



European Myeloma Network perspective on CAR T-cell therapies for multiple myeloma

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ABSTRACT

Chimeric antigen receptor (CAR) T cells (CAR-T) have dramatically changed the treatment landscape of B-cell malignancies, providing a potential cure for relapsed/refractory patients. Long-term responses in patients with acute lymphoblastic leukemia and non-Hodgkin lymphomas have encouraged further development in myeloma. In particular, B-cell maturation antigen (BCMA)-targeted CAR-T have established very promising results in heavily pre-treated patients. Moreover, CAR-T targeting other antigens (*i.e.*, SLAMF7 and CD44v6) are currently under investigation. However, none of these current autologous therapies have been approved, and despite high overall response rates across studies, main issues such as long-term outcome, toxicities, treatment resistance, and management of complications limit as yet their widespread use. Here, we critically review the most important pre-clinical and clinical findings, recent advances in CAR-T against myeloma, as well as discoveries in the biology of a still incurable disease, that, all together, will further improve safety and efficacy in relapsed/refractory patients, urgently in need of novel treatment options.

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Introduction

Recently engineered chimeric antigen receptors (CAR) have greatly increased anti-tumor effects of CAR T cells (CAR-T). Impressive results have been observed with CD19-directed CAR-T in B-cell lymphoproliferative disorders.¹⁻³ In addition, several CAR-T products have been developed for the treatment of multiple myeloma (MM). None has yet been approved, and, despite high overall response (OR) across studies, main issues such as long-term outcomes, toxicities and complications need to be solved to allow their widespread clinical use. In this review, the European Myeloma Network (EMN) group aimed to describe the most important pre-clinical and clinical findings, and recent advances in CAR-T technology against MM that may improve their safety and efficacy. Contents, comments and sugges-

tions have been incorporated in the manuscript after at least three rounds of discussion resulting in the final unanimously approved version.

Target antigens

CAR are artificial fusion proteins with a modular design that confer antigen-specificity to T cells in an human leukocyte antigen (HLA)-independent manner providing intracellular stimulatory signals to enhance survival, proliferation, cytolytic capacity and cytokine production of T cells.⁴ Figure 1 illustrates the components of CAR constructs.⁵⁻⁸ For successful CAR-T therapy, identification of suitable tumor antigens is crucial, since it requires a delicate balance between effectiveness and safety considerations. Ideal antigens should be: (i) highly and homogeneously expressed on tumor cell surface, (ii) expressed at different disease stages, (iii) pivotal in disease pathophysiology, (iv) limited or not shed into the bloodstream, (v) not affected by selective treatment pressure that may cause down-regulation or elimination, and (vi) not

expressed on normal tissues.⁹⁻¹⁰ Great progress has been made to identify potential molecules as CAR targets in MM. In this section, we summarize pre-clinical data on the most relevant MM-associated antigens, while a comprehensive overview is provided in Table 1.

B-cell maturation antigen

The B-cell maturation antigen (*BCMA*) gene is located on chromosome 16 and the *BCMA* (aliases: CD269, TNFRSF17) protein, a transmembrane glycoprotein member of the tumor necrosis factor receptor (TNFR) superfamily, is expressed on subsets of B cells (plasmablasts and plasma cells) and up-regulated during B-cell differentiation. It is not expressed on solid organ tissues, hematopoietic cells or naïve B cells.¹¹⁻¹² Along with two associated receptors (calcium modulator and cyclophilin ligand interactor [TACI] and B-cell activation factor receptor [BAFF-R]) and its ligands (a proliferation inducing ligand [APRIL] and B-cell activating factor [BAFF]) *BCMA* regulates maturation, differentiation, and promotes B-cell survival.¹³⁻¹⁵

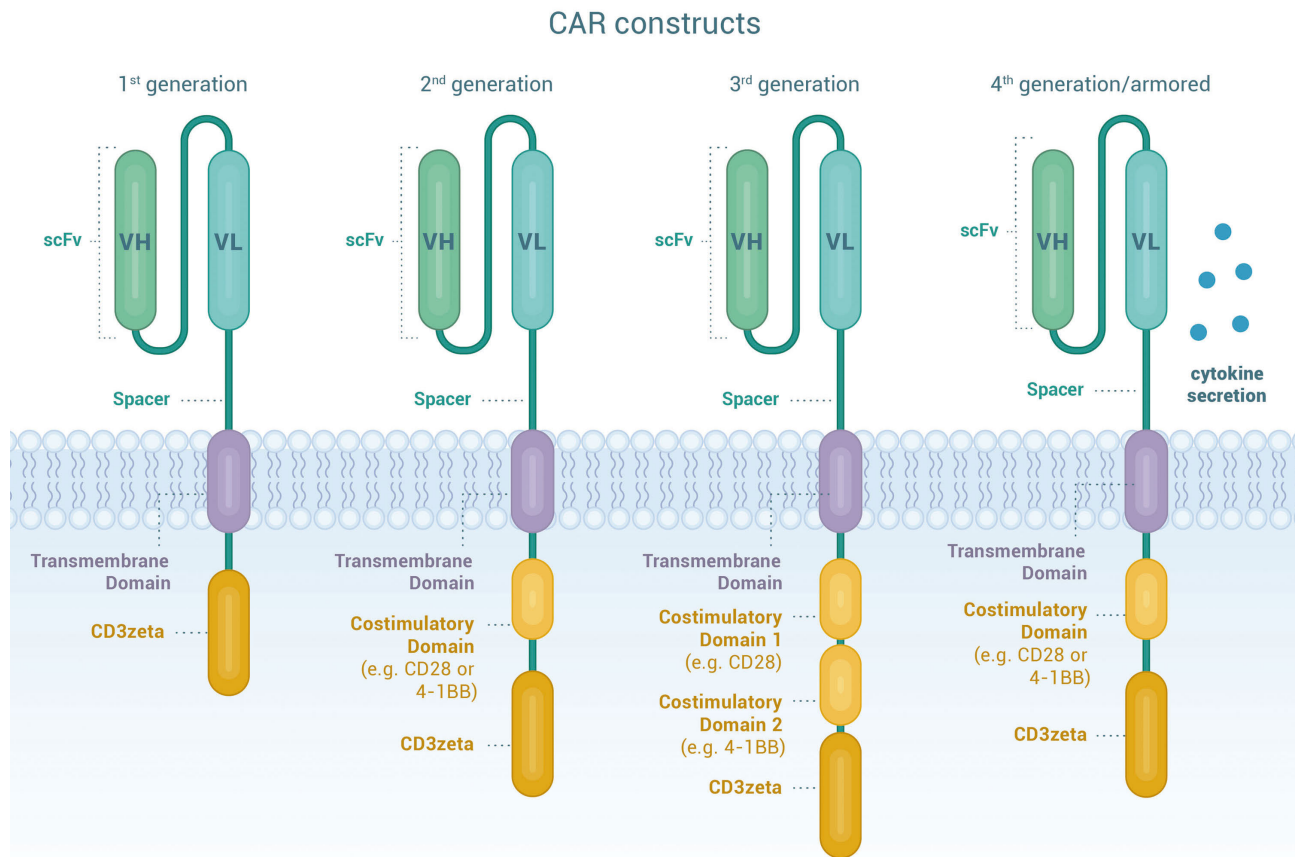


Figure 1. Chimeric antigen receptor T cells. Chimeric antigen receptors (CAR) are designer proteins that redirect T cells towards a defined surface antigen on tumor cells. The CAR construct contains four essential components. The extracellular antigen recognition domain consists of a single chain variable fragment (scFv) commonly derived from the variable domains of the heavy and light chains (VH and VL) of monoclonal antibodies joined by a linker to provide flexibility and solubility and therefore improve antigen recognition and binding capacity. The hinge or spacer moiety based on Ig- (IgG1 or IgG4), CD8- or CD28-derived domains, provides flexibility, stability and the suitable length for optimal access to the target antigen. The transmembrane domain links the extracellular and intracellular domains of the CAR. It is based on CD3 ζ , CD4, CD8 α , CD28 or ICOS moieties, influences CAR stability and signaling and may also be involved in immune synapse arrangement. The last components of the CAR construct are the intracellular signaling domains. The activation domain is typically derived from the CD3 ζ moiety of the T-cell receptor (first generation CAR), whereas co-stimulatory domains are derived from CD28, 4-1BB, OX40, CD27, or ICOS (second and third generation CAR). Co-stimulation results in intracellular signals that further optimize T-cell function, persistence and proliferation. Through additional genetic modifications, so called "armored" CAR T cells (CAR-T) (fourth generation CAR) secrete cytokines or express ligands to bolster CAR-T function or to overcome the immunosuppressive tumor microenvironment. Taken together, the molecular fine-tuning of pre-existing CAR components can greatly improve cellular migration, foster expansion and persistence of the CAR-T and decrease toxicity.

Expression of BCMA in malignant plasma cells is enhanced compared to non-malignant cells, though levels are not homogeneous. Its expression is associated with proliferation and survival of tumor cells and contributes to the immunosuppressive bone marrow (BM) microenvironment.¹⁵⁻¹⁷ BCMA cleavage by γ -secretase sheds soluble BCMA (sBCMA) into the bloodstream.¹⁸ sBCMA may play a role in myeloma pathogenesis, and high sBCMA levels have been associated to worse prognosis.¹⁹ BCMA is currently considered the most compelling antigen for targeted immunotherapy. Carpenter *et al.* reported on the first proof-of-concept using a second generation, CD28 co-stimulated CAR against BCMA in the preclinical setting. BCMA CAR-T specifically recognized the antigen, eradicated *in vivo* tumors and killed primary myeloma cells,¹¹ setting the cornerstone for the first-in-human phase I clinical trial evaluating BCMA CAR-T in MM.²⁰

Transmembrane activator, calcium modulator, and cyclophilin ligand interactor

TACI (TNFRSF13B) is a transmembrane protein that

recognizes ligands APRIL, BAFF and calcium modulator and cyclophilin ligand (CAML). It is expressed on subsets of naïve and memory B cells, plasma cells, non-germinal center cells, monocytes and dendritic cells. TACI supports growth and survival in myeloma cells, though its expression is lower compared to BCMA.²¹⁻²³ A third-generation APRIL-based CAR recognizing both BCMA and TACI antigens has been engineered. Though this construct demonstrated tumor control in an *in vivo* model of tumor escape with BCMA⁺ TACI⁺ cells,²² the AUTO2 trial (clinicaltrials.gov. Identifier: NCT03287804) was, however terminated because of lack of efficacy.²⁴

CD19

In most B-cell malignancies, CD19 is highly and uniformly expressed.²⁵⁻²⁹ MM was traditionally considered mostly CD19 negative with low level CD19 expression attributed to a putative “myeloma stem cell”. However, highly sensitive direct stochastic optical reconstruction microscopy (dSTORM) unveiled expression of CD19 on a considerable subset (10–80%) of myeloma cells in more than two thirds of patients, of whom only one fifth was considered CD19 positive by conventional flow cytometry.

Table 1. Potential target antigens for CAR-T therapy for multiple myeloma.

Antigen	Expression in MM	Expression in normal hematopoietic cells	Expression in healthy solid organ tissues	Development state
BCMA	60-100%	Late memory B cells, plasma cells	No	Clinical trial
TACI	78%	Naïve and memory B cells, plasma cells, monocytes and dendritic cells	No	Clinical trial
CD19	10-80%	B-cells, plasma cells	No	Clinical trial
SLAMF7 (CD319)	High and uniform expression	NK-cells, monocytes, macrophages, dendritic cells, T cells, B cells, plasma cells	No	Clinical trial
CD38	High and uniform expression	Lymphoid and myeloid cells, hematopoietic precursors, thymocytes	Prostatic epithelium, pancreatic islet cells, cerebellar Purkinje cells	Clinical trial
CD44v6	43% in advanced stage	Activated T cells, monocytes	Keratinocytes	Clinical trial
GPRC5D	≥50% in 65% of patients	B-cells, plasma cells	Hair follicles	Clinical trial
CD138	High expression	Plasma cells	Epithelial cells, gastrointestinal tract and hepatocytes	Clinical trial
NKG2D	Heterogenous	NK, T and $\gamma\delta$ T cells	No	Clinical trial
κ light chain	κ -restricted myeloma cells	Mature B cells	No	Clinical trial
CD56	High expression, decreased in extramedullary disease	T and NK cells	Central and peripheral nervous system	Clinical trial
Lewis Y	50%	No	Epithelial cells	Clinical trial
NY-ESO-1	60-100%	No	No	Clinical trial
CD229 (SLAMF3)	High and homogeneous expression, probably in myeloma stem cell	T, NK and B cells	No	Preclinical investigation
Integrin β 7	High expression	High expression in B cells and low to moderate expression in CD34 ⁺ hematopoietic cells	No	Preclinical investigation
CD70	0.2-42%	Activated T and B cells, dendritic cells and plasma cells	No	Preclinical investigation
CD1d	High expression	Antigen-presenting cells, thymocytes, B cells, and hematopoietic stem cells	Epithelial cells	Preclinical investigation

BCMA: B-cell maturation antigen; GPRC5D: G protein-coupled receptor class C group 5 member D; NKG2D: Natural Killer Group 2 member D; NY-ESO-1: New York Esophageal Squamous Cell Carcinoma 1; SLAMF3 and SLAMF7: signaling lymphocytic activation molecules family member 3 and 7; TACI: Transmembrane activator, calcium modulator, and cyclophilin ligand interactor.

try. As CAR-T can eliminate cells expressing less than 100 target antigens/cell, CD19 has become a relevant CAR target antigen. In preclinical models, BCMA-CD19 bispecific CAR-T eliminated myeloma cell lines more potently than BCMA- or CD19-directed CAR-T alone.³⁰ Due to an off-target expression limited to B cells, toxicity concerns of (co-)targeting CD19 are limited and clinical evaluation of bispecific CAR-T is ongoing (clinicaltrials.gov. Identifier: NCT03455972, NCT03549442).

SLAMF7

The elotuzumab target antigen signaling lymphocytic activation molecule (SLAM) family member 7 (SLAMF7, aliases: CD319, CS-1, CRACC) is an immunomodulatory transmembrane receptor, initially identified on the surface of natural killer (NK) cells.³¹ It is expressed on a variety of other innate immune cells,³² but also T cells, B cells and plasma cells.³¹⁻³³ Importantly, SLAMF7 is expressed on aberrant plasma cells and its precursor³⁴ and confers homing of the myeloma cells to the BM niche. While redirecting T cells against a self-antigen may appear difficult, preclinical experiments demonstrated that it is feasible to generate clinically relevant doses of SLAMF7-directed CAR-T, with or without additional inactivation of the endogenous *SLAMF7* gene.³³⁻³⁵ In preclinical models, potent anti-myeloma activity was demonstrated, resulting in rapid, comprehensive and sustained cell depletion.³³ SLAMF7-directed CAR-T eliminated SLAMF7 positive lymphocytes *in vitro*, while SLAMF7 negative lymphocytes were spared and retained their functions.³³ Clinical evaluation of SLAMF7 CAR-T with functional safety switches is currently ongoing (clinicaltrials.gov. Identifier: NCT03958656, EudraCT Nr.2019-001264-30).

CD38

Successfully targeting CD38 (cyclic ADP ribose hydrolase, ADPRC1) with daratumumab and isatuximab has led to the development of anti-CD38 CAR-T. CD38 is a transmembrane glycoprotein that functions as an ectoenzyme, adhesion molecule and regulator of migration and signaling. It is expressed on malignant plasma cells,³⁶ but low expression can be found on lymphoid and myeloid cells, hematopoietic precursors,³⁷ thymocytes, cerebellar Purkinje cells and other tissues. CD38 is an activation marker of T cells at intermediate or late activation stages. As CD38-directed CAR-T demonstrated great antigen-specific efficacy in preclinical myeloma models,³⁸ affinity modification of the CAR was developed as an approach to mitigate on-target, off-tumor toxicity towards other CD38 positive hematopoietic cells. Affinity reduction of the antigen binding domain by a factor of 1,000 enabled selective elimination of myeloma cells with high CD38 expression while sparing normal cells with less pronounced CD38 expression. However, it has been reported that levels of CD38 expression on myeloma cells can decline over the disease course.³⁹ In this regard, agents that induce selective modulation of CD38 expression levels, such as all-trans retinoic acid (ATRA) or histone deacetylase (HDAC) inhibitors,⁴⁰ represent a promising group for combination therapy with CD38-directed CAR-T. In order to address the issue of antigen reduction by increasing the potency of the cell product, a novel construct termed "dimeric antigen receptor" (DAR) was developed. In fact, the DAR T cells that incorporate a fragment antigen-bind-

ing (Fab) moiety instead of the single chain variable fragment (scFv), demonstrated superior preclinical activity. However, their clinical relevance, and the risk of on-target, off-tumor effects, remains to be determined.

CD44v6

CD44 glycoproteins are encoded by a highly conserved gene,⁴¹ but are nevertheless characterized by considerable protein heterogeneity due to post-transcriptional modifications or splicing variants. While CD44 plays a role in physiological processes and is expressed on healthy tissues,⁴² the isoform variant 6 is relatively restricted to malignant cells^{43,44} including plasma cells. In healthy tissues, CD44v6 expression is limited to skin keratinocytes. It is absent on hematopoietic precursor cells, but low level expression can be found on activated T cells and monocytes. While the development of an anti-CD44v6 immunoconjugate was discontinued due to severe skin toxicities,⁴⁵ preclinical investigation of CD44v6-directed CAR-T showed promising efficacy with no impact on keratinocytes that represent potentially immune-privileged sites.⁴⁶ The clinical relevance of the observed monocyte depletion remains unclear. However, as monocyte-derived cytokines play a relevant role for the pathogenesis of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), a beneficial effect is possible and clinical evaluation is ongoing (clinicaltrials.gov. Identifier: NCT04097301).

GPRC5D

The orphan G protein-coupled receptor, class C group 5 member D (GPRC5D), is expressed ubiquitously in malignant bone marrow plasma cells (500 to 1,000 times higher expression than on normal cells), hair follicles, and in the lung. CAR-T targeting GPRC5D have demonstrated promising preclinical activity, and a phase I clinical trial is ongoing (clinicaltrials.gov. Identifier: NCT04555551).

CD229

CD229, a SLAM family receptor ("SALM3"), is generally expressed on myeloma cells and "precursor" myeloma cells. Its high expression suggests a potential as a target for CAR-T studies have shown that this newly designed CD229 CAR-T has high activity against MM cells, memory B cells and MM stem cells *in vitro* and *in vivo*.⁴⁷

Clinical studies

B-cell maturation antigen-specific chimeric antigen receptor T cells

The National Cancer Institute group performed the first study with BCMA-specific CAR-T with a CD28 costimulatory domain (murine scFv) in heavily pretreated patients.²⁰ At the highest CAR-T dose (9×10^6 cells/kg), 13 of 16 patients (81%) achieved at least partial response (PR) with a median event-free survival of 31 weeks. Other studies confirmed high activity of BCMA CAR-T in this patient subset.⁴⁸⁻⁵⁰ Advanced clinical findings have been reported with ide-cel^{50,51} and cilta-cel.⁴⁸ Both therapies received Food and Drug Administration breakthrough designation. In this section, we will discuss these two CAR-T products. CAR-T constructs and main clinical characteristics are summarized in Table 2.

Ide-cel (bb2121)

The first-in-man study with ide-cel (CRB-401) evaluated escalating doses of CAR-T (50×10^6 , 150×10^6 , 450×10^6 , or 800×10^6 in the dose-escalation phase, and 150×10^6 - 450×10^6 in the expansion phase) in extensively pretreated MM (median of six prior therapy lines; 69% triple-class refractory).⁵¹ Sixty-two patients were enrolled. At least PR was achieved by 76% of patients including complete response (CR) in 39%. All 15 patients with \geq CR who had an assessment for minimal residual disease (MRD) were MRD-negative at the level of 10^{-5} . Baseline BCMA expression or sBMCA levels did not affect response. There was a trend towards lower response in patients who received

$\leq 150 \times 10^6$ CAR-T, in those with less *in vivo* CAR-T expansion, and in those with high-risk cytogenetic abnormalities. Median progression free survival (PFS) was 8.8 months for all patients, and 9.0 months for those who received 450×10^6 CAR-T. Median overall survival (OS) was 34.2 months. Based on these promising results of the phase I trial, a second trial (KarMMA, phase II study) was initiated to evaluate the value of ide-cel in larger numbers of patients who were previously exposed to immunomodulatory drugs (IMiD), a proteasome inhibitor, and a CD38 antibody.⁵⁰ In this study 140 patients were enrolled with a manufacturing success of 99%; 128 of 140 (91%) received CAR-T, whereby 88% received bridging therapy prior to

Table 2. Ide-cel and Cilta-cel: CAR-T and clinical characteristics

	Ide-cel (bb2121) / KarMMA (phase II)	Cilta-cel (JNJ-4528) / CARTITUDE-1 (phase IB / II)
Antigen-binding domain	scFv (murine)	Bispecific variable fragments of llama heavy-chain antibodies; two distinct BCMA epitopes are targeted
Signaling domains	CD3 ζ /4-1BB	CD3 ζ /4-1BB
Vector	Lentiviral	Lentiviral
Other features	Bb21217 uses the same CAR construct as used for ide-cel. During <i>ex vivo</i> culture a PI3K inhibitor is added to enrich for CAR-T with memory-like phenotype	Bi-epitope BCMA binding confers high avidity binding
Lymphodepletion	Flu/Cy	Flu/Cy
CAR-T dose	150-450 $\times 10^6$	Median dose: 0.71 $\times 10^6$ /kg
Number of patients	128 (140 patients underwent leukapheresis)	Data presented for first 97 (113 patients were enrolled/apheresed)
Bridging therapy (%)	88	65
Number of prior therapies (median)	6	6
Triple-class refractory (%)	84	88
High-risk cytogenetics (del(17p), t(4;14), or t(14;16) (%))		35 24
Extramedullary disease (%)	39	13
\geq PR	150-450 $\times 10^6$: 73% 150 $\times 10^6$: 50% 300 $\times 10^6$: 69% 450 $\times 10^6$: 82%	97%
\geq CR	150-450 $\times 10^6$: 33% 150 $\times 10^6$: 25% 300 $\times 10^6$: 29% 450 $\times 10^6$: 39%	67%
Median PFS	150-450 $\times 10^6$: 8.8 months 150 $\times 10^6$: 2.8 months 300 $\times 10^6$: 5.8 months 450 $\times 10^6$: 12.1 months	Median PFS: Not reached; 12-month PFS rate: 77%
CRS (all grades) (%)	84	95
CRS (grade ≥ 3) (%)	5	4
Median time to CRS onset (any grade) (days)	1	7
Median duration of CRS (any grade) (days)	5	4
Neurotoxicity (all grades) (%)	18	21 (ICANS: 17%; other neurotoxicity*: 12%)
Neurotoxicity (grade ≥ 3) (%)	3	10 (ICANS: 2%; other neurotoxicity: 9%)
Median time to neurotoxicity onset (any grade) (days)	2	ICANS: 8 days; other neurotoxicities*: 27 days
Median duration of neurotoxicity (any grade) (days)	3	ICANS: 4 days; other neurotoxicities: 75 days
Time to peak CAR-T expansion (days)	11	13
CAR-T persistence 6 months, %	59	42

*Other neurotoxicities are defined as neurotoxicities occurring after resolution of cytokine release syndrome (CRS) and/or immune effector cell-associated neurotoxicity syndrome (ICANS). PR: partial response; CR: complete response; PFS: progression free survival.

CAR-T. Patients were highly pretreated with a median of six prior therapy lines and 84% had triple-class refractory disease (refractory to one protease inhibitor [PI], one IMiD, and a CD38 antibody). At least PR was achieved by 73% including \geq CR in 33%. MRD-negative CR was achieved in 26%. Median time to response was 1 month. Fifty-four patients, who received 450×10^6 CAR-T, had superior response (\geq PR: 82%; \geq CR: 39%; MRD-negative CR: 28%) when compared to lower doses. Revised Multiple Myeloma International Staging System (R-ISS) stage 3 disease at enrollment had inferior response, compared to R-ISS stage 1 or 2. As in the phase I trial, baseline BCMA expression did not affect response to ide-cel. With median follow-up of 13.3 months, overall median PFS was 8.8 months. Median PFS increased with higher CAR-T dose with a median PFS of 12.1 months for patients who received 450×10^6 CAR-T. Patients, who achieved at least CR, also experienced better PFS (\geq CR: median PFS of 20.2 months; very good partial response [VGPR]: median PFS of 11.3 months; PR: median PFS of 5.4 months; no-response: 1.8 months). Median OS was 19.4 months. Durable CAR-T persistence was observed up to 1 year: CAR-T were detected at 1, 3, 6, 9, and 12 months in 99%, 75%, 59%, 37%, and 46% respectively. CAR-T expansion was increased at higher doses. In an ongoing phase III study, ide-cel is compared with standard-of-care regimens in patients with 2-4 prior regimens, including IMiD, PI, and CD38 antibody (KarMMa-3). Ide-cel is also evaluated in the multi-cohort KarMMa-2 study, in patients with early relapse after first-line therapy or patients with sub-optimal response after autografting (<VGPR).

Cilta-cel (JNJ-4528)

The CARTITUDE-1 study evaluates cilta-cel (target dose: 0.75×10^6 CAR⁺ T /kg) in patients exposed to PI, IMiD and CD38 antibody. Preliminary results were presented at the 2020 ASH conference. Sixteen of 113 patients, who underwent apheresis, were not dosed because of consent withdrawal (n=5), progressive disease (n=2) or death (n=9). The remaining 97 patients had received a median of six prior lines of therapy. Ninety-seven percent of patients achieved at least PR with stringent CR in 67%. Forty-eight of the 57 patients evaluable for MRD, 93% were MRD-negative at the level of 10^{-5} . Response was independent of baseline BCMA expression. Median time to first response was 1 month. At a median follow-up of 12.4 months, 12-month PFS was 77%. Peak CAR-T expansion was observed around day 10-14, and CAR-T were observed in 36% of patients at 3 months of follow-up.⁵² Interestingly, response to cilta-cel was independent of CAR-T expansion and persistence.⁵² In the Chinese LEGEND-2 trial, different conditioning regimens were used, as well as variable CAR-T infusion methods (split vs. single infusion). The Xi'an site, which used cyclophosphamide lymphodepletion therapy and three CAR-T cell infusions (dose: $0.07\text{--}2.1 \times 10^6$ /kg; median dose: 0.50×10^6 /kg), enrolled 57 out of 74 patients.⁵³ These patients had received a median of three prior lines of therapy (prior PI and IMiD: 60%). Overall response rate (ORR) was 88% with CR in 74% (median time to response: 1 month). MRD-negative CR was achieved in 68%. At a median follow-up of 25 months, median PFS was 19.9 months for all patients, while it was 28.2 months for those in CR. Median OS was 36.1 months (not reached for patients in CR). Cilta-cel is also being evaluated in a phase

III study (CARTITUDE-4), which compares CAR-T *versus* pomalidomide, bortezomib and dexamethasone or daratumumab, pomalidomide and dexamethasone in relapsed and lenalidomide-refractory MM. In addition, the ongoing CARTITUDE-2 study is evaluating cilta-cel in different patient populations, including those with early relapse after frontline therapy, prior exposure to a BCMA-targeting drug, and those with <CR post-auto-SCT.

Other B-cell maturation antigen-specific chimeric antigen receptor T cells

In order to further improve the activity and/or persistence of CAR-T, several studies are evaluating novel BCMA-targeting CAR-T. Studies include CAR constructs containing a fully human BCMA-specific binding domain to reduce development of humoral and/or cellular immune responses against CAR-T, which may impair CAR-T persistence.^{49,54-56} One of these products with a fully human antigen-binding domain is orva-cel (orvacabtagene autoleucel), which is currently evaluated in the phase I/II EVOLVE study. This study shows promising efficacy of orva-cel in heavily pretreated MM (median of six prior lines of therapy; 94% triple-class refractory). At least PR was achieved in 92% of 62 patients treated at higher dose levels ($300\text{--}600 \times 10^6$ CAR-T), with CR in 36%. Follow-up is ongoing. Treatment was associated with a low incidence of grade ≥ 3 cytokine release syndrome (3%) and grade ≥ 3 neurotoxicity (3%). Following CAR-T, there was robust expansion and durable persistence (69% of patients had detectable CAR-T at 6 months). Moreover, preclinical studies have shown that enrichment for BCMA-targeting CAR-T displaying a memory-like phenotype leads to improved persistence in mouse models,⁵⁷ which may result in more durable disease control. Bb21217 uses the same CAR molecule as ide-cel, but bb21217 is cultured in the presence of a PI3 kinase inhibitor, which leads to enrichment for CAR-T with a memory-like phenotype. Preliminary results showed efficacy in 69 heavily pretreated patients (64% triple-class refractory) with an ORR of 68% (CR of 29%; median response duration: 17.0 months). Interestingly, a high memory-like T-cell count in the drug product was associated with superior CAR-T expansion and less progression at 6 months. The manufacturing process for orva-cel is also designed to produce CAR-T enriched for central memory T-cell phenotype.⁵⁶ Other trials are evaluating combinations of CAR-T with other drugs to improve activity and durability of response. Based on preclinical data showing that the T-cell stimulatory effects of IMiD enhance the efficacy of CAR-T,⁵⁸⁻⁶⁰ several ongoing clinical studies are evaluating the combination of lenalidomide and CAR-T. The combination of BCMA CAR-T with γ -secretase inhibitors is also investigated, because *in vitro* studies show that γ -secretase inhibitors block BCMA cleavage and increase BCMA cell surface expression levels.^{54,61} These results are confirmed in an ongoing clinical study, which shows that gamma secretase inhibition enhances BCMA surface expression on MM cells and reduces soluble BCMA levels.⁵⁴

CD19-specific CAR-T

Recent studies showed that MM cells express ultra-low levels of CD19,³⁰ moreover MM cells with disease-propagating properties also express CD19. This formed the rationale for the evaluation of CD19-specific CAR-T in

MM.⁶² One study evaluated CD19 CAR-T following salvage high-dose therapy and autologous transplantation. All ten patients had shown a PFS shorter than 1 year from a previous transplant. Two experienced a longer PFS after transplant plus CD19 CAR-T therapy, compared with first autologous stem-cell transplantation. Only one patient experienced CRS (grade 1).⁶³

Chimeric antigen receptor T-cell toxicity in multiple myeloma

CAR-T may induce several life-threatening side effects such as CRS and ICANS.⁶⁴ Hemophagocytosis and prolonged cytopenias may also occur. CRS mainly consists of fever, hypotension, hypoxia and organ toxicity, which may result in organ failure. ICANS may include several symptoms: impaired concentration, cognitive disorders, confusion, agitation, tremor, lethargy, aphasia, delirium, somnolence, seizures, motor weakness, paresis or signs of intracerebral pressure. ICANS usually occurs during or after CRS and may manifest a biphasic course, in about 10% of cases up to 4 weeks after CAR-T infusion. A ten-point neurologic assessment, at least twice a day, using the ICE screening tool is recommended for early detection.⁶⁴ Reported toxicity rates of hallmark studies are illustrated in Table 3. Importantly, a broad consensus statement offering updated comprehensive recommendations for the treatment of toxicities associated with immunotherapies has been recently published.⁶⁵ Though many aspects remain unknown, mechanisms underlying CRS and ICANS have become clearer. Several factors contribute to different toxicity rates.⁶⁵ Moreover, incidence and severity of adverse effects vary between diseases. While the incidence of any grade CRS is comparable between diseases, CRS severity (\geq grade 3) is highest in patients with ALL and lowest in MM. This may partly be explained by disease burden and aggressiveness. In addition, earlier treatment of CAR-T side effects with more experience in

recent trials may have contributed to reduce progression to higher grades of toxicity. Unlike CRS, incidence of ICANS is higher in ALL or lymphoma and appears lower in MM patients. Other factors such as tumor burden, prior treatment, CAR-T constructs and dose administered have been described. Several grading systems have been proposed to assess CRS and ICANS. Recently, the American Society for Transplantation and Cellular Therapy grading system was compared to other grading scores in two adult populations.⁶⁶ Interestingly, incidence of CRS and ICANS were similar in all grading systems. By contrast, only 25% and 54% of patients were however assigned to the same severity grade given the discrepancies in scoring adverse symptoms. These differences may also easily lead to inconsistent management guidelines among studies. Overall, efforts should be made to unify grading systems be used across clinical trials.

Improving chimeric antigen receptor T-cell therapies in multiple myeloma

Despite numerous autologous CAR-T products under development^{67,68} and encouraging high response rates,⁶⁹ none have yet been approved, with BCMA remaining the best evaluated target.^{49,51,69} Other limitations are toxicities, resistance mechanisms, availability, and patient management (Table 4). Here we highlight possible strategies for improvement.^{70,71}

Safety

Preventing cytokine release

The pro-inflammatory interleukin-6 (IL-6) is increasingly acknowledged to play a central role in the pathogenesis of CRS.⁴⁷ A recent study designed a non-signaling membrane-bound IL-6 receptor (mbaIL6) which was constituted by a scFv derived from an antibody against IL-6, and linked to a transmembrane anchoring peptide. The study identified expression of mbaIL6 on the surface of T cells

Table 3. Toxicity of CAR-T cell treatment in multiple myeloma

CAR-T	Construct	Cell dose	Trial	Sponsor	N	Cytopenia 3/4	CRS 3/4	ICANS 3/4	Ref
BCMA/CD28		9x10 ⁶ cells / kg bw	First-in-humans	NIH	16	leucopenia 94% neutropenia 88% thrombopenia 63% prolonged 13%	38%	6%	30
BCMA/4-1BB		C1: 1-5x10 ⁸ total cells C2: Cy+1-5x10 ⁷ total cells C3: Cy+1-5x10 ⁸ total cells	Phase I	UPenn	25	leucopenia 44% neutropenia 44% thrombopenia 28% prolonged n.r.	32%	12%	49
BCMA/4-1BB	LCAR-B38M (JNJ-4528)	med. 0.5x10 ⁶ cells / kg bw	LEGEND-2	China (Phase 1)	57	leucopenia 30% neutropenia n.r. thrombopenia 23% prolonged 16%	7%	0%	53
BCMA/4-1BB	JNJ-4528	med. 0.73x10 ⁶ cells / kg bw	CARTITUDE-1 (Phase I/II)	Janssen	29	leucopenia 59% neutropenia 100% thrombopenia 69% prolonged n.r.	7%	3%	48 68
BCMA/CD28	bb2121	50-800 x 10 ⁶ total cells	CRB-401 (Phase I)	BMS / Celgene	33	leucopenia 58% neutropenia 85% thrombopenia 45% prolonged n 3% t 35%	6%	3%	51

CRS: cytokine-release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; Ref.: references; bw: body weight; C: cohort; Cy: cyclophosphamide; n.r.: not reported; n: number of patients.

which was associated with rapid removal of IL-6 from the culture supernatant. Furthermore, T-cell proliferation increased while the signaling and function of IL-6 was neutralized. T cells co-expressing mba1L6 and anti-CD19 CAR, neutralized IL-6 derived from macrophages while maintaining antitumor activity *in vitro* and in a xenograft model.⁷² Thus, CAR-T incorporating the capacity to remove IL-6 may provide new strategies for preventing CRS. Another strategy is the incorporation of “suicide” switches, such as constructs containing a CAR and inducible caspase 9. Administration of a small molecule causing dimerization of inducible caspase 9 resulted in apoptosis and CAR-T-specific depletion.⁷³

Reducing off-tumor on-target toxicity

In order to avoid adverse off-tumor effects, spatial and temporal activity of CAR-T need to be limited. Under this hypothesis, GPRC5D has been proposed as a novel target antigen, expressed on almost all CD138+ cells.⁷⁴ Like BCMA, its expression is restricted to plasma cells, except for hair follicles. Preliminary results of anti-GPRC5D CAR-T showed potent anti-MM efficacy *in vitro* and in a mouse model, with the encouraging finding that these cells also effectively eradicated MM after treatment with anti-BCMA CAR-T. Most recently, it was demonstrated that simultaneous targeting of GPRC5D and BCMA could prevent relapse mediated by BCMA escape. Several multi-target constructs were compared and in BCMA-negative disease, dual-target (bicistronic) and pooled approaches exhibited the highest efficacy, whereas for GPRC5D/BCMA-expressing disease, the dual-target appeared to be more efficacious. Mechanistically, expressing two CAR on one cell enhanced the strength of CAR-T/target interactions.

Reducing immunogenicity and simplifying structures

In order to reduce the immunogenicity of the CAR binding domain, human or humanized scFv have been used more frequently in recent studies, instead of murine sequences.^{68,75} Furthermore, a reduction of immunogenicity

might be achieved by the incorporation of heavy-chain-only binding domains, which subsequently simplify the structure of the CAR antigen-binding domain without having a light-chain domain.⁷⁶ In general, simplified structures may facilitate better gene expression by transduced T cells.^{76,77} Moreover, limiting the size of expressed genes is important for the potential expression of >1 protein.^{78,79} A recent study demonstrated that CAR with antigen-recognition domains consisting of only a fully human heavy-chain variable domain (FHVH33) in addition to 4-1BB and CD3 ζ domains mediated comparable cytokine release, reduction in tumor burden, and degranulation in mice when compared to an identical CAR with a conventional scFv.^{76,80} Further investigations identified a crucial contribution of 4-1BB in reducing activation-induced cell death, enabling survival of T cells expressing FHVH33-CAR.⁷⁶

Efficacy

Understanding antigen loss

Some relapses are either antigen-negative or antigen-low.⁸⁰ One study in leukemia mouse models could dissect evidence for CAR promoting reversible antigen loss through a mechanism called trogocytosis.⁸¹ This mechanism defines an active process of rapid intercellular transfer of membrane fragments and related molecules. The specific target antigen is transferred to T cells resulting in decreased density on tumor cells, leading to declined T-cell activity by boosting fratricide T-cell killing and exhaustion.⁸¹ These cascades affected CAR constructs that included different costimulatory domains (CD28 or 4-1BB), and the effect was dependent on antigen density. Thus, it was hypothesized that multi-target CAR-T could overcome these limitations.⁸¹

Multi-targeting

T-cells expressing single-chain bispecific CAR are able to prevent antigen escape.^{68,82} Moreover, CAR pools combining two single-input CAR-T products have been proposed (Figure 2). Pooling a humanized anti-CD19 and a

Table 4. Limitations and ways to improve CAR-T therapy in multiple myeloma.

	What may limit CAR-T therapy?	How to improve CAR-T therapy?
Toxicity	On-target, on-tumor	Anti-IL6 treatment and prevention Safeguard designs incorporating drugs such as rituximab/cetuximab
	On-target, off-tumor	Tackling immunogenicity Simplified CAR structures (e.g., heavy-chain-only binding domains)
Resistance	Impaired CAR-T expansion/persistence	Multi-targeting therapy (dual-target, OR-target, CARpool) More accurate measurement of expansion/persistence “Suicide switches”
	Immunosuppression induced by BM microenvironment	Combination of immunomodulatory modulation and CAR-T
	Antigen loss or downregulation	Senolytic CAR-T (?) Address trogocytosis Increase antigen density (e.g. γ -secretase-inhibition for anti-BCMA therapy)
Management	Suboptimal recognition and treatment of severe events	Increase comparability and knowledge sharing of intensive care unit management and other care settings Outcome prediction
Availability	Lack of scale-up	Allogeneic CAR-T
	High costs	Optimize supply chain models (e.g., intermediate players for cryopreservation)
	No stockpiling	
	Time	

BCMA: B-cell maturation antigen; IL6: interleukin-6; BM: bone marrow.

murine anti-BCMA CAR-T was investigated in 22 patients.⁶³ The study had a median follow-up of 6 months and reported a high ORR of 95%, with CR of 57%, and relatively low CRS of grades ≥ 3 (4%).⁶³ Preliminary results of two other CD19/BCMA studies showed similar ORR but lower CR (22% and 16%).^{83,84} One study investigated dual-target CAR-T co-expressing two full-length receptors, namely CD38 and BCMA.⁸⁵ Median follow-up was 9 months and the ORR was 88%. PFS was 75% and higher CRS of grades ≥ 3 were noted compared with tandem CAR (25%). OR-gate tandem CAR consist of a single CAR structure targeting two antigens with two distinct antigen recognition domains (scFv) linked consecutively with a single signal transducing intracellular domain.^{82,86} A recent study using CS1/BCMA tandem CAR-T showed superior CAR expression and function in comparison with T cells co-expressing individual CS1 and BCMA CAR. When compared to the OR-gate (tandem) CAR, dual-target CAR require a much larger genetic payload, leading to poorer transduction efficiency and reduced proliferation. A recent Chinese study using BCMA-CD19 dual FasT CAR-T showed an overall response rate of 93.8% with median duration of follow up of 7.3 months at cutoff. Importantly, most patients showed high-risk features.⁸⁷ A much more compact genetic footprint may greatly support viral integration, thus product manufacturing, suggesting an advantage for single-chain tandem CAR in relation to dual-targeting. With respect to CARpools, this strategy could avoid poor transduction efficiency. Among these three approaches, mechanistically, CAR pool may be the least effective.⁸⁰

Targeting the tumor microenvironment

The BM milieu is heavily involved in MM pathogenesis and resistance to treatment. Conflicting data exist on whether monoclonal antibodies against CD38 are effective in the BM microenvironment,⁸⁸ whereas immunomodulatory agents may be able to overcome these inhibitory effects.⁸⁹ Accordingly, combining these drugs with CAR-T therapy may provide synergistic effects.⁵⁹ Conversely, tissue microenvironment itself is

modulated by secretory programs and stable cell-cycle arrest, defined as cellular senescence, which is a tumor-suppressive mechanism. Accumulating aberrant senescent cells create an inflammatory milieu resulting in tissue damage and fibrosis. In order to eliminate these senescent cells, "senolytic" CAR-T have been proposed.⁹⁰ One study discovered the cell-surface protein urokinase-type plasminogen activator receptor (uPAR) being broadly induced during senescence,⁸⁸ and further dissected that anti-uPAR CAR-T efficiently ablated senescent cells *in vitro* and *in vivo*, restoring tissue homeostasis in mice with liver fibrosis.⁹⁰ In MM, it has been shown that u-PAR contributes to the functioning of cancer-associated fibroblasts during MM progression,⁹¹ and that higher expression of u-PAR was associated with disease progression, worse survival and early extramedullary spread of MM cells. Although it has to be noted that a caveat of senolytic CAR-T are the potential off-target toxicities,⁹² these results may encourage the incorporation of cellular strategies specifically addressing the MM microenvironment.

Availability and management

Allogeneic chimeric antigen receptor T cells

Allogeneic CAR-T may decrease cost and enable broader availability.⁹³ Notwithstanding, allogeneic CAR-T bare the risk for graft-*versus*-host disease (GvHD). For this reason, TALEN- and CRISPR-based gene editing has been introduced to produce allogeneic CAR-T with off-the-shelf availability.^{68,93,94} One recent study on allogeneic anti-BCMA CAR-T used gene editing, namely TALEN, to confer resistance to lymphodepletion and to reduce GvHD risk.⁹⁵ By further incorporating a CD20 mimotope-based switch-off within the CAR, rituximab could be given to eliminate the CAR-T in case of adverse events. Another preclinical approach using similar safety features but anti-CS1 CAR-T (UCARTCS1),³⁵ specifically degranulated and proliferated in response to MM cells, supporting further evaluation and testing of this universal therapy. Current investigational studies also include (i) the non-viral piggyBac system, aimed at transposing stem cell memory T cells together with (ii) the Cas-CLOVER™ gene editing

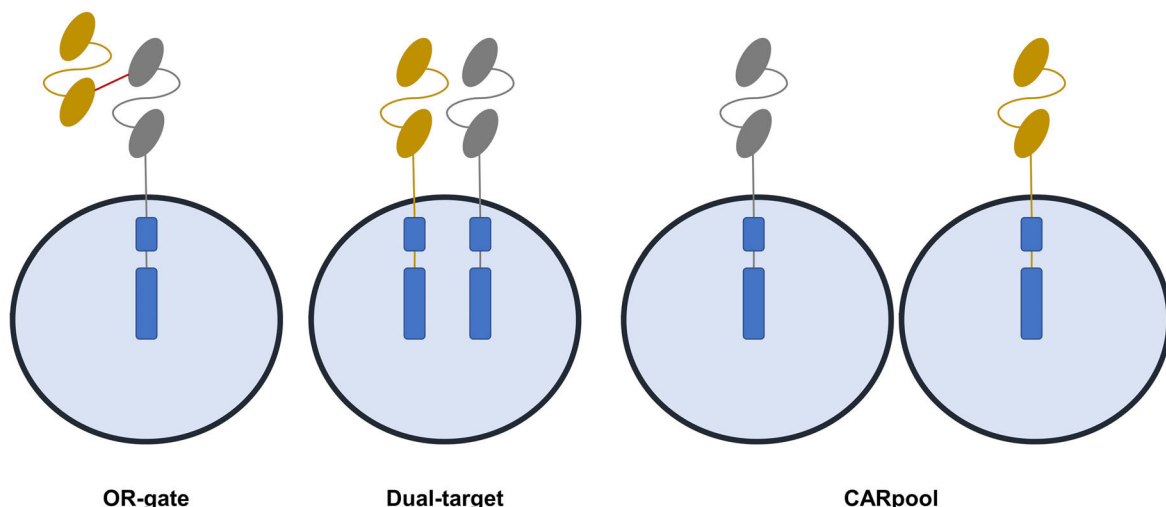


Figure 2. Overview of current multi-targeted chimeric antigen receptor T-cell approaches. Multi-targeting may be a mean to improve efficacy of CAR-T. Three major methods could be exploited: OR-gate (tandem) CAR-T: consist of the expression of two different CAR on the same T cell; dual-target CAR-T: consist of encoding two different target specific single-chain variable fragment antibodies on same CAR protein using a single vector; CARpool: involves production of two separate single target CAR-T products infused together or sequentially.

system consisting of CRISPR guide-RNA and dead Cas9 fused to Clo51 nuclease, and (iii) a nano-particle delivery system carrying the gene for an anti-BCMA Centyrin-based CAR with a fully human binding domain. Rimiducid, a lipid-permeable tacrolimus analogue and protein dimerizer, may be administered for safety switch activation.⁹⁶ Two phase I dose escalation and cohort expansion studies have recently been initiated. The CTX120 (clinicaltrials.gov. Identifier: NCT04244656) is using anti-BCMA allogeneic CRISPR-Cas9-engineered T cells and the PBCAR269A (clinicaltrials.gov. Identifier: NCT04171843) which applies its own gene editing tool (ARCUS) for GvHD risk reduction.

Integration of chimeric antigen receptor T cells into clinical routine - FACT-JACIE* standards and EBMT** guidelines

Since 2018, with version 7.0, the *Joint Accreditation Committee of ISCT and the **European Bone Marrow Transplantation Group (EBMT) (JACIE) prerequisites for cell therapy accreditation have included standards for infusions of immune-effector cells and CAR-T. The current recommendation is that CAR-T should be administered within the framework of an accredited allogeneic transplant program. The Foundation for the Accreditation of Cellular Therapy (FACT)-JACIE do not cover the manufacturing of CAR-T but do include supply chain and handover of responsibilities when the product is provided by third party. Overall, JACIE standards are structured on the basis of three major functional areas in cellular therapy: cell collection, laboratory processing, and clinical program. All areas required dedicated and highly qualified personnel. Accredited programs for cell therapy must implement a product labeling system that guarantees identification and traceability from collection to manufacturing site and return to clinical units. EBMT recommendations further stress the importance of staff training⁹⁷ and of multidisciplinary approaches with teams who include transplant physicians along with qualified internal medicine sub-specialists after a specific education program. Importantly, CAR-T infusions should be coordinated with intensive care specialists. All accredited centers must implement a policy for rapid escalation of care for critically ill patients including availability of specific drugs (*i.e.*, tocilizumab). Though complications may vary among CAR-T products, they tend to follow a predictable timeline contributing to bed-planning decisions. Recent reports allow designing protocols for anti-infective prophylaxis and common post-infusion complications such as infections and tumor lysis syndrome.⁹⁸ Inevitably, the unfortunate COVID-19 pandemic stresses the importance of scrupulous adherence to

recommended hygiene procedures.⁹⁹ Importantly, an EBMT registry, for all transplant centers accredited for cell therapies, has been created to collect data on efficacy, side effects and clinical outcomes for post-marketing surveillance.

Conclusions

The clinical role of CAR-T in the current armamentarium of MM treatments remains as yet to be fully determined. Moreover, other promising forms of antibody-based immunotherapies have been added. Despite some limitations of CAR-T therapy experienced in early studies in MM, one advantage of this cellular therapy is the inherent potential to finetune its design. Simpler structures and multi-target approaches may significantly improve efficacy and safety. Constant learning to handle CAR-T therapy may also enable better patient-centered management. Last, long-term outcome studies and specific detection and analysis of CAR-T dynamics *in vivo* are essential to allow deeper understanding of their inherent functions which will facilitate future designs of improved CAR-T products. However, selecting patients who may most benefit from CAR-T and best timing of their administration still require rather lengthy and thorough clinical investigations. One more challenge that lies ahead will be the cost effectiveness of future commercial products. This issue has already been addressed in patients with lymphoma where cost reductions will be inevitable to make CAR-T sustainable therapies for health care systems.¹⁰⁰ Despite all remaining open questions and issues that still need to be addressed, and hopefully answered and resolved within the next years, we are now, without any doubt, at the dawn of a new era that will significantly improve patient outcome.

Disclosures

No conflicts of interest to disclose.

Contributions

BB, ME, NWCJD designed the review and wrote the manuscript; LG, MD, FG, ET, LGRL, SD, NG, NK, RP and ET provided data and interpretation; MAD, PS, HE and MB reviewed the manuscript.

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