapy (*Table 1*). In contrast to ZUMA-1, cryopreserved apheresis products were utilized,

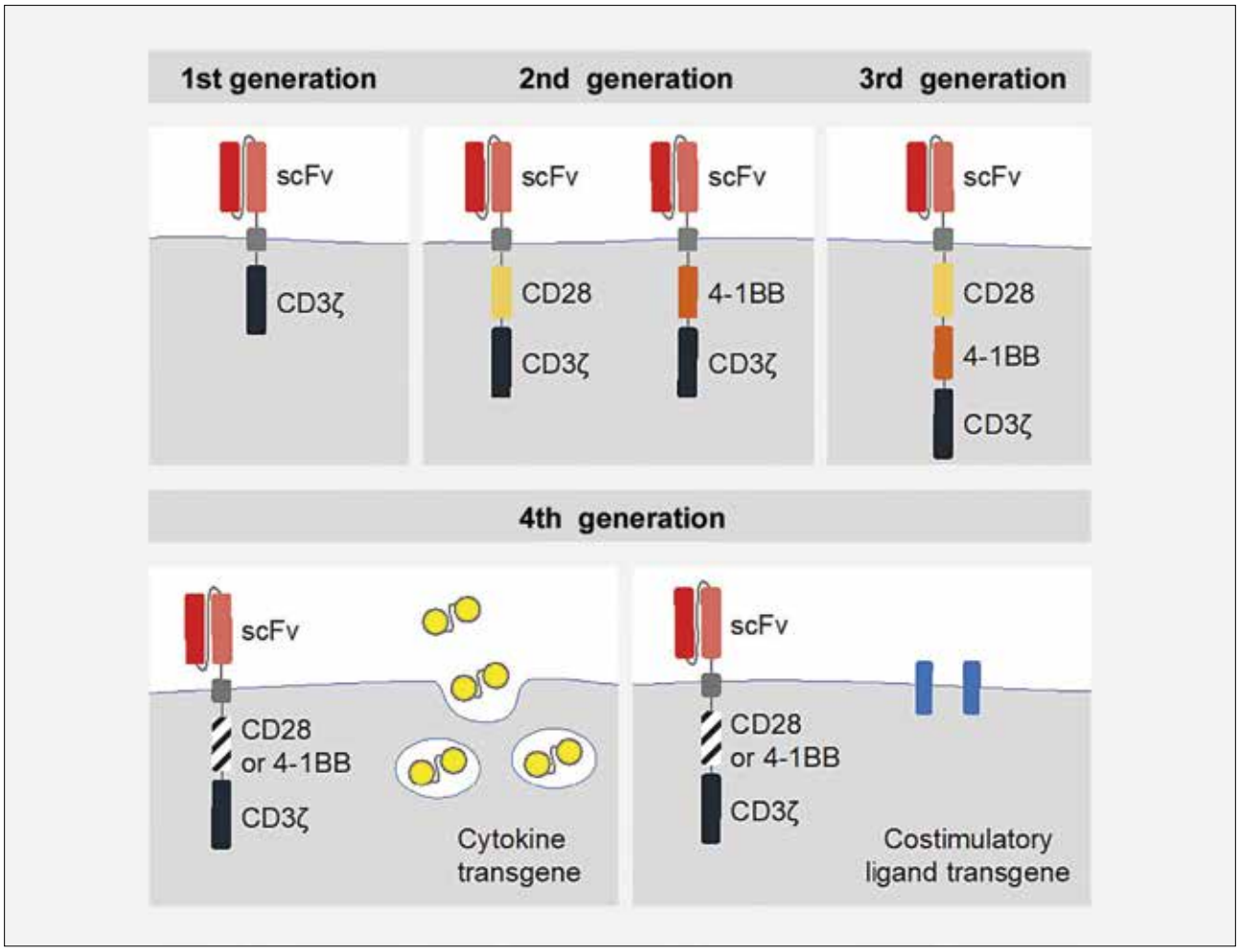

FIGURE 1. Evolution of CAR constructs (Adapted from Brentjens and Curran).[7]

and bridging chemotherapy was allowed for patients with rapidly progressive disease.[19] Overall, 92% of the patients received bridging chemotherapy. LD chemotherapy consisted of cyclophosphamide and fludarabine, or bendamustine. Similar to the ZUMA-1 trial, the primary endpoints of the trial were ORR and the rate of CR. A total of 165 patients were enrolled and 111 patients were infused with tisagenlecleucel. In JULIET, about half of the patients had refractory disease with at least three prior lines of therapy (including ASCT in 49% of the patients). In the 93 response-evaluable patients (at least 3 months of follow-up), the reported ORR and CR rates were 52% and 40%, respectively. More efficacy details are shown in *Table 2*; Kaplan Meier curve for OS is shown in *Figure 2*.[19,20]

Based on the promising results of ZUMA-1 and JULIET, the US FDA approved axi-cel and tisagenlecleucel for r/r DLBCL in October 2017 and May 2018, respectively. A couple of months later, both agents were also approved by the EMA. Tisagenlecleucel is reimbursed in Belgium since June 1st, 2019 for fit (performance status 0-1), adult patients with r/r DLBCL after a minimum of 2 prior lines of therapy. With the approval of axi-cel and tisagenlecleucel, interest in reporting the efficacy of this therapy in real clinical practice grew. "Real-world" data on the use of axi-cel were reported at ASH 2018 by *Nastoupil et al*. and others (excellently reviewed by *Viardot et al*. in reference 23).[21-23] Overall, 43% of the patients in the study by *Nastoupil et al*. did not meet the inclusion criteria of ZUMA-1. Moreover, 55% received bridging therapy whereas this was not allowed in ZUMA-1. Of the 294 leukapheresed patients, 274 were actually infused. Best ORR (81%) and CR (57%) rates were similar to those reported in ZUMA-1 (83% and 58%, respectively). This essentially confirms that the efficacy of axi-cel in r/r B-cell NHL (including DLBCL, TFL and PMBCL) could be replicated outside the strict eligibility criteria of clinical trials.[21-23]

In the multicentre TRANSCEND-001 study, the 4-1BB CAR T-cell construct lisocabtagene maraleucel (liso-cel) was evaluated in 134 patients with r/r B-cell NHL, including DLBCL, TFL, FL grade 3b, mantle cell lymphoma (MCL), DLBCL arising from marginal zone lymphoma (MZL) and

TABLE 3. Published clinical results of MM CAR T-cell clinical trials targeting BCMA.[31-36]

Car T cell product	N	ORR (N)	Median PFS (95%CI)
bb2121 [31]	33	85% (28)	11.8 months (6.2-n.e.)
CAR T-BCMA UPenn [32]	25	48% (12)	2.0 months (ND)
NCI CAR BCMA-T [33]	10	20% (2)	1.5 months (ND)
NCI CAR BCMA-T [34]	16	81% (13)	7.25 months (ND)
LCAR-B38M [35]	17	88% (15)	12.2 months (ND)
LCAR-B38M [36]	57	88% (50)	15.0 months (11.0-n.e.)

n.e.: not estimable; ND: no data; PFS: progression-free survival; ORR: objective response rate.

PMBCL.[24-26] In total, 114 patients were infused in this trial, but 12 patients received a nonconforming product resulting in 102 evaluable patients. In the CORE sub-cohort, which only included the r/r DLBCL and TFL patients (N=73), the median age was 60 years and at least 50% of the patients were refractory to three or more lines of therapy (38% failed a prior ASCT) (*Table 1*). At a median follow up of 12 months, the best ORR and CR rates in the CORE sub-cohort were 80% and 59%, respectively.[26] The efficacy data are summarized in *Table 2*.

B-CELL PRECURSOR ALL

The phase II ELIANA trial investigated the CD19-directed genetically modified autologous T-cell product tisagenlecleucel as a single infusion for r/r paediatric and young adult B-cell ALL. From the 107 patients who were screened, 92 were enrolled; 17 patients could not be infused for a variety of reasons: death (N=7), serious adverse events (N=3) or CAR T-cell production failure (N=7). Of the 75 patients who received tisagenlecleucel, 65 (87%) received bridging chemotherapy between enrolment and infusion, and 72 (96%) received LD chemotherapy (mostly fludarabine plus cyclophosphamide). Patients in the study received a median of 3 prior therapies, and 61% of patients previously underwent an alloSCT. The overall remission rate within 3 months (CR/CRi) was reported at 81% and the median duration of the remission was not reached at a median follow-up of 1 year. All patients with a treatment response were negative for minimal residual disease (MRD). The event-free survival (EFS) and OS rates at 6 months were 73% and 90%, respectively, dropping to 50% and 76% at the 1-year landmark.[27] Long-term *in vivo* persistence was demonstrated. All patients with a response to treatment had B-cell aplasia, and most patients in the study received immunoglobulin replacement in accordance with local practice. Grade 3/4 adverse events (AEs) with a suspected relation to tisagenlecleucel occurred in 73% of patients. Cytokine release syndrome (CRS) occurred in 77% of patients, of whom 48% received the IL-6 blocker tocilizumab. Neurologic events occurred in 40% of patients; all these events occurred within the first 2 months.[27]

In August 2018, the EMA approved tisagenlecleucel for the treatment of paediatric and young adult patients up to 25 years of age with B-cell ALL that is refractory, in relapse after alloSCT or in second or later relapse. Tisagenlecleucel is reimbursed for this indication in Belgium since June 1st, 2019.

MULTIPLE MYELOMA

Multiple myeloma is a haematological cancer formed by malignant plasma cells. Over the last decade, we have witnessed enormous progress in the treatment of MM, but despite these advances, the disease remains incurable. Therefore, the development of new therapeutic drugs is needed, and CAR T-cell therapy is considered promising. B-cell maturation antigen (BCMA) is the most widely used target antigen in CAR T-cell studies for MM.[28-30] BCMA expression is largely restricted to (malignant) plasma cells and some mature B cells.[31,32] BCMA appears to play an important role in the promotion of MM cell survival, proliferation, and was also found to be involved in the development of drug resistance.[33] As shown in *Table 3*, BCMA CAR T-cell therapy produces an ORR of up to 88%. However, the observed therapeutic effect was often transient and relapses are frequently observed. Over the different CAR T-cell trials in MM, the median PFS observed with BCMA CAR T-cell

therapy is in the range of 12 months.[34-39] Downregulation or loss of BCMA expression is likely an important mechanism underlying these relapses.[40] Therefore, alternatives for BCMA are now under intensive investigation in the field of CAR T-cell therapy for MM. In light of this, small studies evaluating CAR T-cell therapies directed against CD19 or CD138 yielded varying results.[41,42] However, whether or not CAR T-cell therapy will ultimately revolutionise the treatment of MM will largely depend on how we will be able to improve response durability. One promising strategy in this respect, consists of dual antigen targeting, for example by combining BCMA and CD19 CAR T-cells.[43] CD19 is a rather unconventional target antigen in MM, because myeloma cells are mostly CD19-negative by flow cytometry. Nevertheless, more sensitive techniques have recently revealed that CD19 is expressed at ultra-low levels on MM cells, and that these levels are sufficient for recognition of MM cells by CD19 CAR T-cells.[44] Moreover, it appears that CD19+ MM cells bear features of a cancer stem cell (i.e. self-renewal and drug resistance), making it an attractive target for immunotherapy.[45] The results of an ongoing randomised study comparing BCMA/CD19 CAR-T cells with BCMA CAR-T cells alone (NCT03549442) are eagerly awaited. Another strategy to avoid BCMA relapses involves the combination of BCMA CAR T-cells with gamma-secretase inhibitors which prevent cleavage of BCMA from the MM cell surface.[46] In addition to this, other studies are looking into the potential of CAR T-cell therapies targeting other antigens, including CD38, SLAMF7, CD44v6, CD56, GPRC5D, amongst other.[47]

CHRONIC LYMPHOCYTIC LEUKAEMIA

CLL was one of the first diseases in which CAR T-cells were used. Since the first report of the efficacy of second-generation CAR T-cells against CLL in 2011, results have been reported of CD19-targeted CAR T-cell therapy in a total of 134 CLL patients.[48,49] Overall, the CLL patients who were treated with CAR T-cell therapy had a particularly poor prognosis, with most of them being in relapse after a large number of treatment lines. In total, 74 of the 108 patients evaluated patients in these studies (68.5%) had p53 alterations, and 41 of the evaluated 70 patients (58.6%) had a complex karyotype.[49] A second observation from the different CAR T-cell reports in CLL is that the efficacy is lower for CLL than for B-ALL and DLBCL: a complete response (CR), according to the IWCLL criteria, was obtained in only a minority (20–30%) of patients with an estimated 18-month PFS of 25%.[50-52] Interestingly, responses appeared to be weaker in the lymph nodes than in the bone marrow and blood. In fact, in some series, a substantial proportion of patients treated with CAR T-cells obtained undetectable MRD in the bone marrow.[51,53,54] For example, in a study by *Turtle et al.* including 24 r/r CLL patients who previously received ibrutinib, an ORR of 71% (21% CR) was reported four weeks after the CAR T-cell infusion, with bone marrow negativity in 58%. Among these MRD negative patients, the PFS and OS rate was almost 100% at a median follow-up of 6.6 months.[51]

The lower efficacy of CAR T-cells in CLL may be partly due T-cell exhaustion in CLL patients resulting in decreased CAR T-cell functionality.[55] To overcome this, several research groups are looking into ways to optimise the CAR constructs in CLL. In addition to this, studies are underway looking into the potential of combining CAR T-cell therapy with other anti-CLL therapies. In this respect, data suggest that ibrutinib may improve the outcome in CLL patients receiving CAR T-cells. In fact, results from two different series (N=19 for both) receiving injections of structurally different CAR T-cells, in combination with ibrutinib demonstrated MRD negative bone marrow responses in more than 90% of the patients.[53,54] Based on these observations, a prospective study will further evaluate the efficacy of ibrutinib maintenance at the time of injection of the CAR T-cell therapy (NCT03331198).

MANAGING CAR T-CELL TOXICITY

The most common acute toxicities observed after CAR T-cell therapy are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS, previously termed CAR T-cell-related encephalopathy syndrome [CRES]), either of which can be lethal. Other, less common, acute toxicities include tumour lysis syndrome and macrophage activation syndrome, but these will be outside the scope of this review.

CYTOKINE RELEASE SYNDROME

CRS is caused by cytokine elevations as a result of immune activation of large numbers of lymphocytes. Interleukin-6 (IL-6) has been implicated as a central mediator of toxicity in CRS.[56] The predictive value of various biomarkers (e.g., high serum levels of IL-6, soluble gp130, IFN-γ, IL-15, IL-8, and/or IL-10) has been studied, but this seems to vary depending on the type of CAR T-cell product used. The cytokine release pattern after CAR T-cell administration appears to be product-specific as well as patient-dependent but usually peaks within the first 2 weeks.[57-59] The cardinal symptoms of CRS include fever, hypotension and hypoxaemia. The median time to onset of CRS was 2-3 days with tisagenlecleucel and axi-cel.[60] The incidence and severity of CRS is greater in heavily pre-treated patients and in those with higher disease burden at the start of therapy (especially

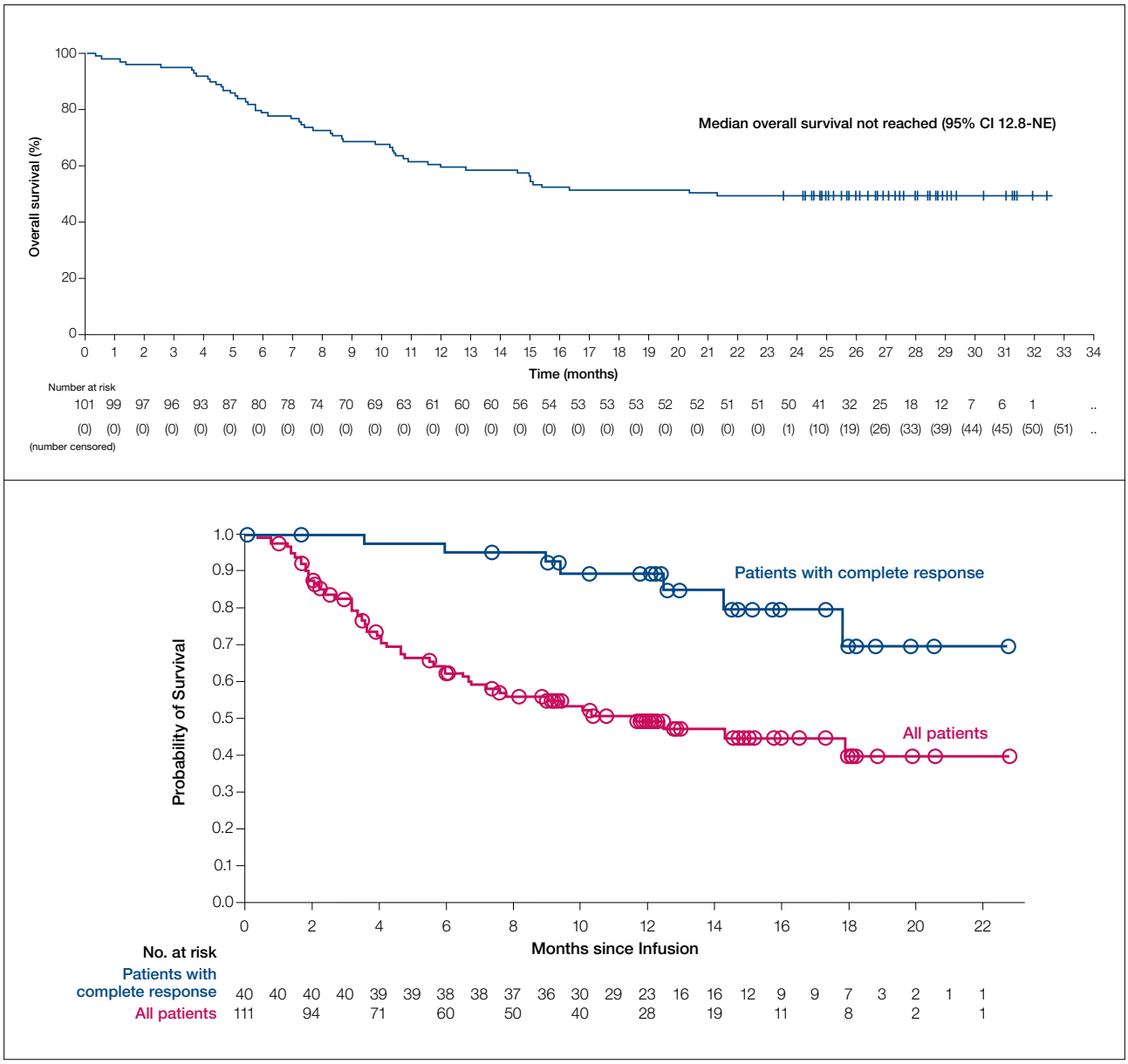

FIGURE 2. Overall survival in ZUMA-1 **(top)** and JULIET **(bottom)**. Both survival curves seem to plateau at 40% from month 20 onwards.[17,20]

in ALL).[57] Tocilizumab, a therapeutic antibody blocking IL-6 receptors, has become the drug of choice for the management of moderate to severe CRS. It induces near-immediate reversal of CRS symptoms in most patients. Importantly, tocilizumab does not seem to affect the efficacy of CAR T-cell therapy in terms of ORR, CR rate, or the durability of responses.[58,27] In ZUMA-1 (axi-cel), JULIET (tisagenlecleucel) and TRANSCEND (liso-cel), tocilizumab was used in 43%, 14% and 17% of the patients, respectively.[17-20,24-26] In the real world, tocilizumab is far more frequently used (in 63% of the cases in the study with axi-cel by *Nastoupil et al.*).[21-23] Until recently, corticosteroids were generally considered only when the toxicities of CAR T-cell therapy are refractory to anti-IL-6 therapy due to concerns regarding their suppressive action on T-cell function.[52] However, it is becoming increasingly clear that corticosteroids can be used safely to treat CAR T-cell-related toxicities without limiting efficacy. This statement is further strengthened by the real-world data on the use of axi-cel in r/r B-cell NHL (i.e. similar efficacy in ZUMA-1 and real-world study by *Nastoupil et al.*, despite the proportionally higher use of corticosteroids to treat CRS [55% *vs.* 27% in ZUMA-1]).[21,23]

In recent years, guidelines for the uniform grading of CRS have been published, of which the guidelines by the American Society for Transplantation and Cellular Therapy

(ASTCT) have become the most widely adopted.[61] CRS is graded with a score of 1 (mild) to 4 (life-threatening).[61] In ZUMA-1, JULIET and TRANSCEND, the incidence of grade ≥3 CRS was 11%, 22% and 1%, respectively.[17-20,24-26] In the real-world study by *Nastoupil et al*., 7% of the patients developed severe CRS.[21,23]

IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXICITY SYNDROME

Neurotoxicity, termed ICANS or CRES, is the second most common serious adverse reaction after administration of CAR T-cell therapy. Affected patients develop toxic encephalopathy with confusion, aphasia, ataxia, delirium, seizures, and cerebral oedema. The causative pathophysiology of these neurological side effects is still not fully understood. IL-6 does not seem to play an important role in ICANS/CRES; in mouse models, it was elegantly shown that anti-IL-6 therapy with tocilizumab did not have a major impact on the development and evolution of ICANS/CRES.[62] Nevertheless, tocilizumab will often be used, especially if the neurotoxicity co-occurs with CRS. Otherwise, corticosteroids are the preferred treatment or, if available, the IL-1 blocker anakinra. The severity of ICANS can fluctuate rapidly, necessitating close patient monitoring. This is especially important for the very rare, but life-threatening cerebral oedema, for which anti-IL-6 therapy is not effective.[58] Similar to CRS, management of ICANS is based on the severity of the neurological symptoms. The 10-point "Immune Effector Cell-Associated Encephalopathy (ICE)" scoring tool is now the gold standard for screening and grading of ICANS.[61] Grade ≥3 neurotoxicity appears to be more common with axi-cel (32% in ZUMA-1 and 33% in *Nastoupil et al.*), as compared to tisagenlecleucel (12% in JULIET) and liso-cel (13% in TRANCEND).[17-26]

OTHER CAR T-CELL-ASSOCIATED ADVERSE EVENTS

CD19 CAR T-cell therapies can result in short or long-term B-cell aplasia, which is also a marker of functional persistence of the CAR T-cells. While short-term B-cell aplasia may not require treatment, persistent B-cell aplasia may require immunoglobulin replacement, especially in children in case they develop infections.

CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, CAR T-cell therapy has revolutionised the treatment of r/r B-cell malignancies. Clinical trials with these agents have demonstrated encouraging therapeutic activity in patients with r/r B-ALL and r/r B-cell NHL (DLBCL, PMBCL, TFL). These initial successes provide a foundation to also develop this treatment strategy in other cancers types, such as MM or CLL. However, the intense immune activation induced by CAR T-cell therapy may also result in severe adverse reactions, which need to be managed appropriately to allow successful clinical use of CAR T-cells. Also, there remain several unresolved issues related to the characterisation of effective T-cell subtypes, number of cells to be infused, and predictive markers of toxicity and resistance. Relapses are common and the focus must now be placed on unravelling the mechanisms of disease relapse after CAR T-cell therapy and on developing treatment strategies for these patients. Possible strategies to improve long-term efficacy include the combined use with immune checkpoint inhibitors,[63] or by multi-targeted CAR T-cell approaches.[64] Finally, the financial burden of CAR T-cell therapy is huge, which is an important challenge in times of ever-increasing pressure on healthcare budgets. Nevertheless, despite these remaining challenges, it is clear that CAR T-cell therapy represents a very promising new therapeutic modality for a variety of haematological malignancies.

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