Determinants of response and mechanisms of resistance of CAR T-cell therapy in multiple myeloma

Niels W.C.J. van de Donk¹, Maria Themeli¹, Saad Z. Usmani².

¹Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Hematology, Cancer Center Amsterdam, Amsterdam, The Netherlands

²Levine Cancer Institute, Carolinas Healthcare System, Charlotte, NC, United States.

Correspondence to: N.W.C.J. van de Donk, MD, PhD
Amsterdam UMC, Vrije Universiteit Amsterdam, Cancer Center Amsterdam, Department of Hematology, De Boelelaan 1117, 1081HV Amsterdam, The Netherlands. Phone: +31(0)20.4442604, fax: +31(0)20.44442601

e-mail: n.vandedonk@vumc.nl

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Abstract

BCMA-specific CAR T-cells have substantial therapeutic potential in multiple myeloma (MM), but most patients eventually relapse. Determinants of response and mechanisms of resistance are most likely multifactorial and include MM-related factors, premanufacturing T-cell characteristics, CAR T-cell-related features, and several components of the immunosuppressive microenvironment. Efforts to improve the potency and safety of CAR T-cell therapy include optimizing CAR design, combinatorial approaches to enhance persistence and activity, treatment of less heavily pretreated patients, and dual-antigen targeting to prevent antigen escape. We expect that these rationally designed strategies will contribute to further improvement in the clinical outcome of MM patients.

Statement of significance

Although BCMA-specific CAR T-cell therapies are highly effective in heavily pretreated MM patients, there is until now no indication of a plateau in the survival curves. In this review, we will provide an overview of the determinants of response and the mechanisms that contribute to the development of treatment failure after initial remission (acquired resistance). A better understanding of these mechanisms, underlying lack of disease response and acquired resistance, may lead to further improvements in the effectiveness of CAR T-cell therapy.
Introduction

Although the introduction of new anti-MM agents markedly improved the survival of multiple myeloma (MM) patients, there is an unmet need for new drugs for patients, who develop resistance to immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), and CD38-targeting antibodies (triple-class refractory disease), which carries a poor prognosis(1). Also newly diagnosed patients with high-risk disease (e.g. presence of del(17p), t(4;14), or t(14;16)) or suboptimal response have an impaired outcome, and these patients may benefit from the incorporation of new drugs with novel mechanisms of action in first-line regimens.

A promising new strategy is the reprogramming of T-cells to target MM cells by introducing genes encoding chimeric antigen receptors (CARs). CARs are fusion proteins, combining an antigen-recognition moiety (commonly a monoclonal antibody-derived single-chain variable fragment (scFv), but other formats such as natural ligands are also possible(2)) with a T-cell activation domain, typically CD3ζ. These 2 parts are connected via an extracellular spacer region (hinge) and a transmembrane-spanning element. Second generation CARs incorporating a costimulatory domain, such as CD28, 4-1BB, OX40, or ICOS, into the CAR endodomain, result in enhanced anti-tumor activity of the modified T-cells, when compared to first-generation CARs without such domain (Figure 1)(3). Importantly, CAR T-cells eliminate tumor cells in a non-major histocompatibility complex-restricted manner.

Most of the CAR T-cell products, currently evaluated in clinical trials for MM patients, target B-cell maturation antigen (BCMA), which is uniformly expressed on the cell surface of MM cells, normal plasma cells, and a subset of mature B-cells. Characteristics, as well as key efficacy and safety data from several studies, evaluating BCMA-targeted CAR T-cells, are provided in Tables 1-2. CAR T-cells specific for other MM-associated antigens, such as CD19, SLAMF7, CD38, GPRC5D, are also investigated in MM. BCMA-specific CAR T-cells have significant therapeutic potential in MM, as evidenced by the high quality responses with a substantial rate of complete response (CR) and...
Minimal-residual disease (MRD) negativity, obtained in heavily pretreated, often triple-class refractory, patients (4-11). Similar to what is observed with other therapies, depth of response is associated with improved PFS in patients treated with CAR T-cell therapy, with best outcomes in patients achieving CR or MRD-negativity (10,12). Most advanced in clinical development are the BCMA-targeting CAR T-cell products idecabtagene vicleucel (ide-cel, bb2121) and ciltacabtagene autoleucel (cilta-cel, JNJ4528), both of which have received FDA breakthrough designation based on promising results in heavily pretreated patients (6,10,11). However, not all patients achieve a remission after CAR T-cell therapy. Furthermore, there is, until now, no indication of a plateau in the survival curves, which contrasts with results obtained with CD19 CAR T-cells in acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL). In this review, we will provide an overview of the determinants of response and the mechanisms that contribute to the development of treatment failure after initial remission (acquired resistance). A better understanding of these mechanisms underlying lack of disease response and acquired resistance, may lead to new strategies to improve the effectiveness of CAR T-cell therapy.

**Determinants of response and mechanisms of resistance to CAR T-cells**

Mechanisms that influence CAR T-cell efficacy are multifactorial and include tumor-, host- (tumor microenvironment and T-cells), and product-related factors (Figure 2). However, the precise impact of these characteristics on primary and acquired resistance is difficult to assess, because of the limited number of patients enrolled in individual studies.

**Tumor-related resistance mechanisms**

*(Soluble) BCMA*
The impact of the marked heterogeneity in BCMA antigen density among MM patients on clinical outcomes following CAR T-cell therapy is not completely clear(2). Several studies show that response, progression-free survival (PFS) and overall survival (OS) after BCMA CAR T-cell therapy are not associated with baseline BCMA expression levels on tumor cells(6,7,9,10,13,14), while in other trials pre-treatment BCMA levels have an impact on depth or durability of response(5,15,16).

Discrepancies between these studies may be explained in part by differences in assays used to quantify BCMA expression with flow cytometry being more sensitive than immunohistochemistry(17). Membrane-bound BCMA can also be shed from the tumor cell surface, leading to circulation of soluble BCMA (sBCMA). The effect of sBCMA on binding of CAR T-cells to BCMA on the tumor cell surface, is controversial, with some preclinical studies showing that high levels of sBCMA impair cytolytic activity of BCMA-directed CAR T-cells(2,18,19), while in other preclinical studies sBCMA did not affect CAR T-cell activity(20-22). More importantly, in clinical trials, baseline sBCMA levels had no effect on response(4,6,9,23).

Changes in antigen expression over time may also affect the effectiveness of CAR T-cell treatment. Cohen et al. showed that following BCMA CAR T-cell infusions BCMA expression levels decreased on residual MM cells in 67% of patients, both in responding and in non-responding patients(9). In some of these patients BCMA levels were restored back to baseline levels at later time points(9). Several other studies also demonstrated reduced BCMA expression at the time of progression(4,5,15,24-26). The mechanism whereby BCMA CAR T-cells reduce BCMA cell surface expression levels probably includes selection of cells with lower BCMA levels, while tumor cells with higher BCMA expression are eliminated. Furthermore, biallelic BCMA deletions, resulting in lack of BCMA expression, have also recently been found to trigger resistance to BCMA CAR T-cells(27-29). Although BCMA antigen loss seems to be an uncommon mechanism of escape from BCMA-directed CAR T-cell therapy (4% in the KarMMa study(10)), it may have major therapeutic implications because biallelic loss of BCMA will also confer resistance to retreatment with similar or other BCMA-targeted therapies(28). This highlights the need to examine for BCMA gene alterations or to assess BCMA expression, when
treatment with another BCMA-directed immunotherapy is considered. Deletion of 16p, including the BCMA locus, is present in approximately 6-7% of newly diagnosed or relapsed/refractory patients, frequently co-occurring with del(17p)(28,29). It is currently unknown whether these patients are at an increased risk for BCMA loss after immunotherapy. Loss of BCMA expression was also associated with the absence of an increase in sBCMA levels at the time of progression(28). CAR T-cell studies should incorporate serial sBCMA assessments to investigate the potential value of sBCMA as an indicator of BCMA loss at relapse(28). In addition, preclinical MM models showed that transfer of BCMA from the tumor cell surface to CAR T-cells (trogocytosis) may also contribute to antigen loss and antigen-low tumor relapse(30). At the same time, trogocytosis also leads to CAR T-cell fratricide with a negative effect on CAR T-cell activity(30).

The use of CAR T-cells with high BCMA binding affinity may prevent the outgrowth of BCMA low tumor clones and may be more effective in patients with low target antigen density at baseline or with substantial heterogeneity in BCMA expression. Such patients, with potential resistant clones, may also benefit from CAR T-cells targeting other MM-associated antigens. Based on preclinical studies showing substantial anti-MM activity of CAR T-cells targeting SLAMF7(31), CD38(32), CD138(33), GPRC5D(34), integrinβ(7)(35), and CD44v6(36), various clinical studies are currently ongoing to evaluate the efficacy and safety of CAR T-cell products targeting these alternative MM-associated antigens. However, because some of these targets, such as CD38 and CD138, are also expressed in critical normal tissues, there is a potential risk of on-target, off-tumor toxicity. CD19 CAR T-cells are also evaluated in MM, based on the identification of a small subset of CD19+ MM cells with a less differentiated phenotype and possibly disease-propagating properties(37,38). In addition, super-resolution microscopy revealed that a substantial fraction of MM cells has low or ultra-low levels of CD19, that triggers elimination by CD19 CAR T-cells(39). CD19 CAR T-cells were clinically evaluated directly after treatment with a second course of melphalan and autologous stem cell transplantation (auto-SCT) in MM patients who previously received auto-SCT with a PFS of less than 1 year(37,38).
Two out of 10 patients exhibited more durable responses, compared with the first transplantation\(^{(37,38)}\).

However, probably most effective will be the use of combinatorial approaches to prevent antigen-loss relapses and to address antigenic heterogeneity. This includes the use of pooled CAR T-cells (co-infusion of 2 CAR T-cell products, each expressing a different CAR). Disadvantages of this strategy include the possibility of selective expansion of one of these CAR T-cell products and the requirement of manufacturing 2 clinical products\(^{(40)}\). Growth competition can be avoided by using dual-targeted CAR T cells (single CAR T-cells expressing 2 distinct CARs with different binding domains, or CAR T-cells expressing a single CAR molecule with two separate binding domains in tandem [tandem CAR])\(^{(40-42)}\). Dual-targeted CAR T-cells can be more effective than pooled CAR T-cells, possibly because of enhanced bivalent immune synapse formation, resulting in improved activation and expansion\(^{(41-43)}\). Several preclinical studies demonstrated superior tumor control and prevention of BCMA escape-mediated relapse by simultaneous targeting of BCMA and SLAMF7, BCMA and GPRC5D, or BCMA and TACi\(^{(2,19,43-45)}\). Similarly, \textit{ex vivo} treatment of MM cells with a mixture of both CD19 CAR T-cells and BCMA CAR T-cells was more effective in reducing colony formation capability, than either CD19 CAR T-cells or BCMA CAR T-cells alone\(^{(38,46)}\). Several clinical studies in RRMM patients demonstrate a high response rate with the combination of CD19- and BCMA-targeting CAR T-cells\(^{(47-49)}\). On-target/off-tumor toxicity consisted of B-cell aplasia and hypogammaglobulinemia\(^{(47)}\). Also dual BCMA and CD19-targeted CAR T-cells show promising activity and a favorable safety profile in RRMM\(^{(46)}\). A limitation of these studies is the single-arm design, which makes it difficult to assess the added value of CD19 targeting. Randomized studies are needed to answer this question\(^{(47,48)}\). Also a tandem CAR T-cell product, targeting CD38 and BCMA, shows promising activity with acceptable toxicity in RRMM\(^{(50)}\). However, the phase 1 study (AUTO2) evaluating APRIL-based CAR T-cells (dual targeting of BCMA and TACi) in RRMM was stopped because of insufficient activity\(^{(51)}\).
Combination approaches that increase antigen density may also enhance CAR T-cell efficacy. Small molecule inhibitors of γ-secretase (GSI) reduce shedding of BCMA and increase BCMA expression, resulting in enhanced CAR T-cell activity in MM mouse models(18). A limitation of GSI is their possible negative impact on CAR T-cell function because of Notch pathway inhibition(18). Preliminary results from a clinical study (NCT03502577) show high activity of the combination of BCMA CAR T-cells and GSI, also in patients that previously failed BCMA-targeted therapy, which may be related to the GSI-mediated increase in BCMA expression and reduction of sBCMA(52). However, there was also a high rate of CRS and neurotoxicity(52). Inhibitors of HDAC7 or the Sec61 complex also increase BCMA cell surface expression(53).

**Tumor load**

Although all studies clearly demonstrate that CAR T-cell therapy is effective in patients with high tumor load, there was a trend towards a moderately lower CR rate with ide-cel in patients with high disease burden (≥50% bone marrow (BM)-localized MM cells), when compared to patients with a relatively low tumor load (29 versus 37% in the KarMMa study)(6,10,54). A high tumor burden with chronic antigen exposure may result in CAR T-cell exhaustion, and thereby impaired antitumor activity(55). Immune checkpoint blockade may reverse the hyporesponsiveness of exhausted T-cells. A better understanding of the impact of tumor burden in studies evaluating other CAR T-cell products is essential, because this may translate into more effective treatment strategies (e.g. reinduction therapy prior to cell therapy to debulk the disease).

**High-risk cytogenetics and extramedullary disease**

The precise impact of cytogenetic abnormalities on the clinical outcome of heavily pretreated patients is currently unknown because of small numbers of patients and limited data on response
duration in high-risk subgroups. Nevertheless, across all studies deep and durable responses were also achieved in RRMM patients with high-risk cytogenetics.(5,6,9-11,54). Extramedullary disease seems to confer a poor outcome in some studies(8,16,26,56,57), while response was similar in others(10,54). The strongest predictor for clinical activity of ide-cel was the revised ISS staging system (reflecting a combination of risk factors including high tumor load, high-risk cytogenetic abnormalities and/or elevated LDH [≥PR: 48 versus 80%; ≥CR: 10 versus 39%; median PFS: 4.9 versus 11.3 months for R-ISS stage 3 versus R-ISS stage 1 or 2 in the KarMMa study])(10,54).

Inhibitory receptors and ligands

MM cells have several properties that enable immune evasion. This includes the expression of inhibitory ligands on the MM cell surface (PD-L1 and PD-L2: ligands for PD-1; galectin-9: ligand for TIM-3; and MHC class II: ligand for LAG-3), which contribute to suppression of T-cell responses. There is increasing evidence that these ligands also impair CAR T-cell activity (for details see section “Nature of CAR T-cells infused in patients”).

In addition, a CRISPR-based screen in a MM cell line identified several novel mechanisms that control the response to BCMA CAR T-cells(53). Knockdown of genes in the sialic acid biosynthesis pathway sensitized MM cells to BCMA CAR T-cells(53). This is in line with prior studies showing that sialic acids, present on the tumor cell surface, impair T-cell mediated tumor immunity(58). This study also showed that ICAM-1 expression is important for BCMA CAR T-cell-mediated tumor cell lysis, while knockdown of genes belonging to the family of diacylglycerol kinases (DGK) increased sensitivity to BCMA CAR T-cells(53).

Resistance to the effector mechanisms of T-cells
T-cells kill their targets through exocytosis of cytotoxic granules that contain pore-forming perforin, as well as serine proteases such as granzyme B. Also induction of apoptosis via cross-linking of death receptors (such as Fas, TRAIL-R1, and TRAIL-R2) results in target cell lysis. Tumor cells can be resistant to T-cell-mediated killing by increased expression of several anti-apoptotic molecules, including serine protease inhibitors (serpins), which inactivate granzyme B(59). Furthermore, death receptor-mediated apoptosis can be prevented by overexpression of the anti-apoptotic protein c-FLIP, death receptor downregulation, cleavage of death receptors, or increased expression of decoy receptors(60,61). It is currently unknown whether resistance of tumor cells to the effector mechanisms of T-cells contributes to escape from CAR T-cell therapy in MM. However, a recent report showed that baseline death receptor expression on leukemic cells, correlates with response after CD19 CAR T-cell therapy in ALL(62). Other defense mechanisms against T-cell-mediated lysis, such as downregulation of MHC class 1 or 2 molecules, or defects in the antigen-processing machinery, will not impair CAR T-cell-mediated tumor cell killing.

Characteristics of the T-cells collected from the patient

Mechanisms of relapses with retained target expression, include decreased persistence and/or decreased function of CAR T-cells. However, the optimal duration of CAR T-cell persistence is unknown, and may also differ between CAR T-cell products. In this section we will discuss several baseline characteristics of the pre-manufacturing T-cells, which have an impact on CAR T-cell persistence and activity, as well as strategies to improve CAR T-cell fitness.

Baseline T-cell characteristics

MM is characterized by a broad range of active immune evasion strategies, that result in qualitative and quantitative abnormalities in immune cells, including T-cells. In addition, there is marked
variability between MM patients in T-cell subset composition, including frequencies of CD4$^+$ and CD8$^+$ T-cells, and proportions of the different T-cell differentiation subsets, which can be explained by differences in age, pathogen exposure, and extent of treatment with immunosuppressive (alkylating drugs, proteasome inhibitors and dexamethasone) or immunostimulating anti-MM therapies (IMiDs) (Figure 3)(63,64). There is increasing evidence that the heterogeneity of T-cell subsets in the apheresis product explains part of the variability of the activity of the CAR T-cells infused to the patients in clinical trials. First, several BCMA CAR T-cell studies show that MM patients with a high frequency of early memory T-cells in the leukapheresis product experience a higher response rate and superior peak CAR T-cell expansion, when compared to patients with a low frequency of these cells(9,27,65,66). Similarly, the presence of early memory T-cells in the leukapheresis product was correlated with response in chronic lymphocytic leukemia (CLL), ALL, and lymphoma patients treated with CD19 CAR T-cells(65,67,68). These findings can be explained by the ability of T-cells with memory properties to undergo self-renewal and by their superior proliferative response, compared to more differentiated T-cells(69). In addition, a higher CD4/CD8 ratio in the leucapheresis product was associated with greater *in vivo* BCMA CAR T-cell expansion and response in MM(9,27). CD4$^+$ T-cells promote the proliferation, survival and activity of CD8$^+$ T-cells by providing a variety of cytokines including IL-2, which explains the synergy between CD4$^+$ and CD8$^+$ CAR T-cells in mediating antitumor responses(70,71). Furthermore, T-cells from BCMA CAR T-cell resistant patients, were enriched with terminally exhausted and senescent cells with high expression of checkpoint inhibitors, such as LAG-3, TIGIT, and PD-1(27). Altogether, this indicates that premanufacturing T-cell characteristics are important determinants of response to CAR T-cell therapy.

*Effect of prior therapy on the nature of T-cells collected from the patient*

Cumulative exposure to several anti-MM drugs, will reduce T-cell numbers or induce functional T-cell defects (Figure 3)(72,73). Interestingly, the frequency of early memory T-cells and CD4/CD8 ratio...
were higher in apheresis samples from MM patients who were early in their disease course, compared to heavily pretreated patients(74). This translated in significantly higher capacity for ex vivo proliferation during manufacturing(74). This is similar to what is observed in other malignancies where chemotherapy leads to depletion of naïve and early memory T-cells over time, and thereby poor in vitro T-cell expansion(75).

Also type of treatment administered prior to leucapheresis may affect the quality and phenotype of the harvested T cells. In ALL it has been shown that clofarabine treatment directly before leukapheresis contributes to inadequate T-cell function and probably suboptimal response to CD19 CAR T-cell therapy(76). Conversely, CLL patients treated with ibrutinib before T-cell collection had improved CD19 CAR T-cell expansion(77). Furthermore, early memory T-cells are depleted by cyclophosphamide and cytarabine in ALL and NHL patients(68). There is currently only limited data available in MM. One study showed that type of therapy prior to apheresis was not associated with response or CAR T-cell expansion(9), while another study demonstrated that patients with daratumumab as part of last line or as bridging therapy had a modestly higher response rate following ide-cel infusion when compared to patients without daratumumab as part of last line treatment (≥PR: 91% versus 75%)(6). The impact of prior therapy on T-cell fitness should be studied in larger cohorts of patients, with a focus on the potential beneficial effects of early collection of T-cells and of immunostimulatory drugs directly prior to T-cell collection. Alternatively, allogeneic T-cells obtained from healthy donors can be used to improve CAR T-cell fitness. Because of the “off-the-shelf” availability, allogeneic therapy may also overcome the logistical challenges of autologous CAR T-cell therapy. Preliminary results from the UNIVERSAL study show promising activity and a manageable safety profile (no graft-versus-host disease or neurotoxicity) of the allogeneic BCMA CAR T-cell product, ALLO-715, in patients with heavily pretreated MM(78). Approximately 90% of patients started treatment within 5 days of study enrollment(78).
BCMA also forms the target for antibody-drug conjugates (e.g. belantamab mafodotin) and bispecific antibodies (e.g. teclistamab, AMG-701, and CC-93269). A small case series showed that serial treatment with different BCMA-targeting agents is feasible(79). Ongoing studies are evaluating in a larger number of patients the efficacy of BCMA CAR T-cell therapy after prior BCMA-directed therapy.

**CAR T-cell-related factors**

*Nature of CAR T-cells infused in patients*

Extent of CAR T-cell expansion is dependent on the number of CAR T-cells administered to patients(5,6,10,12,80). In addition, several studies have demonstrated that peak expansion as well as CAR T-cell persistence are important determinants of response to BCMA CAR T-cell therapy(5,6,9,10,80-83). However, CAR T-cell expansion and persistence were not correlated with best response to cilta-cel, which may be explained by the high affinity binding of these CAR T-cells resulting in rapid elimination of disease(11,13).

Next to quantitative aspects, several qualitative characteristics of the CAR T-cell product, including T-cell functionality, extent of T-cell exhaustion, frequency of less differentiated T-cells, and CD4/CD8 ratio, may influence the efficacy of CAR T-cell therapy. In the bb21217, orva-cel (JCARH125), and P-BCMA-101 studies, patients with a higher proportion of memory-like T-cells in the infused BCMA CAR T-cell product experienced superior expansion, and had a higher probability of response and reduced risk of progression(66,82-84), which was also observed in a CLL trial with CD19 CAR T-cells(67). Preliminary evidence from clinical studies demonstrates that qualitative characteristics of the expanding CAR T-cells are also predictive for response. For example, cell expansion in patients responding to ide-cel was characterized by an increased proportion of CAR T-cells with an effector memory phenotype for both CD4+ and CD8+ subsets.(81) In addition, preclinical studies show that upon repeated antigen encounter, CAR T-cells upregulate inhibitory receptors such as PD-1, TIM-3,
and LAG-3 (45, 85, 86). Similarly, BCMA-targeted CAR T-cells acquire higher PD-1 expression after infusion in patients, which may lead to immune exhaustion and contribute to progression (4, 5). Indeed, it was recently shown that expanding CAR T-cells from patients with sustained response following bb21217 treatment, expressed lower levels of PD-1 and LAG-3, compared to patients who experienced disease progression (66).

Importantly, PD-1 checkpoint blockade with antibodies has the ability to improve CAR T-cell activity and promote tumor cell death (45, 85-87). CAR T-cells can also be engineered to secrete PD-1 or PD-L1 antibodies at the tumor site (88, 89). Moreover, interference with signaling through the endogenous PD-1 receptor by co-transducing a PD-1 dominant negative receptor or a PD-1/CD28 chimeric receptor, enhanced CAR T cell function (85, 90). Similarly, knockdown or knockout of PD-1 in CAR T-cells improved their anti-tumor efficacy (86, 91). Preliminary results show that PD-1 inhibitor-based combination therapy may result in CAR T-cell expansion and anti-MM activity in a subset of patients progressing after BCMA CAR T-cell therapy (92). Other strategies to revert CAR T-cell exhaustion are also explored, including inhibition of different inhibitory immune checkpoints (e.g. LAG-3 or TIM-3) or use of co-stimulatory receptor agonists (e.g., utomilumab).

Manufacturing process

The manufacturing process includes different procedures such as T-cell activation, T-cell expansion, transduction, and storage, which may all affect the characteristics of the CAR T-cell product. Several strategies are explored to improve CAR T-cell fitness by optimizing the manufacturing process (93). Manufacturing can be adapted to generate cell products enriched for specific subsets of T-cells with superior intrinsic abilities for survival and proliferation after infusion in patients (e.g. early memory cells). One strategy is the transduction and expansion of CAR T-cells in the presence of PI3K inhibitors (e.g. idelalisib or bb007), which results in an increased frequency of less differentiated CAR T-cells, decreased expression of PD-1 and TIM-3, improved in vivo persistence, and enhanced activity in
preclinical leukemia and MM models(82,94,95). The CAR T-cell product bb21217 uses the same CAR molecule as ide-cel, but cells are cultured in the presence of bb007, resulting in enrichment for T-cells displaying a memory-like phenotype(82). In the first-in-human study, prolonged CAR T-cell persistence was observed in patients treated with escalating doses of bb21217. However, longer follow-up is required to determine whether this will translate into improved PFS(82). The manufacturing process for orva-cel and P-BCMA-101 is also designed to produce CAR T-cells enriched for central memory T-cell phenotype, but details are not disclosed(23).

Increased understanding of molecular, epigenetic, and metabolic factors that are critical for the formation and maintenance of stem cell-like memory T-cells, may also lead to novel strategies to improve CAR T-cell therapy(96-98). For example, disruption of TET2, depletion of REGNASE-1, or increasing c-Myb levels may also promote the development of memory CAR T-cells and improve CAR T-cell persistence(96-98). Generation of CAR T-cells with optimal differentiation potential and effector activity may also be achieved by using alternative cytokines during manufacturing(67,75). Furthermore, application of modified antigen presenting cells, that provide optimal signals to the CAR T-cells during manufacturing, may enhance overall CAR T-cell expansion or enable the preferential expansion of CAR T-cells with memory phenotype(99).

In addition, the variability of CD4⁺ and CD8⁺ T-cells in the apheresis product results in the production of heterogeneous CAR T-cell products with a large variation in CD4/CD8 ratio, which may contribute to differences in toxicity and activity among patients. Because CD4⁺ T-cell help is essential for durable T-cell immunity, several studies administer CAR T-cells with a consistent CD4/CD8 ratio after separate production of CD4⁺ and CD8⁺ CAR T-cells(24,52). However, it remains an important open research question whether generation of products with homogeneous characteristics will lead to more consistent results in clinical trials. Furthermore, “off-the-shelf” healthy donor-derived CAR T-cells with defined release criteria and minimal inter-donor variability may also lead to more consistent outcomes.
The starting material used to manufacture CAR T-cells may also contribute to manufacturing outcome. For example, high levels of myeloid cells in the starting material results in lower yields of CAR T-cells and increases the risk of product failure (100). This issue can be addressed by applying a T-cell separation strategy.

**CAR structure**

CAR structure affects CAR T-cell fitness, highlighting the importance of improving CAR engineering (Figure 1). CAR T-cell function may be enhanced by changing the antigen-binding domain or costimulatory domains (101). In addition, the design of the hinge region and transmembrane domains of the CAR construct may contribute to the efficiency of immune synapse formation (40).

T-cells expressing a first-generation CAR with the CD3ζ intracellular signaling domain alone have limited activity due to suboptimal activation leading to development of anergy and failure to persist. These limitations can be overcome by the incorporation of additional signaling domains from either CD28, 4-1BB, or OX40, which results in improved CAR T-cell expansion, activation, persistence and anti-tumor activity. Type of costimulatory signaling, has an impact on activity and persistence of CAR T-cells. CD28 costimulatory domains are associated with more rapid expansion and effector cell differentiation and cytotoxic ability of CAR T-cells, while 4-1BB domains may lead to superior persistence with better maintenance of a memory phenotype and reduced exhaustion (55,102-106). Distinct activation of signaling pathways and differential effects on cellular metabolism (with CD28 leading to increased glycolysis and 4-1BB to enhanced mitochondrial oxidative phosphorylation) conferred by these co-receptors, can explain these differences in CAR T-cell function (105). Most BCMA CAR T-cell products, including ide-cel and celta-cel, use a CAR construct with 4-1BB as costimulatory molecule. Application of third generation CARs containing two costimulatory domains may further contribute to improved persistence and enhanced antitumor effects (Figure 1)(101,102).
Excessive CAR signaling as a result of high antigen burden or persistent antigen-independent (tonic) CAR signaling can induce CAR T-cell differentiation and exhaustion, resulting in poor activity \((55,95,101)\). The incorporation of a 4-1BB endodomain instead of CD28 reduced T-cell exhaustion induced by antigen-independent signaling or by persistent antigen exposure, which may explain better persistence of CAR T-cells incorporating 4-1BB in clinical trials \((22,55)\). Tonic signaling can also be reduced by optimizing the length of the spacer, which links the antigen-binding and transmembrane domains, or by targeting of the CAR to the T-cell receptor α constant (TRAC) locus as opposed to random insertion during conventional CAR T-cell manufacturing \((107,108)\). The targeting of CARs to the TRAC locus with CRISPR/Cas9, places the CAR under the control of endogenous regulatory elements, leading to optimal basal and dynamic CAR expression, which improves T-cell potency by preventing tonic CAR signaling, reducing exhaustion, and delaying effector T-cell differentiation \((108)\). Transient rest from CAR signaling has also been shown to protect against T-cell exhaustion \((109)\). In this respect, several innovative strategies are explored to rapidly and reversibly control CAR expression at the cell surface. Both transcriptional \((110)\) and post-transcriptional \((111)\) approaches are currently evaluated in preclinical models. Beyond preventing CAR T-cell exhaustion, controlling CAR expression also has the potential to improve the safety profile of CAR T-cell therapy.

**Immune-mediated rejection**

Immune-mediated rejection may contribute to limited CAR T-cell persistence. In solid tumors and B-cell malignancies non-human antigen-recognition domains or suicide domains can induce humoral or cellular immune responses directed against CAR T-cells, which may result in reduced CAR T-cell counts and loss of activity \((76,112,113)\). Similarly, in the Chinese study with LCAR-B38M, progression was associated with reduced BCMA CAR T-cell numbers and emergence of anti-CAR antibodies \((8)\). Immune-mediated rejection may also limit the ability to treat patients with repeat CAR T-cell infusions. Indeed, development of CAR-specific immune responses explained the limited efficacy of a
second infusion with CD19 CAR T-cells containing a murine scFv(114). Similarly, effectiveness of retreatment with ide-cel is limited (≥PR: 21%; median PFS: 1.0 month), which may in part be related to immune-mediated CAR T-cell rejection(10). All 6 patients, who had a response to retreatment with ide-cel, were antidrug antibody (ADA)-negative, while 73% of the 22 non-responders were ADA-positive(10).

The lymphodepleting conditioning regimen is important to suppress the development of anti-CAR immune responses(113,115). In addition, the immunogenicity of CARs may be reduced by using fully human or humanized CAR constructs(57,112,115). Orva-cel has a fully human BCMA-binding domain. In a phase 1/2 study orva-cel induced a high response rate (≥PR: 92% and ≥CR in 36%) and had an acceptable toxicity profile in 62 patients (94% triple-class refractory), who were treated with 300-600 x10⁶ CAR T-cells(23). Several other BCMA CAR T-cell products with fully human antigen-binding domains are currently evaluated in clinical studies(24,52,56,116), including MCARH171(80,117), FCARH143 (same CAR construct as used for orva-cel)(117), P-BCMA-101(83), CT103A(26), and the CAR T-cell product developed by UPenn(9). Interestingly, deep and durable responses were observed in 4 patients, who received CT103A after failure of a murine BCMA CAR T-cell product(26).

Furthermore, less complex binding domains, such as heavy-chain-only domains, have the potential to decrease immunogenicity(22,25).

CAR T-cells are usually generated by retro- or lentiviral transduction. Non-viral vectors are also explored as a mode of gene transfer, which may decrease immunogenicity and reduce the cost of CAR T-cell production. In this context, transposon vectors (e.g. Sleeping Beauty and PiggyBac DNA transposons) have been shown to mediate stable integration and expression of CAR genes(83). In addition, CAR T-cells can be engineered by mRNA transfection, which eliminates the risk of transgene-mediated mutagenesis(118). However, the transient CAR expression with this method, may require repetitive CAR T-cell dosing(118).
**Bridging therapy**

Bridging therapy is administered to the majority of patients to control disease during the manufacturing process (Figure 3). Ideally, bridging therapy should not interfere with subsequent CAR T-cell expansion and persistence. Therefore, the half-life of the anti-MM agents should be taken into account. In addition, a better understanding is needed to what extent certain bridging therapies can reshape the immune suppressive BM microenvironment into a more permissive microenvironment for CAR T-cell therapy.

**Lymphodepleting conditioning regimen**

The lymphodepleting conditioning regimen (typically fludarabine/cyclophosphamide) prior to CAR T-cell infusion, is important for CAR T-cell expansion and persistence as a result of elimination of immune suppressor cells, prevention of CAR-directed immune responses, and through increased availability of homeostatic cytokines to newly infused cells (Figure 3)(9,114). Although lymphodepletion with fludarabine/cyclophosphamide is effective in patients with MM and other hematological malignancies, this lymphodepleting regimen is also associated with toxicity, such as long-lasting cytopenias and infections(119). Therefore further investigations are warranted to define the most optimal lymphodepleting conditioning regimen prior to CAR T-cell immunotherapy in MM.

**Immune resistance conferred by the tumor microenvironment**

The MM microenvironment, which consists of several components, including BM stromal cells (BMSCs), immune suppressor cells and immunosuppressive molecules, promotes tumor growth and impairs immune responses. Importantly, BMSCs also protect MM cells against CAR T-cells through various mechanisms including secretion of TGF-β and induction of anti-apoptotic proteins in MM
BMSC-mediated resistance can be overcome by increasing the avidity of CAR T-cells or through combination of immunotherapy with inhibitors of anti-apoptotic mediators.

Immune suppressor cells impair CAR T-cell activity in different types of cancer. Although Treg expansion has been described in MM patients without response to BCMA CAR T-cell therapy, the precise role of Tregs in mediating CAR T-cell resistance remains unclear. The impact of other immune suppressor cells, such as myeloid-derived suppressor cells (MDSCs) and immunosuppressive macrophages, on CAR T-cell activity is currently unknown in MM, and therefore all ongoing CAR T-cell trials should be accompanied by immune monitoring studies to increase our understanding of the potential ability of immune suppressor cells to impair both CAR T-cell function and persistence. The MM microenvironment is also rich in immunosuppressive cytokines and molecules. Interestingly, pretreatment levels of IL-10 are elevated in MM patients with suboptimal response following ide-cel treatment. Other immunosuppressive molecules (e.g. TGF-β, indoleamine 2,3-dioxygenase (IDO), arginase and adenosine) have been shown to confer resistance to CAR T-cells in various malignancies, but their role in MM is unclear.

Inhibitory effects from the tumor microenvironment can be partly reversed by engineering “armored” CAR T-cells that have improved ability to withstand the tumor milieu (Figure 1). Such genetic modification strategies include 1) CAR T-cells engineered to release immune stimulatory cytokines upon CAR engagement, 2) neutralization of immune suppressive signals (e.g. incorporation of dominant negative TGF-β receptor), 3) transforming an immunosuppressive signal into an immunostimulatory one by introducing a hybrid receptor, or 4) by removing genes encoding inhibitory immune checkpoints (e.g. PD-1). Furthermore, strategies aiming at depleting, deactivating, or inducing the differentiation of immune suppressor cells, may improve the efficacy of CAR T-cells to eliminate tumor cells. The fludarabine/cyclophosphamide lymphodepletion regimen has the ability to induce non-selective Treg depletion. In contrast, low-dose continuous cyclophosphamide has been shown to selectively deplete Tregs in MM and solid...
tumors, while sparing conventional T-cells, resulting in enhanced conventional T-cell and NK cell functions\cite{130,131}. This suggests that low-dose cyclophosphamide may improve the activity of cellular therapy. Furthermore, all-trans retinoic acid is capable of reducing MDSCs numbers as well as their suppressive capacity\cite{125}. Combination therapy with inhibitors of IDO, adenosine, or arginase may also be a promising strategy to overcome the immunosuppression conferred by the tumor microenvironment. In addition, CAR T-cells simultaneously targeting tumor cells as well as components of the supportive microenvironment, may lead to CAR T-cells that are resistant to microenvironment-induced immunosuppression. For example, CD38-specific CAR T-cells or BCMA/CD38 dual-targeted CAR T-cells have the ability to eliminate CD38$^+$ immune suppressor cells, such as Bregs, in MM patients\cite{50,132}. Bregs were also eradicated by CD19-specific CAR T-cells\cite{49}. Furthermore, in the face of Treg-mediated inhibition, superior functionality of CD28 over 4-1BB signaling was reported, which is possibly explained through enhanced secretion of proinflammatory cytokines in the presence of Tregs by CD28-based CAR T-cells\cite{123}.

Additionally, more advanced and already evaluated in clinical trials, are combination strategies with approved anti-MM agents to improve CAR T-cell function and overcome the immunosuppressive effects of the BM microenvironment. First, CD38-targeting antibodies, such as daratumumab, have the ability to eliminate CD38$^+$ immune suppressor cells such as CD38$^+$ Tregs, Bregs, and MDSCs, which makes this class of anti-MM agents, potential combination partners for CAR T-cell therapy, or an important component of bridging therapy to reshape the tumor microenvironment\cite{133}. CD38 is also contributing to T-cell immunosuppression through the generation of adenosine. Reducing adenosine production with CD38-targeting antibodies may further improve CAR T-cell function\cite{134}. On the other hand, CD38-targeting antibodies may also have a negative effect on CAR T-cell therapy, because activated T-cells have increased CD38 expression. However, we recently demonstrated that CD38 expression on the T-cell surface is rapidly reduced following daratumumab exposure, which prevents their elimination\cite{135}. Second, IMiDs may also be a valuable adjunct to CAR T-cells, because of their broad immunomodulatory effects, including the inhibition of Treg development in MM.
Furthermore, IMiDs enhance T-cell function through the cereblon-dependent degradation of the T-cell repressors Ikaros and Aiolos(136). There is substantial preclinical evidence that the T-cell stimulatory effects of IMiDs can be used in concert with CAR T-cell therapy. Indeed, lenalidomide enhances T-helper (Th) 1-associated cytokine production, decreases secretion of Th2-associated cytokines, and improves immune synapse formation between CAR T-cells and tumor cells, resulting in enhanced cytotoxic activity of CAR T-cells(31,137,138). In MM mouse models, lenalidomide also enhanced the activity and persistence of SLAMF7- and BCMA-targeting CAR T-cells(31,137). Based on these preclinical data, several ongoing clinical studies are evaluating the combination of lenalidomide and CAR T-cells.

Conclusions

Approximately 30 years after the first reports describing engineered T-cells with chimeric scFv receptors(139), CAR T-cell therapy holds great promise in MM with regulatory approval of the first BCMA CAR T-cell products expected in the nearby future. Despite promising results, new strategies are needed to further improve the outcome of CAR T-cell therapy. A better understanding of tumor, host, and product-related features has already resulted into the design of next-generation CAR T-cell products with enhanced cytotoxic ability and improved persistence, as well as better protection against the immunosuppressive microenvironment. Also the application of immunostimulatory anti-MM agents, as opposed to drugs with immunosuppressive effects, prior to T-cell collection, may contribute to improved CAR T-cell activity. Furthermore, several clinical studies are currently evaluating the combination of CAR T-cells with therapies that are able to reduce the impact of the immunosuppressive microenvironment. However, attention should also be paid to increased toxicities such as cytokine release syndrome, that may occur in combination therapies. Finally, the introduction of new targets for CAR T-cell therapy will allow for combinatorial treatment to prevent antigen escape.
Other strategies to redirect T-cells to MM cells are also explored in MM, with promising activity of “off-the-shelf available” bispecific antibodies in patients with triple-class refractory MM(140-145). As opposed to a single infusion of CAR T-cells, bispecific antibodies are typically administered until disease progression. BCMA is also the target for antibody-drug conjugates, such as belantamab mafodotin(146-148). While cross-trial comparisons are challenging because of differences in patient characteristics, design and follow-up duration, single agent activity of CAR T-cells and bispecific antibodies is substantially higher than that of antibody-drug conjugates(146). On the other hand, depth of response with several CAR T-cell products is superior to what has been achieved with bispecific antibodies(11,14). However, studies evaluating bispecific antibodies have relatively short follow-up duration, and therefore depth of response may still improve over time. In addition, the CAR T-cell manufacturing period delays administration, which may be problematic for patients with rapidly progressive disease. Such patients may therefore be underrepresented in CAR T-cell studies, which should be taken into account when CAR T-cell therapy is compared with other types of immunotherapy. Besides efficacy, choice of modality is also dependent on other factors including patient characteristics, disease features, safety profile, availability (approval status and costs), and practical considerations (see Table 3). The safety profile of bispecific antibodies compares favorably with CAR T-cell therapy, with a lower frequency of grade ≥3 neurotoxicity and CRS, and therefore elderly patients may also benefit from treatment with bispecific antibodies(141-145). Although anti-MM activity of antibody-drug conjugates is modest in triple-class refractory MM, CRS and neurotoxicity are not observed(146,147). Hence, these agents may be applied to a larger and more diverse patient population. A limitation of belantamab mafodotin is the frequent development of keratopathy, which may substantially impair quality of life(146). Other cell types, with different killing mechanisms, such as NK cells, invariant NKT cells, γδ T-cells, or myeloid cells, can also be engineered to express a CAR(149,150). However, at this moment, in the absence of clinical data, it is unknown whether adoptive therapy with alternative cell types will be able to overcome resistance conferred
by the tumor microenvironment. Altogether, we expect that these strategies will contribute to
further improvement in survival of MM patients with preserved quality of life.

Acknowledgements

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and 3.
REFERENCES


### Table 1. Selected studies evaluating BCMA-specific CAR T-cells: CAR T-cell features and patient characteristics.

<table>
<thead>
<tr>
<th>NCI</th>
<th>UPenn (Novartis)</th>
<th>Ide-cel (BMS)</th>
<th>bb21217 (BMS)</th>
<th>LCAR-B38M</th>
<th>Citla-cel (Janssen)</th>
<th>Orva-cel (JCARH125; BMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of trial; Clinicaltrials.gov Identifier; reference</td>
<td>Phase 1; NCT0215967(5)</td>
<td>Phase 1; NCT02546167(9)</td>
<td>Phase 1; NCT02658929 (CRB-401)(12)</td>
<td>Phase 2; NCT03361748 (KarMMA)(10)</td>
<td>Phase 1; NCT03090659 (LEGEND-2)(14)</td>
<td>Phase 1b/2; NCT03548207 (CARTITUDE-1)(11)</td>
</tr>
<tr>
<td>Antigen-binding domain</td>
<td>scFv (murine)</td>
<td>scFv (human)</td>
<td>scFv (murine)</td>
<td>scFv (murine)</td>
<td>Bispecific variable fragments of llama heavy-chain antibodies; two distinct BCMA epitopes are targeted</td>
<td>Bispecific variable fragments of llama heavy-chain antibodies; two distinct BCMA epitopes are targeted</td>
</tr>
<tr>
<td>Signaling domains</td>
<td>CD3ζ/CD28</td>
<td>CD3ζ/4-1BB</td>
<td>CD3ζ/4-1BB</td>
<td>CD3ζ/4-1BB</td>
<td>No</td>
<td>CD3ζ/4-1BB</td>
</tr>
<tr>
<td>Lymphodepletion</td>
<td>Flu/Cy</td>
<td>+/ Cy</td>
<td>Flu/Cy</td>
<td>Flu/Cy</td>
<td>Flu/Cy</td>
<td>Cy</td>
</tr>
<tr>
<td>Bridging therapy</td>
<td>0%</td>
<td>84%</td>
<td>52%</td>
<td>88%</td>
<td>NR</td>
<td>Not allowed</td>
</tr>
<tr>
<td>BCMA expression required</td>
<td>yes</td>
<td>no</td>
<td>In dose escalation phase BCMA expression on ≥50% MM cells required, not in dose expansion cohort</td>
<td>No</td>
<td>In dose escalation phase BCMA expression on ≥50% MM cells required, not in dose expansion cohort</td>
<td>yes</td>
</tr>
<tr>
<td>Number of patients included in analysis</td>
<td>24 (16 patients received 9x10⁶ CAR T cells/kg (highest dose-level))</td>
<td>25</td>
<td>62</td>
<td>128</td>
<td>69</td>
<td>57</td>
</tr>
<tr>
<td>Number of prior therapies (median)</td>
<td>10 in highest dose-level</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>IMiD and PI- refractory</td>
<td>NR</td>
<td>96%</td>
<td>81%</td>
<td>89%</td>
<td>80%</td>
<td>NR (60% exposed to prior PI and IMiD)</td>
</tr>
<tr>
<td>CD38 antibody refractory</td>
<td>NR</td>
<td>72%</td>
<td>≥69%</td>
<td>94%</td>
<td>74%</td>
<td>NR, probably 0%</td>
</tr>
<tr>
<td>CAR T-cell dose</td>
<td>0.3-9x10⁶/kg</td>
<td>Cohort 1: 1.5x10⁶</td>
<td>Cohort 2: Cy+1.5x10⁶</td>
<td>Cohort 3: Cy+1.5x10⁶</td>
<td>50-800x10⁶</td>
<td>150-450x10⁶</td>
</tr>
</tbody>
</table>

Abbreviations: NR, not reported.
Twelve out of 97 patients treated with cilta-cel in the CARTITUDE-1 study experienced neurotoxicity which occurred after resolution of cytokine release syndrome and/or immune effector cell-associated neurotoxicity syndrome (ICANS). Five patients experienced movement and/or neurocognitive changes, and 7 had adverse events including nerve palsy and peripheral motor neuropathy.

Table 2. Selected studies evaluating BCMA-specific CAR T-cells: efficacy and safety data.

<table>
<thead>
<tr>
<th>Stage of trial; Clinicaltrials.gov Identifier; reference</th>
<th>NCI</th>
<th>UPenn (Novartis)</th>
<th>Ide-cel (BMS)</th>
<th>bb21217 (BMS)</th>
<th>LCAR-B38M</th>
<th>Cita-cel (Janssen)</th>
<th>Orva-cel (JCARH125; BMS)</th>
</tr>
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<tbody>
<tr>
<td>Phase 1; NCT02215967(5)</td>
<td></td>
<td>Phase 1; NCT02546167(9)</td>
<td>Phase 2; NCT02658929 (CRB-401)(12)</td>
<td>Phase 2; NCT0361748 (KarMMa)(10)</td>
<td>Phase 1; NCT03274219 (CRB-402)(82)</td>
<td>Phase1; NCT03090659 (LEGEND-2)(14)</td>
<td>Phase 1b/2; NCT03548207 (CARTITUDE-1)(11)</td>
</tr>
<tr>
<td>≥PR</td>
<td>0.3-3x10^7/kg; 20% 9x10^5/kg; 81%</td>
<td>48%</td>
<td>50-800x10^5; 76% 450x10^5 (n=38); 90%</td>
<td>150-450x10^5; 73% 150x10^5; 50% 300x10^5; 69% 450x10^5; 81%</td>
<td>150-450x10^5; 68% 150x10^5; 83% 300x10^5; 43% 450x10^5; 73%</td>
<td>88%</td>
<td>97%</td>
</tr>
<tr>
<td>≥CR</td>
<td>0.3-3x10^5/kg; 0% 9x10^5/kg; 13%</td>
<td>8%</td>
<td>50-800x10^5; 39% 450x10^5 (n=38); 37%</td>
<td>150-450x10^5; 33% 150x10^5; 25% 300x10^5; 29% 450x10^5; 39%</td>
<td>150-450x10^5; 29% 150x10^5; 42% 300x10^5; 14% 450x10^5; 30%</td>
<td>74%</td>
<td>67%</td>
</tr>
</tbody>
</table>

MRD-negativity (assay used to assess MRD is also provided)
-100% of 11 evaluable patients treated with 9x10^6 CAR T-cells/kg and with ≥PR
-NGF (1x10^5 depth)
-57% of 7 patients with ≥VGPR
-NGF (1x10^5 depth)
-100% of 15 evaluable patients with ≥CR
-NGS (1x10^5 depth)
-75% of the 67 patients with ≥VGPR
-79% of the 42 patients with ≥CR
-NGS (1x10^5 depth)
-89% of 28 evaluable patients with ≥PR
-100% of 13 evaluable patients with ≥CR
-NGS (1x10^5 depth)
-93% of the 42 patients with ≥CR
-NGF (~1x10^5 depth)

Median PFS
-9x10^7/kg; 31 weeks (EFS)
Cohort 1: 65 days
Cohort 2: 57 days
Cohort 3: 125 days
-50-800x10^5 cells: 8.8 months
450x10^5 cells: 9.0 months
150-450x10^5; 8.8 months
150x10^5; 2.8 months
300x10^5; 5.8 months
450x10^5; 12.1 months
PFS not reported;
median duration of response: 17.0 months
19.9 months
Not reached; 12-month PFS rate: 77%
300x10^7; 9.3 months
450x10^7; not reached
600x10^7; not reached

Cytokine release syndrome (any grade)
• 0.3-3x10^7/kg; 40% 9x10^5/kg; 94%
88%
76%
84%
65%
90%
95%
89%

Grade ≥3 cytokine release syndrome
• 0.3-3x10^5/kg; 0% 9x10^5/kg; 38%
32%
7%
5%
4%
7%
4%
3%

Neurotoxicity (any grade)
NR
32%
36%
18%
16%
2%
21%
13%

Grade ≥3 neurotoxicity
NR
12%
2%
3%
4%
0%
10%
3%
Abbreviations: NR, not reported; NGS, next-generation sequencing; NGF, next-generation flow cytometry; PR, partial response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response.
Table 3. Comparison of BCMA-directed immunotherapies for patients with advanced MM (mostly triple-class refractory MM).

<table>
<thead>
<tr>
<th></th>
<th>Autologous CAR T-cell therapy(10,11,23,82)</th>
<th>Bispecific antibodies(140-145)</th>
<th>Antibody-drug conjugates(146-148)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥PR</td>
<td>≥70-97%</td>
<td>60-80% at higher dose levels</td>
<td>Around 30%</td>
</tr>
<tr>
<td>≥CR</td>
<td>30-67%</td>
<td>Around 20% at higher dose levels</td>
<td>Around 3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depth of response may still improve given the relatively short follow-up</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRS</td>
<td>65-95% (grade ≥3: 3-5%)</td>
<td>39-77% (grade ≥3 in 0-9%)</td>
<td>Not observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRS is generally confined to step-up and first full doses</td>
<td></td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>13-21% (grade ≥3: 3-10%)</td>
<td>0-20% (grade ≥3: 0-1%)</td>
<td>Not observed</td>
</tr>
<tr>
<td>Ocular toxicity</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Ocular toxicity/keratopathy common</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Belantamab mafodotin 2.5 mg/kg: grade ≥3 keratopathy: 27% [any grade: 71%]</td>
</tr>
<tr>
<td><strong>Practical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>considerations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Availability</td>
<td>Currently only available in clinical trials</td>
<td>Currently only available in clinical trials</td>
<td>Belantamab mafodotin is approved</td>
</tr>
<tr>
<td>Number of</td>
<td>Generally a single infusion, followed by drug holiday</td>
<td>Treatment until progression</td>
<td>Treatment until progression</td>
</tr>
<tr>
<td>administrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization</td>
<td>Inpatient treatment</td>
<td>Hospitalization often required during step-up and first full doses</td>
<td>Fully outpatient</td>
</tr>
<tr>
<td>Route of administration</td>
<td>IV</td>
<td>IV or SC</td>
<td>IV</td>
</tr>
<tr>
<td>Infrastructure</td>
<td>Requires dedicated facilities (e.g. cell therapy unit), and input from dedicated infectious disease specialists, intensive care physicians, and neurologists</td>
<td>Can be offered in most hospitals, but intensive care unit should be present for management of severe CRS</td>
<td>Baseline and follow-up assessments by ophthalmologist are required to manage ocular toxicity</td>
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<tr>
<td>Off-the-shelf available</td>
<td>No (but allogeneic CAR T-cells are off-the-shelf available)</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Other features</td>
<td>Gene editing will contribute to next-generation CAR T-cell products with enhanced killing capacity and improved persistence</td>
<td>Several other targets explored</td>
<td>Several other targets explored</td>
</tr>
<tr>
<td></td>
<td>Several other targets explored</td>
<td>Trispecific antibodies and bi/trispecific NK cell engagers in (pre)clinical development</td>
<td>Other types of immunoconjugates are in clinical development, such as immunocytokines and immunotoxins</td>
</tr>
</tbody>
</table>
**Figure legends**

**Figure 1. Evolution of CAR design.** The 1st generation CARs mediate antigen recognition and T-cell activation through the fusion of an extracellular antigen-binding single-chain-variable region (scFv) with an intracellular signaling domain from the CD3ζ chain. In this way surface antigens can be recognized by CAR T cells independent of major-histocompatibility-complex (HLA)-mediated presentation. Second generation CARs provide combined activation and costimulatory signals through the addition of the intracellular domain of costimulatory receptors. The 3rd generation CARs consist of two costimulatory domains. In the latest 4th generation design, CARs are co-expressed with enzymes, cytokines and costimulatory ligands or receptors transferred with the same vector construct.

Abbreviations: scFv, single-chain fragment variable; TM: transmembrane.

**Figure 2. Determinants of response to CAR T-cell therapy.**

Various factors, including tumor-related features, MM microenvironment-related factors, premanufacturing T-cell characteristics and CAR T-cell characteristics, have an impact on response to CAR T-cell therapy.

**Figure 3. Impact of therapy on CAR T-cell activity.**

Treatment prior to leucapheresis, as well as bridging therapy administered to the patient during the production of CAR T-cells, and lymphodepleting chemotherapy prior to CAR T-cell infusion, can have an impact on the anti-tumor effect of CAR T-cells. In addition, in the setting of clinical trials, several
agents are administered post CAR T-cell infusion (e.g., IMiDs and CD38-targeting antibodies) to enhance CAR T-cell efficacy or improve CAR T-cell persistence.
Chimeric antigen receptors (CARs)

1st generation
- scFv
- Costimulation: CD3ζ

2nd generation
- 4-1BB
- OX-40
- ICOS
- CD28
- CD3ζ

3rd generation
- CD28
- 4-1BB
- OX-40
- ICOS
- CD3ζ

4th generation
- Cytokines
- Enzymes
- scFv
- Costimulatory ligands/receptors
**MM microenvironment-related factors**
- BM stromal cells
- Immune suppressor cells
- Immunosuppressive molecules
- Type of bridging therapy
- Lymphodepleting conditioning regimen

**Tumor-related features**
- Antigen expression levels
- Presence of soluble antigen
- Antigen loss or diminished antigen expression
- Tumor load
- High-risk cytogenetic aberrations
- Extramedullary disease
- Resistance to effector mechanisms of T-cells
- Inhibitory receptors and ligands, which suppress T-cell function

---

**Premanufacturing T-cell characteristics**
- Absolute T-cell count
- Proportion of T-cells with an early memory phenotype
- CD4/CD8 ratio
- Impact of prior therapy on T-cell fitness

**CAR T-cell characteristics**
- Qualitative characteristics of the CAR T-cell product (frequency of less differentiated CAR T-cells, extent of CAR T-cell exhaustion, CD4/CD8 ratio)
- CAR T-cell dose
- Manufacturing process
- CAR-related factors:
  - Affinity of the antigen-binding domain
  - Type of costimulatory domain
  - Design of the hinge region and transmembrane domains
  - Immunogenicity of CARs (fully human/humanized CAR constructs or non-human domains)
**Figure 3**

**Treatment prior to CAR T-cell therapy**
- Impact on T-cell numbers, frequency of T-cell subsets, and T-cell fitness
- Impact on BM microenvironment
- Possible impact prior BCMA-targeted therapy on BCMA cell surface expression on MM cells

**Combination therapy**
- Clinical Studies are evaluating combination therapy with agents, which improve CAR T-cell activity or persistence by direct effects on CAR T-cells or by overcoming the immunosuppressive effects of the BM microenvironment

**Leucapheresis**
- Disease control during CAR T-cell manufacturing
- Possible impact on CAR T-cell function, depending on half-life of the administered agents
- Possible impact on BM microenvironment

**CAR T-cell infusion**
- Elimination of immune suppressor cells
- Prevention of CAR-directed immune responses
- Increased availability of homeostatic cytokines to infused CAR T-cells

**Bridging therapy during CAR T-cell manufacturing**
- Lympophodelting conditioning regimen

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Niels W.C.J. van de Donk, Maria Themeli and Saad Zafar Usmani

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