REVIEW

Determinants of Response and Mechanisms of Resistance of CAR T-cell Therapy in Multiple Myeloma

Niels W.C.J. van de Donk[1], Maria Themeli[1], and Saad Z. Usmani[2]

ABSTRACT

B-cell maturation antigen (BCMA)–specific chimeric antigen receptor (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 patients with MM.

Significance: Although BCMA-specific CAR T-cell therapies are highly effective in heavily pretreated patients with MM, there has been, until now, no indication of a plateau in the survival curves. In this review, we 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–multiple myeloma (MM) agents has markedly improved the survival of patients with MM, there is an unmet need for new drugs for patients who develop resistance to immunomodulatory drugs (IMiD), proteasome inhibitors (PI), 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 (CAR). 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; ref. 2] with a T-cell activation domain, typically CD3ζ. These two 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 antitumor activity of the modified T cells compared with first-generation CARs without such domain (Fig. 1; ref. 3). Importantly, CAR T cells eliminate tumor cells in a non–major histocompatibility complex (MHC)–restricted manner.

Most CAR T-cell products, currently evaluated in clinical trials for patients with MM, 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 and 2. CAR T cells specific for other MM-associated antigens, such as CD19, SLAMF7, CD38, and GPRC5D, are also being 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

[1]Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands. [2]Levine Cancer Institute, Carolinas Healthcare System, Charlotte, North Carolina.

Corresponding Author: Niels W.C.J. van de Donk, Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081HV Amsterdam, the Netherlands. Phone: 31-20-4442604; Fax: 31-20-44442601; E-mail: n.vandedonk@vumc.nl

Blood Cancer Discov 2021;2:302–18

doi: 10.1158/2643-3230.BCD-20-0227

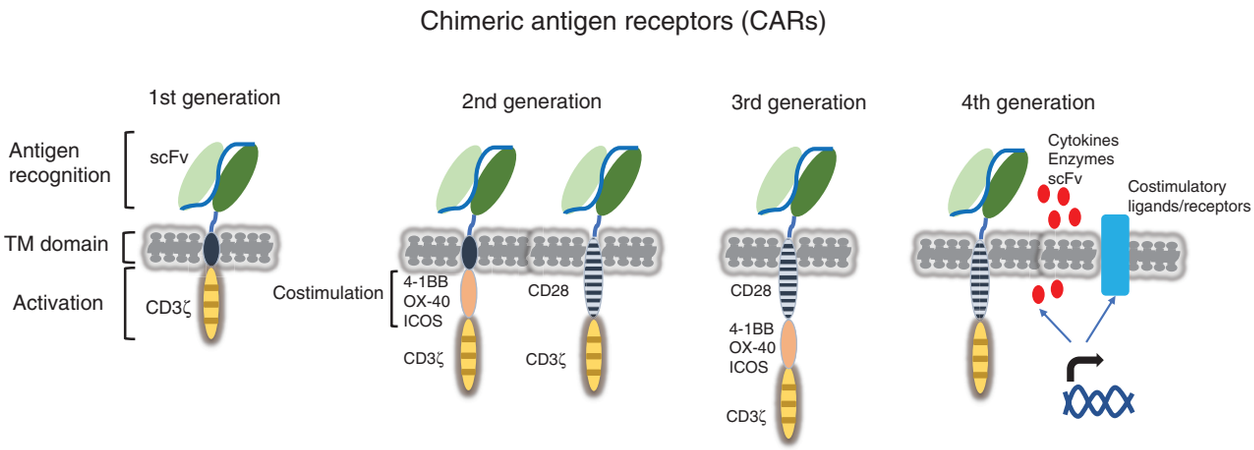

Figure 1. Evolution of CAR design. First-generation CARs mediate antigen recognition and T-cell activation through the fusion of an extracellular antigen-binding single-chain variable region (scFv) with an intracellular signaling domain from the CD3ζ chain. In this way, surface antigens can be recognized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

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 progression-free survival (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, Abecma, bb2121) and ciltacabtagene autoleucel (cilta-cel, JNJ4528; refs. 6, 10, 11). The FDA approved ide-cel in March 2021 for the treatment of relapsed/refractory MM (RRMM) patients after four or more prior therapies, including an IMiD, a PI, and a CD38-targeting antibody (6, 10). In addition, cilta-cel received FDA breakthrough designation based on promising results in heavily pretreated patients (11). However, not all patients achieve a remission after CAR T-cell therapy. Furthermore, there has been, 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 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 (Fig. 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 density among patients with MM on clinical outcomes following CAR T-cell therapy is not completely clear (2). Several studies show that response, PFS, and overall survival 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 pretreatment 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 IHC (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), whereas 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 and colleagues 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 nonresponding patients (9). In some of these patients, BCMA levels were restored 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

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

	NCI	UPenn (Novartis)	Ide-cel (BMS)		bb21217 (BMS)	LCAR-B38M	Cilta-cel (Janssen)	Orva-cel (JCARH125; BMS)
Stage of trial; ClinicalTrials.gov Identifier; reference	Phase I; NCT02215967 (5)	Phase I; NCT02546167 (9)	Phase I; NCT02658929 (CRB-401; ref. 12)	Phase II; NCT03361748 (KarMMa; ref. 10)	Phase I; NCT03274219 (CRB-402; ref. 82)	Phase I; NCT03090659 (LEGEND-2; ref. 14)	Phase Ib/II; NCT03548207 (CARTITUDE-1; ref. 11)	Phase I/II; NCT03430011 (EVOLVE; ref. 23)
Antigen-binding domain	scFv (murine)	scFv (human)	scFv (murine)	scFv (murine)	scFv (murine)	Bispecific variable fragments of llama heavy-chain antibodies; two distinct BCMA epitopes are targeted	Bispecific variable fragments of llama heavy-chain antibodies; two distinct BCMA epitopes are targeted	scFv (human)
Signaling domains	CD3ζ/CD28	CD3ζ/4-1BB	CD3ζ/4-1BB	CD3ζ/4-1BB	CD3ζ/4-1BB	CD3ζ/4-1BB	CD3ζ/4-1BB	CD3ζ/4-1BB
Lymphodepletion	Flu/Cy	± Cy	Flu/Cy	Flu/Cy	Flu/Cy	Cy	Flu/Cy	Flu/Cy
Bridging therapy	0%	84%	52%	88%	NR	Not allowed	65%	63%
BCMA expression required	Yes	No	In dose-escalation phase, BCMA expression on ≥50% MM cells required, not in the dose-expansion cohort	No	In dose-escalation phase, BCMA expression on ≥50% MM cells required, not in the dose-expansion cohort	Yes	No	No
Number of patients included in analysis	24 (16 patients received 9×10^6 CAR T cells/kg (highest dose level)	25	62	128	69	57	97	62
Number of prior therapies (median)	10 in highest dose level	7	6	6	6	3	6	6
IMiD and PI refractory	NR	96%	81%	89%	80%	NR (60% exposed to prior PI and IMiD)	NR; 88% triple-class refractory	NR; 94% triple-class refractory
CD38 antibody refractory	NR	72%	≥69%	94%	74%	NR; probably 0%	99%	≥94%
CAR T-cell dose	$0.3–9\times10^6$/kg	Cohort 1: $1–5\times10^8$ Cohort 2: Cy+$1–5\times10^7$ Cohort 3: Cy+$1–5\times10^8$	$50–800\times10^6$	$150–450\times10^6$	$150–450\times10^6$	Median dose: 0.5×10^6/kg	Median dose: 0.71×10^6/kg	$300–600\times10^6$

Abbreviations: BMS, Bristol Myers Squibb; cilta-cel, ciltacabtagene autoleucel; Cy, cyclophosphamide; Flu, fludarabine; ide-cel, idecabtagene vicleucel; NR, not reported; orva-cel, orvacabtagene autoleucel; UPenn, University of Pennsylvania.

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

	NCI	UPenn (Novartis)	Ide-cel (BMS)		bb21217 (BMS)	LCAR-B38M	Cilta-cel (Janssen)	Orva-cel (JCARH125; BMS)
Stage of trial; ClinicalTrials.gov Identifier; reference	Phase I; NCT02215967 (5)	Phase I; NCT02546167 (9)	Phase I; NCT02658929 (CRB-401; ref. 12)	Phase II; NCT03361748 (KarMMa; ref. 10)	Phase I; NCT03274219 (CRB-402; ref. 82)	Phase I; NCT03090659 (LEGEND-2; ref. 14)	Phase Ib/II; NCT03548207 (CARTITUDE-1; ref. 11)	Phase I/II; NCT03430011 (EVOLVE; ref. 23)
≥PR	$0.3–3\times10^6$/kg: 20% 9×10^6/kg: 81%	48%	$50–800\times10^6$: 76% 450×10^6 ($n=38$): 90%	$150–450\times10^6$: 73% 150×10^6: 50% 300×10^6: 69% 450×10^6: 81%	$150–450\times10^6$: 68% 150×10^6: 83% 300×10^6: 43% 450×10^6: 73%	88%	97%	92%
≥CR	$0.3–3\times10^6$/kg: 0% 9×10^6/kg: 13%	8%	$50–800\times10^6$: 39% 450×10^6 ($n=38$): 37%	$150–450\times10^6$: 33% 150×10^6: 25% 300×10^6: 29% 450×10^6: 39%	$150–450\times10^6$: 29% 150×10^6: 42% 300×10^6: 14% 450×10^6: 30%	74%	67%	36%
MRD negativity (assay used to assess MRD is also provided)	• 100% of 11 evaluable patients treated with 9×10^6 CAR T cells/kg and with ≥PR • NGF (0.0007% depth)	• 57% of 7 patients with ≥VGPR • NGF (1×10^{-5} depth)	• 100% of 15 evaluable patients with ≥CR • NGS (1×10^{-4} depth)	• 75% of 67 patients with ≥VGPR • 79% of 42 patients with ≥CR • NGS (1×10^{-5} depth)	• 89% of 28 evaluable patients with ≥PR • 100% of 13 evaluable patients with ≥CR • NGS (1×10^{-5} depth)	• 93% of the 42 patients with CR • NGF (~10^{-4} depth)	• 93% of 57 evaluable patients • NGS (1×10^{-5} depth)	• 84% of 25 evaluable patients with ≥PR at month 3 • NGS (1×10^{-5} depth)
Median PFS	9×10^6/kg: 31 weeks (EFS)	Cohort 1: 65 days Cohort 2: 57 days Cohort 3: 125 days	$50–800\times10^6$ cells: 8.8 months 450×10^6 cells: 9.0 months	$150–450\times10^6$: 8.8 months 150×10^6: 2.8 months 300×10^6: 5.8 months 450×10^6: 12.1 months	PFS not reported; median duration of response: 17.0 months	19.9 months	Not reached; 12-month PFS rate: 77%	300×10^6: 9.3 months 450×10^6: not reached 600×10^6: not reached
Cytokine release syndrome (any grade)	$0.3–3\times10^6$/kg: 40% 9×10^6/kg: 94%	88%	76%	84%	65%	90%	95%	89%
Grade ≥3 cytokine release syndrome	$0.3–3\times10^6$/kg: 0% 9×10^6/kg: 38%	32%	7%	5%	4%	7%	4%	3%
Neurotoxicity (any grade)	NR	32%	36%	18%	16%	2%	21%[a]	13%
Grade ≥3 neurotoxicity	NR	12%	2%	3%	4%	0%	10%[a]	3%

Abbreviations: BMS, Bristol Myers Squibb; cilta-cel, ciltacabtagene autoleucel; CR, complete response; EFS, event-free survival; ide-cel, idecabtagene vicleucel; NGF, next-generation flow cytometry; NGS, next-generation sequencing; NR, not reported; orva-cel, orvacabtagene autoleucel; PFS, progression-free survival; sCR, stringent complete response; UPenn, University of Pennsylvania; VGPR, very good partial response.

[a]Twelve 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. Five patients experienced movement and/or neurocognitive changes, and seven had adverse events including nerve palsy and peripheral motor neuropathy.

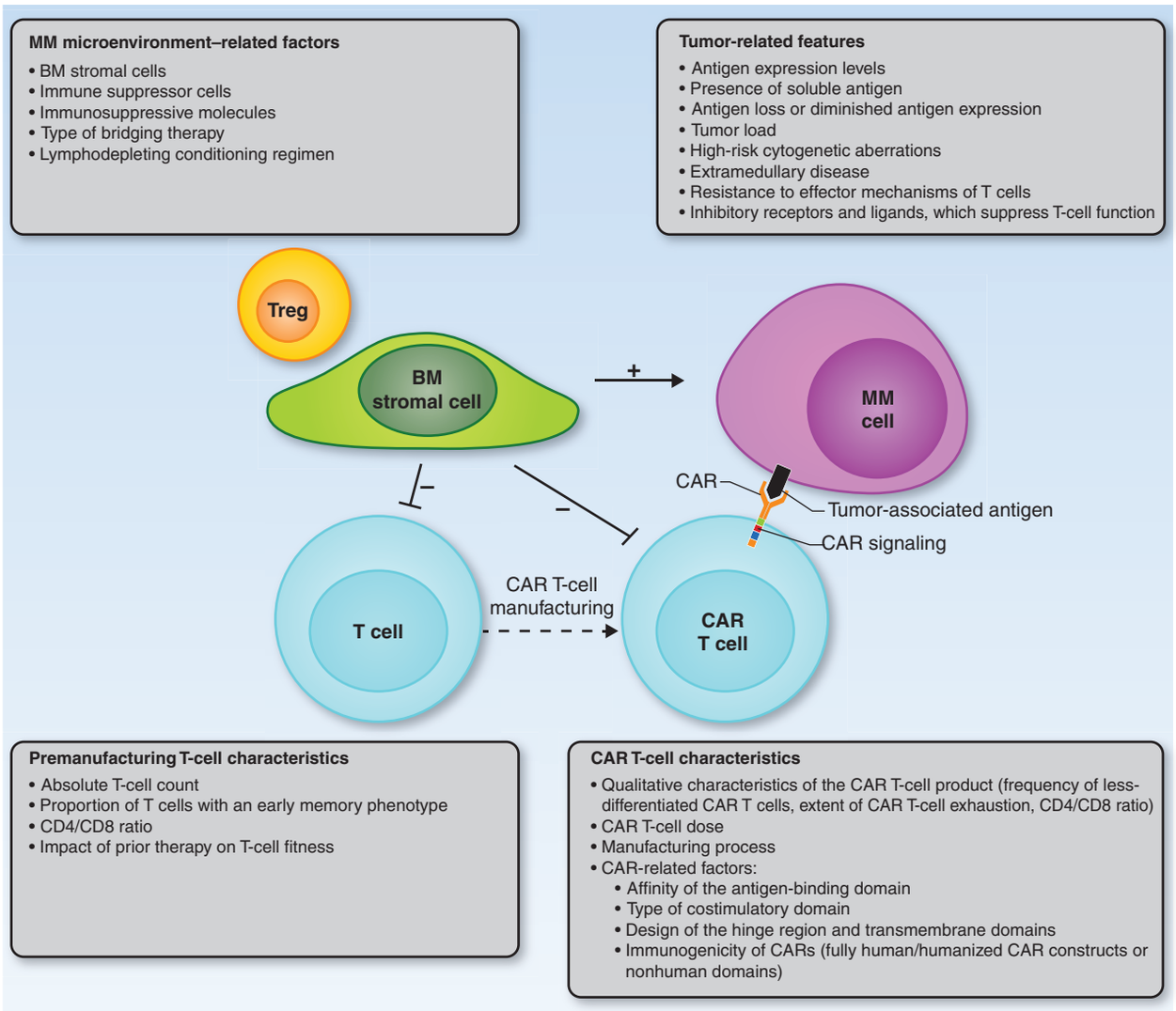

Figure 2. Determinants of response to CAR T-cell therapy. Various factors, including tumor-related features, MM microenvironment–related factors, premanufacturing T-cell characteristics, and CAR T-cell characteristics, have an impact on response to CAR T-cell therapy. BM, bone marrow; Treg, regulatory T cell.

levels, whereas 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; ref. 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% to 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 being 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 have low or ultralow levels of CD19, which 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 patients with MM who previously received auto-SCT with PFS of less than 1 year (37, 38). Two 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 (coinfusion of two 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 two clinical products (40). Growth competition can be avoided by using dual-targeted CAR T cells [single CAR T cells expressing two distinct CARs with different binding domains or CAR T cells expressing a single CAR molecule with two separate binding domains in tandem (tandem CAR); refs. 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, *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 patients with RRMM have demonstrated 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 I 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 who 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 cytokine release syndrome (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 toward a moderately lower CR rate with ide-cel in patients with high disease burden [≥50% bone marrow (BM)–localized MM cells] when compared with patients with a relatively low tumor load (29% vs. 37% in the KarMMa study; refs. 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 patients with RRMM 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), whereas response was similar in others (10, 54). The strongest predictor for clinical activity of ide-cel was the Revised International Staging System (R-ISS), reflecting a combination of risk factors including high tumor load, high-risk cytogenetic abnormalities, and/or elevated lactate dehydrogenase [≥partial response (PR): 48% vs. 80%; ≥CR: 10% vs. 39%; median PFS: 4.9 vs. 11.3 months for R-ISS stage 3 vs. R-ISS stage 1 or 2 in the KarMMa study; refs. 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 the section "Nature of CAR T-cells Infused in Patients").

In addition, a CRISPR-based screen in an 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, whereas 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 antiapoptotic molecules, including serine protease inhibitors (serpins), which inactivate granzyme B (59). Furthermore, death receptor–mediated apoptosis can be prevented by overexpression of the antiapoptotic 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 I or II molecules, or defects in the antigen-processing machinery will not impair CAR T-cell–mediated tumor cell killing.

CHARACTERISTICS OF T CELLS COLLECTED FROM PATIENTS

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 discuss several baseline characteristics of the premanufacturing 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 patients with MM 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, PIs, and dexamethasone) or immunostimulating anti-MM therapies (IMiDs; Fig. 3; refs. 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 patients in clinical trials. First, several BCMA CAR T-cell studies show that patients with MM 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 with 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 patients with chronic lymphocytic leukemia (CLL), ALL, and lymphoma 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 with more differentiated T cells (69). In addition, a higher CD4/CD8 ratio in the leukapheresis 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 IL2, 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 inhibitory immune checkpoint receptors, 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 Patients

Cumulative exposure to several anti-MM drugs will reduce T-cell numbers or induce functional T-cell defects (Fig. 3; ref. 72, 73). Interestingly, the frequency of early memory T cells and CD4/CD8 ratio was higher in apheresis samples from patients with MM who were early in their disease course compared with 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 leukapheresis 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, patients with CLL 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 patients with ALL and non-Hodgkin lymphoma (68). Currently, only limited data are available in MM. One study showed that type of therapy prior to apheresis was not associated with response or CAR T-cell expansion (9), whereas 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 with patients without daratumumab as part of last line treatment (≥PR: 91% vs. 75%; ref. 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

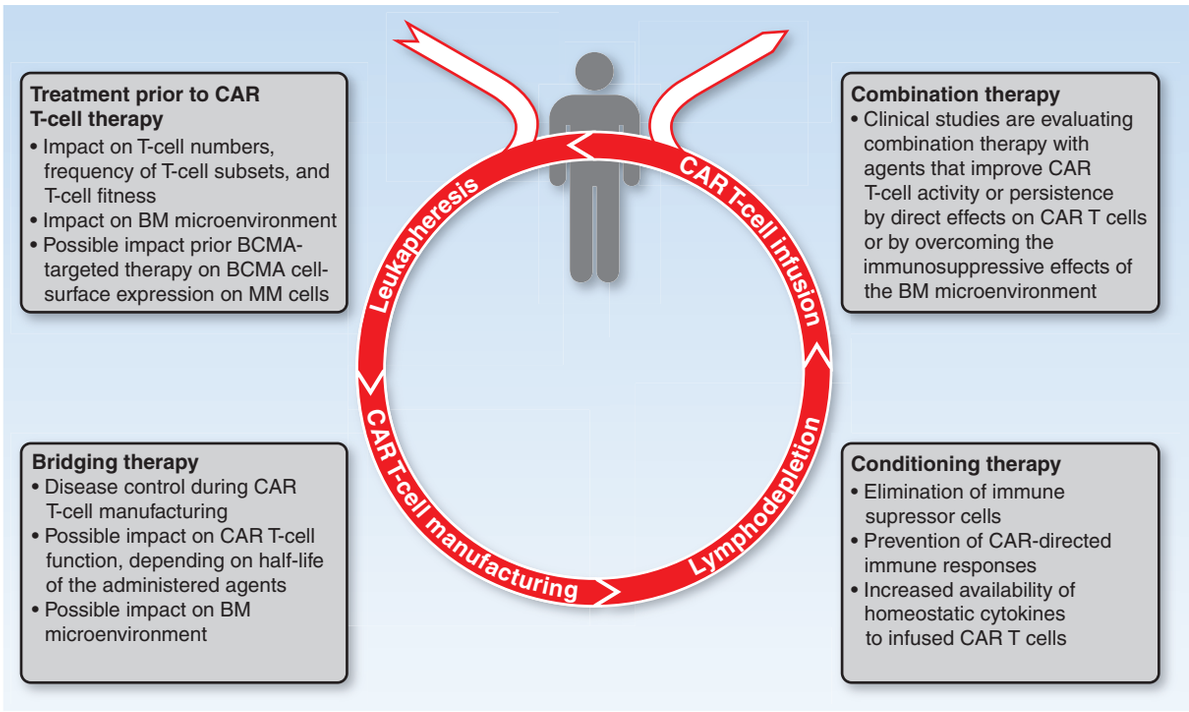

Figure 3. Impact of therapy on CAR T-cell activity. Treatment prior to leukapheresis, bridging therapy administered to the patient during the production of CAR T cells, and lymphodepleting chemotherapy prior to CAR T-cell infusion can have an impact on the antitumor effect of CAR T cells. In addition, in the setting of clinical trials, several agents are administered after CAR T-cell infusion (e.g., IMiDs and CD38-targeting antibodies) to enhance CAR T-cell efficacy or improve CAR T-cell persistence.

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 the 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

The 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 and 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, orvacabtagene autoleucel (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 idecel 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 with 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 cotransducing 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 antitumor 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 being explored, including inhibition of different inhibitory immune checkpoints (e.g., LAG-3 or TIM-3) or use of costimulatory receptor agonists (e.g., utomilumab).

Manufacturing Process

The manufacturing process includes different procedures such as T-cell activation, T-cell expansion, transduction, and storage, all of which may affect the characteristics of the CAR T-cell product. Several strategies are being 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 a central memory T-cell phenotype, but details have not been 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, which 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 are administering 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 interdonor 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 result in lower yields of CAR T cells and increase 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 (Fig. 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 antitumor 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, whereas 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 coreceptors can explain these differences in CAR T-cell function (105). Most BCMA CAR T-cell products, including ide-cel and cilta-cel, use a CAR construct with 4-1BB as a costimulatory molecule. Application of third-generation CARs containing two costimulatory domains may further contribute to improved persistence and enhanced antitumor effects (Fig. 1; refs. 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 being explored to rapidly and reversibly control CAR expression at the cell surface. Both transcriptional (110) and posttranscriptional (111) approaches are currently being 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, nonhuman 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, whereas 73% of the 22 nonresponders 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 I/II study, orva-cel induced a high response rate (≥PR: 92%; ≥CR: 36%) and had an acceptable toxicity profile in 62 patients (94% triple-class refractory) who were treated with 300–600 × 10^6 CAR T cells (23). Several other BCMA CAR T-cell products with fully human antigen–binding domains are currently being evaluated in clinical studies (24, 52, 56, 116), including MCARH171 (80, 117), FCARH143 (same CAR construct as used for orva-cel; ref. 117), P-BCMA-101 (83), CT103A (26), and the CAR T-cell product developed by the University of Pennsylvania (9). Interestingly, deep and durable responses were observed in four 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. Nonviral vectors are also being 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 (Fig. 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 as 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 increased availability of homeostatic cytokines to newly infused cells (Fig. 3; refs. 9, 114). Although lymphodepletion with fludarabine/cyclophosphamide is effective in patients with MM and other hematologic 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 (BMSC), 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 TGFβ and induction of antiapoptotic proteins in MM cells (120, 121). BMSC-mediated resistance can be overcome by increasing the avidity of CAR T cells or through combination of immunotherapy with inhibitors of antiapoptotic mediators (120).

Immune suppressor cells impair CAR T-cell activity in different types of cancers (122–125). Although regulatory T cell (Treg) expansion has been described in patients with MM without response to BCMA CAR T-cell therapy (27), 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 (MDSC) 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 IL10 are elevated in patients with MM with suboptimal response following ide-cel treatment (81). Other immunosuppressive molecules [e.g., TGFβ, indoleamine 2,3-dioxygenase (IDO), arginase, and adenosine] have been shown to confer resistance to CAR T cells in various malignancies (126), 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 (Fig. 1). Such genetic modification strategies include (i) CAR T cells engineered to release immune-stimulatory cytokines upon CAR engagement, (ii) neutralization of immune-suppressive signals (e.g., incorporation of dominant-negative TGFβ receptor), (iii) transforming an immunosuppressive signal into an immunostimulatory one by introducing a hybrid receptor, or (iv) removing genes encoding inhibitory immune checkpoints (e.g., PD-1; refs. 87, 107, 127–129). Furthermore, strategies aimed at depleting, deactivating, or inducing the differentiation of immune suppressor cells may improve the efficacy of CAR T cells to eliminate tumor cells (125). The fludarabine/cyclophosphamide lymphodepletion regimen has the ability to induce nonselective 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 natural killer (NK) cell functions (130, 131). This suggests that low-dose cyclophosphamide may improve the activity of cellular therapy. Furthermore, all-trans retinoic acid is capable of reducing MDSC numbers as well as their suppressive capacity (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 regulatory B cells (Breg), in patients with MM (50, 132). Bregs were also eradicated by CD19-specific CAR T cells (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 (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 a potential combination partner for CAR T-cell therapy or an important component of bridging therapy to reshape the tumor microenvironment (133). CD38 also contributes to T-cell immunosuppression through the generation of adenosine. Reducing adenosine production with CD38-targeting antibodies may further improve CAR T-cell function (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 T-cell elimination (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 recent FDA approval of the first BCMA CAR T-cell product ide-cel, and expected regulatory approval of cilta-cel in the near 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 in 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 CRS, that may occur in combination therapies. Finally, the introduction of new targets for CAR T-cell therapy will allow for combinatorial treatments to prevent antigen escape.

Other strategies to redirect T cells to MM cells are also being 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). Although cross-trial comparisons are challenging because of differences in patient characteristics, design, and follow-up duration, single-agent activity of CAR T cells

Table 3. Comparison of BCMA-directed immunotherapies for patients with advanced MM (mostly triple-class refractory MM)

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

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 the survival of patients with MM, with preserved quality of life.

Authors' Disclosures

N.W.C.J. van de Donk reports grants and other support from Janssen Pharmaceuticals, Amgen, Celgene, Bristol Myers Squibb; grants from Cellectis; and other support from Adaptive, Novartis, Roche, Takeda, Bayer, and Servier outside the submitted work. M. Themeli reports grants from Multiple Myeloma Research Foundation and Foundation Cancer Center Amsterdam during the conduct of the study, as well as a patent for effective generation of tumor-targeted T cells derived from pluripotent stem cells licensed and with royalties paid from Fate Therapeutics. S.Z. Usmani reports grants and personal fees from Amgen, Bristol Myers Squibb, Celgene, Sanofi, Seattle Genetics, SkylineDX, Takeda, Janssen, Merck, GlaxoSmithKline, and Teneobio; personal fees from AbbVie and Mundi Pharma; and grants from Pharmacyclics outside the submitted work.

Acknowledgments

The authors thank Victor Muñoz Sanz (Sanz Serif Research + Design Agency) for creating Figs. 2 and 3.

Received December 17, 2020; revised February 18, 2021; accepted March 28, 2021; published first April 26, 2021.

REFERENCES

1. Gandhi UH, Cornell RF, Lakshman A, Gahvari ZJ, McGehee E, Jagosky MH, et al. Outcomes of patients with multiple myeloma refractory to CD38-targeted monoclonal antibody therapy. Leukemia 2019;33:2266–75.
2. Lee L, Draper B, Chaplin N, Philip B, Chin M, Galas-Filipowicz D, et al. An APRIL-based chimeric antigen receptor for dual targeting of BCMA and TACI in multiple myeloma. Blood 2018;131:746–58.
3. Maher J, Brentjens RJ, Gunset G, Riviere I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. Nat Biotechnol 2002;20:70–5.
4. Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, et al. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. Blood 2016;128:1688–700.
5. Brudno JN, Maric I, Hartman SD, Rose JJ, Wang M, Lam N, et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. J Clin Oncol 2018;36:2267–80.
6. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 2019;380:1726–37.
7. Zhao WH, Liu J, Wang BY, Chen YX, Cao XM, Yang Y, et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. J Hematol Oncol 2018;11:141.
8. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc Natl Acad Sci U S A 2019;116:9543–51.
9. Cohen AD, Garfall AL, Stadtmauer EA, Melenhorst JJ, Lacey SF, Lancaster E, et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. J Clin Invest 2019;129:2210–21.
10. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med 2021;384:705–16.
11. Madduri D, Berdeja J, Usmani S, Jakubowiak A, Agha M, Cohen A, et al. CARTITUDE-1: phase 1b/2 study of ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy, in relapsed/refractory multiple myeloma. Blood 2020;136:177.
12. Lin Y, Raje N, Berdeja J, Siegel D, Jagannath S, Madduri D, et al. Idecabtagene vicleucel (ide-cel, bb2121), a BCMA-directed CAR T cell therapy, in patients with relapsed and refractory multipe myeloma: updated results from phase 1 CRB-401 study. Blood 2020;136:131.
13. Zudaire E, Madduri D, Usmani S, Jakubowiak A, Berdeja J, Geng D, et al. Translational analysis from CARTITUDE-1, an ongoing phase 1b/2 study of JNJ-4528 BCMA-targeted CAR-T cell therapy in relapsed and/or refractory multiple myeloma (R/R MM), indicates preferential expansion of $CD8^+$ T cell central memory cell subset. Blood 2019;134:928.
14. Wang B, Zhao W, Liu J, Chen Y, Cao X, Yang Y, et al. Long-term follow-up of a phase 1, first-in-human open-label study of LCAR-B38M, a structurally differentiated chimeric antigen receptor T (CAR-T) cell therapy targeting B-cell maturation antigen (BCMA), in patients (pts) with relapsed/refractory multiple myeloma (RRMM). Blood 2019;134:579.
15. Green D, Pont M, Cowan A, Cole G, Sather B, Nagengast A, et al. Response to Bcma CAR-T cells correlates with pretreatment target antigen density and is improved by small molecule inhibition of gamma secretase. Blood 2019;134:1856.
16. Li C, Wang Q, Zhu H, Mao X, Wang Y, Zhang Y. T cells expressing anti B-cell maturation antigen chimeric antigen receptors for plasma cell malignancies. Blood 2018;132:1013.
17. Salem DA, Maric I, Yuan CM, Liewehr DJ, Venzon DJ, Kochenderfer J, et al. Quantification of B-cell maturation antigen, a target for novel chimeric antigen receptor T-cell therapy in myeloma. Leuk Res 2018;71:106–11.
18. Pont MJ, Hill T, Cole GO, Abbott JJ, Kelliher J, Salter AI, et al. gamma-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. Blood 2019;134:1585–97.
19. Schmidts A, Ormhoj M, Choi BD, Taylor AO, Bouffard AA, Scarfo I, et al. Rational design of a trimeric APRIL-based CAR-binding domain enables efficient targeting of multiple myeloma. Blood Adv 2019;3:3248–60.
20. Carpenter RO, Evbuomwan MO, Pittaluga S, Rose JJ, Raffeld M, Yang S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. Clin Cancer Res 2013;19:2048–60.
21. Bu DX, Singh R, Choi EE, Ruella M, Nunez-Cruz S, Mansfield KG, et al. Pre-clinical validation of B cell maturation antigen (BCMA) as a target for T cell immunotherapy of multiple myeloma. Oncotarget 2018;9:25764–80.
22. Lam N, Trinklein ND, Buelow B, Patterson GH, Ojha N, Kochenderfer JN. Anti-BCMA chimeric antigen receptors with fully human heavy-chain-only antigen recognition domains. Nat Commun 2020;11:283.
23. Mailankody S, Jakubowiak A, Htut M, Costa L, Lee K, Ganguly S, et al. Orvacabtagene autoleucel (orva-cel), a B-cell maturation antigen

(BCMA)-directed CAR T cell therapy for patients (pts) with relapsed/refractory multiple myeloma (RRMM): update of the phase 1/2 EVOLVE study (NCT03430011). J Clin Oncol 38, 2020 (suppl; abstr 8504).
24. Green D, Pont M, Sather B, Cowan A, Turtle CJ, Till B, et al. Fully human BCMA targeted chimeric antigen receptor T cells administered in a defined composition demonstrate potency at low doses in advanced stage high risk multiple myeloma Blood 2018;132:1011.
25. Mikkilineni L, Manasanch EE, Vanasse V, Brudno JN, Mann J, Sherry R, et al. Deep and durable remissions of relapsed multiple myeloma on a first-in-humans clinical trial of T cells expressing an anti-B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) with a fully-human heavy-chain-only antigen recognition domain. Blood 2020;136:498.
26. Wang D, Wang J, Hu G, Wang W, Xiao Y, Cai H, et al. A phase I study of a novel fully human BCMA-targeting CAR (CT103A) in patients with relapsed/refractory multiple myeloma. Blood 2021 Jan 21 [Epub ahead of print].
27. Leblay N, Maity R, Barakat E, McCulloch S, Duggan P, Jimenez-Zepeda VH, et al. Cite-seq profiling of T-cells in multiple myeloma patients undergoing BCMA targeting CAR T or Bites immunotherapy. Blood 2020;136:719.
28. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai YT, Prabhala R, et al. Biallelic loss of BCMA as a resistance mechanism to CAR T cell therapy in a patient with multiple myeloma. Nat Commun 2021;12:868.
29. Da Vià MC, Dietrich O, Truger M, Arampatzi P, Duell J, Heidemeier A, et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. Nat Med 2021; 27:616–9.
30. Hamieh M, Dobrin A, Cabriolu A, van der Stegen SJC, Giavridis T, Mansilla-Soto J, et al. CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. Nature 2019;568:112–6.
31. Wang X, Walter M, Urak R, Weng L, Huynh C, Lim L, et al. Lenalidomide enhances the function of CS1 chimeric antigen receptor-redirected T cells against multiple myeloma. Clin Cancer Res 2018; 24:106–19.
32. Drent E, Groen RW, Noort WA, Themeli M, Lammerts van Bueren JJ, Parren PW, et al. Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma. Haematologica 2016;101:616–25.
33. Globerson Levin A, Rawet Slobodkin M, Waks T, Horn G, Ninio-Many L, Deshet Unger N, et al. Treatment of multiple myeloma using chimeric antigen receptor T cells with dual specificity. Cancer Immunol Res 2020;8:1485–95.
34. Smith EL, Harrington K, Staehr M, Masakayan R, Jones J, Long TJ, et al. GPRC5D is a target for the immunotherapy of multiple myeloma with rationally designed CAR T cells. Sci Transl Med 2019;11:eaau7746.
35. Hosen N, Matsunaga Y, Hasegawa K, Matsuno H, Nakamura Y, Makita M, et al. The activated conformation of integrin β(7) is a novel multiple myeloma-specific target for CAR T cell therapy. Nat Med 2017;23:1436–43.
36. Casucci M, Nicolis di Robilant B, Falcone L, Camisa B, Norelli M, Genovese P, et al. CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. Blood 2013;122:3461–72.
37. Garfall AL, Maus MV, Hwang WT, Lacey SF, Mahnke YD, Melenhorst JJ, et al. Chimeric antigen receptor T cells against CD19 for multiple myeloma. N Engl J Med 2015;373:1040–7.
38. Garfall AL, Stadtmauer EA, Hwang WT, Lacey SF, Melenhorst JJ, Krevvata M, et al. Anti-CD19 CAR T cells with high-dose melphalan and autologous stem cell transplantation for refractory multiple myeloma. JCI Insight 2018;3:e120505.
39. Nerreter T, Letschert S, Götz R, Doose S, Danhof S, Einsele H, et al. Super-resolution microscopy reveals ultra-low CD19 expression on myeloma cells that triggers elimination by CD19 CAR-T. Nat Commun 2019;10:3137.
40. Zah E, Lin MY, Silva-Benedict A, Jensen MC, Chen YY. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells. Cancer Immunol Res 2016;4:498–508.
41. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest 2016;126:3814–26.
42. Hegde M, Mukherjee M, Grada Z, Pignata A, Landi D, Navai SA, et al. Tandem CAR T cells targeting HER2 and IL13Ralpha2 mitigate tumor antigen escape. J Clin Invest 2016;126:3036–52.
43. Fernández de Larrea C, Staehr M, Lopez AV, Ng KY, Chen Y, Godfrey WD, et al. Defining an optimal dual-targeted CAR T-cell therapy approach simultaneously targeting BCMA and GPRC5D to prevent BCMA escape–driven relapse in multiple myeloma. Blood Cancer Discov 2020;1:146–54.
44. Chen KH, Wada M, Pinz KG, Liu H, Shuai X, Chen X, et al. A compound chimeric antigen receptor strategy for targeting multiple myeloma. Leukemia 2018;32:402–12.
45. Zah E, Nam E, Bhuvan V, Tran U, Ji BY, Gosliner SB, et al. Systematically optimized BCMA/CS1 bispecific CAR-T cells robustly control heterogeneous multiple myeloma. Nat Commun 2020;11:2283.
46. Jiang H, Dong B, Gao L, Liu L, Ge J, He A, et al. Clinical results of a multicenter study of the first-in-human dual BCMA and CD19 targeted novel platform fast CAR-T cell therapy for patients with relapsed/refractory multiple myeloma. Blood 2020;136:178.
47. Yan Z, Cao J, Cheng H, Qiao J, Zhang H, Wang Y, et al. A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial. Lancet Haematol 2019;6:e521–e9.
48. Yan L, Yan Z, Shang J, Shi X, Jin S, Kang L, et al. Sequential CD19- and Bcma-specific chimeric antigen receptor T cell treatment for RRMM: report from a single center study. Blood 2019;134:578.
49. Yan L, Qu S, Shang J, Shi X, Kang L, Xu N, et al. Sequential CD19 and BCMA-specific CAR T-cell treatment elicits sustained remission of relapsed and/or refractory myeloma. Cancer Med 2021;10: 563–74.
50. Li C, Mei H, Hu Y, Guo T, Liu L, Jiang H, et al. A bispecific CAR T-cell therapy targeting BCMA and CD38 for relapsed/refractory multiple myeloma: updated results from a phase 1dose-climbing trial. Blood 2019;134:930.
51. Popat R, Zweegman S, Cavet J, Yong K, Lee L, Faulkner J, et al. Phase 1 first-in-human study of AUTO2, the first chimeric antigen receptor (CAR) T cell targeting APRIL for patients with relapsed/refractory multiple myeloma (RRMM). Blood 2019;134:3112.
52. Cowan A, Pont M, Sather B, Turtle CJ, Till B, Nagengast A, et al. Efficacy and safety of fully human Bcma CAR T cells in combination with a gamma secretase inhibitor to increase Bcma surface expression in patients with relapsed or refractory multiple myeloma. Blood 2019;134:204.
53. Ramkumar P, Abarientos AB, Tian R, Seyler M, Leong JT, Chen M, et al. CRISPR-based screens uncover determinants of immunotherapy response in multiple myeloma. Blood Adv 2020;4:2899–911.
54. Raje N, Siegel D, Jagannath S, Lonial S, Munshi N, Moreau P, et al. Idecabtagene vicleucel (ide-cel, bb2121) in relapsed and refractory multiple myeloma: analyses of high-risk subgroups in the KarMMa study. Blood 2020;136:3234.
55. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med 2015;21:581–90.
56. Jie J, Hao S, Jiang S, Li Z, Yang M, Zhang W, et al. Phase 1 trial of the safety and efficacy of fully human anti-BCMA CAR T-cells in relapsed/refractory multiple myeloma. Blood 2019;134:4435.
57. Hao S, Jin J, Jiang S, Li Z, Zhang W, Yang M, et al. Two-year follow-up of investigator-initiated phase 1 trials of the safety and efficacy of fully human anti-Bcma CAR T cells (CT053) in relapsed/refractory multiple myeloma. Blood 2020;136:132.
58. Büll C, Boltje TJ, Balneger N, Weischer SM, Wassink M, van Gemst JJ, et al. Sialic acid blockade suppresses tumor growth by enhancing T-cell-mediated tumor immunity. Cancer Res 2018;78:3574–88.

59. Medema JP, de Jong J, Peltenburg LT, Verdegaal EM, Gorter A, Bres SA, et al. Blockade of the granzyme B/perforin pathway through overexpression of the serine protease inhibitor PI-9/SPI-6 constitutes a mechanism for immune escape by tumors. Proc Natl Acad Sci U S A 2001;98:11515–20.
60. Pitti RM, Marsters SA, Lawrence DA, Roy M, Kischkel FC, Dowd P, et al. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature 1998;396:699–703.
61. Medema JP, de Jong J, van Hall T, Melief CJ, Offringa R. Immune escape of tumors in vivo by expression of cellular FLICE-inhibitory protein. J Exp Med 1999;190:1033–8.
62. Singh N, Lee YG, Shestova O, Ravikumar P, Hayer KE, Hong SJ, et al. Impaired death receptor signaling in leukemia causes antigen-independent resistance by inducing CAR T-cell dysfunction. Cancer Discov 2020;10:552–67.
63. Kay NE, Leong TL, Bone N, Vesole DH, Greipp PR, Van Ness B, et al. Blood levels of immune cells predict survival in myeloma patients: results of an Eastern Cooperative Oncology Group phase 3 trial for newly diagnosed multiple myeloma patients. Blood 2001;98:23–8.
64. Rytlewski J, Madduri D, Fuller J, Campbell T, Mashadi-Hossein A, Thompson E, et al. Effects of prior alkylating therapies on preinfusion patient characteristics and starting material for CAR T-cell product manufacturing in late-line multiple myeloma. Blood 2020;136:1405.
65. Wang M, Pruteneau-Malinici I, Cohen A, Garfall AL, Milone MC, Tian L, et al. Identification and validation of predictive biomarkers to CD19- and BCMA-specific CAR T-cell responses in CAR T-cell precursors. Blood 2019;134:622.
66. Finney O, Yeri A, Mao P, Pandya C, Alonzo E, Hopkins G, et al. Molecular and phenotypic profiling of drug product and post-infusion samples from CRB-402, an ongoing: phase I clinical study of bb21217 a BCMA-directed CAR T cell therapy. Blood 2020;136:1401.
67. Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med 2018;24:563–71.
68. Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med 2016;8:320ra3.
69. Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, et al. Central memory self/tumor-reactive $CD8^+$ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl Acad Sci U S A 2005;102:9571–6.
70. Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, et al. Chimeric antigen receptor-modified T cells derived from defined $CD8^+$ and $CD4^+$ subsets confer superior antitumor reactivity in vivo. Leukemia 2016;30:492–500.
71. Adusumilli PS, Cherkassky L, Villena-Vargas J, Colovos C, Servais E, Plotkin J, et al. Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. Sci Transl Med 2014;6:261ra151.
72. Heider U, Rademacher J, Kaiser M, Kleeberg L, von Metzler I, Sezer O. Decrease in $CD4^+$ T-cell counts in patients with multiple myeloma treated with bortezomib. Clin Lymphoma Myeloma Leuk 2010;10:134–7.
73. Chung DJ, Pronschinske KB, Shyer JA, Sharma S, Leung S, Curran SA, et al. T-cell exhaustion in multiple myeloma relapse after autotransplant: optimal timing of immunotherapy. Cancer Immunol Res 2016;4:61–71.
74. Garfall AL, Dancy EK, Cohen AD, Hwang WT, Fraietta JA, Davis MM, et al. T-cell phenotypes associated with effective CAR T-cell therapy in postinduction vs relapsed multiple myeloma. Blood Adv 2019;3:2812–5.
75. Das RK, Vernau L, Grupp SA, Barrett DM. Naïve T-cell deficits at diagnosis and after chemotherapy impair cell therapy potential in pediatric cancers. Cancer Discov 2019;9:492–9.
76. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015;385:517–28.
77. Fraietta JA, Beckwith KA, Patel PR, Ruella M, Zheng Z, Barrett DM, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. Blood 2016;127:1117–27.
78. Mailankody S, Matous J, Liedtke M, Sidana S, Malik S, Nath R, et al. First-in-human study of the allogeneic anti-BCMA ALLO-715 CAR T-cell therapy and the anti-CD52 mab ALLO-647 in relapsed/refractory multiple myeloma (UNIVERSAL Study). Blood 2020;136:129.
79. Cohen AD, Garfall AL, Dogan A, Lacey SF, Martin C, Lendvai N, et al. Serial treatment of relapsed/refractory multiple myeloma with different BCMA-targeting therapies. Blood Adv 2019;3:2487–90.
80. Mailankody S, Ghosh A, Staehr M, Purdon TJ, Roshal M, Halton E, et al. Clinical responses and pharmacokinetics of MCARH171, a human-derived BCMA targeted CAR T cell therapy in relapsed/refractory multiple myeloma: final results of a phase 1 clinical trial. Blood 2018;132:959.
81. Martin M, Thompson E, Brown W, Finney O, Rytlewski J, Jiang Y, et al. Idecabtagene vicleucel (ide-cel, bb2121) responders are characterized by early and temporally consistent activation and expansion of CAR T cells with a T effector phenotype. Blood 2020;136:2315.
82. Alsina M, Shah N, Raje N, Jagannath S, Madduri D, Kaufman JL, et al. Updated results from the phase 1 CRB-402 study of anti-BCMA CAR T-cell therapy bb21217 in patients with relapsed and refractory multiple myeloma: correlation of expansion and duration of response with T-cell phenotypes [abstract]. In: Proceedings of the 62nd ASH Annual Meeting and Exposition; 2020 Dec 5–8. Washington (DC): American Society of Hematology; 2020. Abstract nr 130. Available from: https://ash.confex.com/ash/2020/webprogram/Paper140410.html.
83. Costello C, Cohen A, Patel K, Ali S, Berdeja J, Shah N, et al. Phase 1/2 study of the safety and response of P-BCMA-101 CAR-T cells in patients with relapsed/refractory (r/r) multiple myeloma (MM). Blood 2020;136:134.
84. Colonna M, Navarro G, DeVries T, Beckett V, Amsberry A, Radhakrishnan A, et al. Orvacabtagene autoleucel (orva-cel; JCARH125): a fully human BCMA-targeted second-generation CAR T cell product characterized by a predominant central memory phenotype with high in vitro and in vivo proliferative potential and sustained in vivo persistence. Blood 2020;136:2358.
85. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 2016;126:3130–44.
86. Gargett T, Yu W, Dotti G, Yvon ES, Christo SN, Hayball JD, et al. GD2-specific CAR T cells undergo potent activation and deletion following antigen encounter but can be protected from activation-induced cell death by PD-1 blockade. Mol Ther 2016;24:1135–49.
87. Yeku OO, Purdon TJ, Koneru M, Spriggs D, Brentjens RJ. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. Sci Rep 2017;7:10541.
88. Suarez ER, Chang de K, Sun J, Sui J, Freeman GJ, Signoretti S, et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. Oncotarget 2016;7:34341–55.
89. Li S, Siriwon N, Zhang X, Yang S, Jin T, He F, et al. Enhanced cancer immunotherapy by chimeric antigen receptor-modified T cells engineered to secrete checkpoint inhibitors. Clin Cancer Res 2017;23:6982–92.
90. Liu X, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. Cancer Res 2016;76:1578–90.
91. Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. Sci Rep 2017;7:737.
92. Bernabei L, Garfall AL, Melenhorst JJ, Lacey SF, Stadtmauer E, Vogl DT, et al. PD-1 inhibitor combinations as salvage therapy for relapsed/refractory multiple myeloma (MM) patients progressing after Bcma-directed CAR T cells. Blood 2018;132:1973.

93. Gargett T, Truong N, Ebert LM, Yu W, Brown MP. Optimization of manufacturing conditions for chimeric antigen receptor T cells to favor cells with a central memory phenotype. Cytotherapy 2019;21:593–602.
94. Stock S, Ubelhart R, Schubert ML, Fan F, He B, Hoffmann JM, et al. Idelalisib for optimized CD19-specific chimeric antigen receptor T cells in chronic lymphocytic leukemia patients. Int J Cancer 2019;145:1312–24.
95. Zheng W, O'Hear CE, Alli R, Basham JH, Abdelsamed HA, Palmer LE, et al. PI3K orchestration of the in vivo persistence of chimeric antigen receptor-modified T cells. Leukemia 2018;32:1157–67.
96. Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature 2018;558:307–12.
97. Gautam S, Fioravanti J, Zhu W, Le Gall JB, Brohawn P, Lacey NE, et al. The transcription factor c-Myb regulates CD8(+) T cell stemness and antitumor immunity. Nat Immunol 2019;20:337–49.
98. Wei J, Long L, Zheng W, Dhungana Y, Lim SA, Guy C, et al. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. Nature 2019;576:471–6.
99. Hasan AN, Selvakumar A, O'Reilly RJ. Artificial antigen presenting cells: an off the shelf approach for generation of desirable T-cell populations for broad application of adoptive immunotherapy. Adv Genet Eng 2015;4:130.
100. Stroncek DF, Ren J, Lee DW, Tran M, Frodigh SE, Sabatino M, et al. Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptor T cells. Cytotherapy 2016;18:893–901.
101. Guedan S, Posey AD Jr, Shaw C, Wing A, Da T, Patel PR, et al. Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. JCI Insight 2018;3:e96976.
102. Drent E, Poels R, Ruiter R, van de Donk N, Zweegman S, Yuan H, et al. Combined CD28 and 4-1BB costimulation potentiates affinity-tuned chimeric antigen receptor-engineered T cells. Clin Cancer Res 2019;25:4014–25.
103. Zhao Z, Condomines M, van der Stegen SJC, Perna F, Kloss CC, Gunset G, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell 2015;28:415–28.
104. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther 2009;17:1453–64.
105. Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey AD Jr, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. Immunity 2016;44:380–90.
106. Finney HM, Akbar AN, Lawson AD. Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. J Immunol 2004;172:104–13.
107. Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science 2020;367:eaba7365.
108. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 2017;543:113–7.
109. Weber EW, Lynn RC, Parker KR, Anbunathan H, Lattin J, Sotillo E, et al. Transient "rest" induces functional reinvigoration and epigenetic remodeling in exhausted CAR-T cells. BioRxiv 920496 [Preprint]. 2020. Available from: https://doi.org/10.1101/2020.01.26.920496.
110. Drent E, Poels R, Mulders MJ, van de Donk N, Themeli M, Lokhorst HM, et al. Feasibility of controlling CD38-CAR T cell activity with a Tet-on inducible CAR design. PLoS One 2018;13:e0197349.
111. Jan M, Scarfò I, Larson RC, Walker A, Schmidts A, Guirguis AA, et al. Reversible ON- and OFF-switch chimeric antigen receptors controlled by lenalidomide. Sci Transl Med 2021;13:eabb6295.
112. Lamers CH, Willemsen R, van Elzakker P, van Steenbergen-Langeveld S, Broertjes M, Oosterwijk-Wakka J, et al. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. Blood 2011;117:72–82.
113. Turtle CJ, Hanafi LA, Berger C, Hudecek M, Pender B, Robinson E, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of $CD8^+$ and $CD4^+$ CD19-specific chimeric antigen receptor-modified T cells. Sci Transl Med 2016;8:355ra116.
114. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR-T cells of defined $CD4^+$:$CD8^+$ composition in adult B cell ALL patients. J Clin Invest 2016;126:2123–38.
115. Gorovits B, Koren E. Immunogenicity of chimeric antigen receptor T-cell therapeutics. BioDrugs 2019;33:275–84.
116. Li C, Wang J, Wang D, Hu G, Yang Y, Zhou X, et al. Efficacy and safety of fully human BCMA targeting CAR T-cell therapy in relapsed/refractory MM. Blood 2019;134:929.
117. Smith EL, Staehr M, Masakayan R, Tatake IJ, Purdon TJ, Wang X, et al. Development and evaluation of an optimal human single-chain variable fragment-derived BCMA-targeted CAR T cell vector. Mol Ther 2018;26:1447–56.
118. Lin L, Cho SF, Xing L, Wen K, Li Y, Yu T, et al. Preclinical evaluation of $CD8^+$ anti-BCMA mRNA CAR T cells for treatment of multiple myeloma. Leukemia 2021;35:752–63.
119. Bechman N, Maher J. Lymphodepletion strategies to potentiate adoptive T-cell immunotherapy – what are we doing; where are we going? Expert Opin Biol Ther 2020;21:627–37.
120. Holthof L, van der Horst H, Poels R, van der Schans J, Gelderloos A, Fenghzi L, et al. The impact and modulation of microenvironment-induced immune resistance against CAR T cell and antibody treatments in multiple myeloma. Blood 2019;134:137.
121. Sakemura R, Cox M, Hansen M, Hefazi M, Roman C, Schick K, et al. Targeting cancer associated fibroblasts in the bone marrow prevents resistance to chimeric antigen receptor T cell therapy in multiple myeloma. Blood 2019;134:865.
122. Reiss D, Do T, Kuo D, Gray V, Olson N, Lee C, et al. Multiplexed immunofluorescence (IF) analysis and gene expression profiling of biopsies from patients with relapsed/refractory (R/R) diffuse large B cell lymphoma (DLBCL) treated with lisocabtagene maraleucel (liso-cel) in transcend NHL 001 reveal patterns of immune infiltration associated with durable response Blood 2019;134:202.
123. Kegler A, Koristka S, Bergmann R, Berndt N, Arndt C, Feldmann A, et al. T cells engrafted with a UniCAR 28/z outperform UniCAR BB/z-transduced T cells in the face of regulatory T cell-mediated immunosuppression. Oncoimmunology 2019;8:e1621676.
124. Ruella M, Klichinsky M, Kenderian SS, Shestova O, Ziober A, Kraft DO, et al. Overcoming the immunosuppressive tumor microenvironment of Hodgkin lymphoma using chimeric antigen receptor T cells. Cancer Discov 2017;7:1154–67.
125. Long AH, Highfill SL, Cui Y, Smith JP, Walker AJ, Ramakrishna S, et al. Reduction of MDSCs with all-trans retinoic acid improves CAR therapy efficacy for sarcomas. Cancer Immunol Res 2016;4:869–80.
126. Ninomiya S, Narala N, Huye L, Yagyu S, Savoldo B, Dotti G, et al. Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. Blood 2015;125:3905–16.
127. DeRenzo C, Gottschalk S. Genetic modification strategies to enhance CAR T cell persistence for patients with solid tumors. Front Immunol 2019;10:218.
128. Avanzi MP, Yeku O, Li X, Wijewarnasuriya DP, van Leeuwen DG, Cheung K, et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Rep 2018;23:2130–41.
129. Golumba-Nagy V, Kuehle J, Hombach AA, Abken H. CD28-zeta CAR T cells resist TGF-beta repression through IL-2 signaling, which can be mimicked by an engineered IL-7 autocrine loop. Mol Ther 2018;26:2218–30.
130. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes $CD4^+CD25^+$ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007;56:641–8.

131. Franssen LE, Nijhof IS, Bjorklund CC, Chiu H, Doorn R, van Velzen J, et al. Lenalidomide combined with low-dose cyclophosphamide and prednisone modulates Ikaros and Aiolos in lymphocytes, resulting in immunostimulatory effects in lenalidomide-refractory multiple myeloma patients. Oncotarget 2018;9:34009–21.
132. Drent E, Themeli M, Poels R, de Jong-Korlaar R, Yuan H, de Bruijn J, et al. A rational strategy for reducing on-target off-tumor effects of CD38-chimeric antigen receptors by affinity optimization. Mol Ther 2017;25:1946–58.
133. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38^{+} immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood 2016;128:384–94.
134. Chen L, Diao L, Yang Y, Yi X, Rodriguez BL, Li Y, et al. CD38-mediated immunosuppression as a mechanism of tumor cell escape from PD-1/PD-L1 blockade. Cancer Discov 2018;8:1156–75.
135. Krejcik J, Frerichs KA, Nijhof IS, van Kessel B, van Velzen JF, Bloem AC, et al. Monocytes and granulocytes reduce CD38 expression levels on myeloma cells in patients treated with daratumumab. Clin Cancer Res 2017;23:7498–511.
136. Gandhi AK, Kang J, Havens CG, Conklin T, Ning Y, Wu L, et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). Br J Haematol 2014;164:811–21.
137. Works M, Soni N, Hauskins C, Sierra C, Baturevych A, Jones JC, et al. Anti-B-cell maturation antigen chimeric antigen receptor T cell function against multiple myeloma is enhanced in the presence of lenalidomide. Mol Cancer Ther 2019;18:2246–57.
138. Kuramitsu S, Ohno M, Ohka F, Shiina S, Yamamichi A, Kato A, et al. Lenalidomide enhances the function of chimeric antigen receptor T cells against the epidermal growth factor receptor variant III by enhancing immune synapses. Cancer Gene Ther 2015;22:487–95.
139. Gross G, Gorochov G, Waks T, Eshhar Z. Generation of effector T cells expressing chimeric T cell receptor with antibody type-specificity. Transplant Proc 1989;21:127–30.
140. Harrison B, Minnema M, Lee HC, Spencer A, Kapoor P, Madduri D, et al. A phase 1 first in human (FIH) study of AMG 701, an anti-B-cell maturation antigen (BCMA) half-life extended (HLE) BiTE® (bispecific T-cell engager) molecule, in relapsed/refractory (RR) multiple myeloma (MM). Blood 2020;136:28.
141. Garfall AL, Usmani S, Mateos MV, Nahi H, Van de Donk N, San-Miguel J, et al. Updated phase 1 results of teclistamab, a B-cell maturation antigen (BCMA) x CD3 bispecific antibody, in relapsed and/or refractory multiple myeloma (RRMM). Blood 2020;136:27.
142. Costa L, Wong S, Bermudez A, de la Rubia J, Mateos MV, Ocio E, et al. Interim results from the first phase 1 clinical study of the B-cell maturation antigen (BCMA) 2+1 T-cell engager (TCE) CC-93269 in patients (pts) with relapsed/refractory multiple myeloma (RRMM) [abstract]. In: Proceedings of the 25th EHA Annual Congress; 2020 Jun 11–21. The Hague (the Netherlands): European Hematology Association; 2020. Abstract nr S205. Available from: https://library.ehaweb.org/eha/2020/eha25th/295025/luciano.j.costa.interim.results.from.the.first.phase.1.clinical.study.of.the.
143. Madduri D, Rosko A, Brayer J, Zonder J, Bensinger W, Li J, et al. REGN5458, a BCMA x CD3 bispecific monoclonal antibody, induces deep and durable responses in patients with relapsed/refractory multiple myeloma (RRMM). Blood 2020;136:291.
144. Lesokhin A, Levy M, Dalovisio A, Bahlis N, Solh M, Sebag M, et al. Preliminary safety, efficacy, pharmacokinetics, and pharmacodynamics of subcutaneously (SC) administered PF-06863135, a B-cell maturation antigen (BCMA)-CD3 bispecific antibody, in patients with relapsed/refractory multiple myeloma (RRMM). Blood 2020;136:3206.
145. Rodriguez C, D'Souza A, Shah N, Voorhees P, Buelow B, Vij R, et al. Initial results of a phase I study of TNB-383B, a BCMA x CD3 bispecific T-cell redirecting antibody, in relapsed/refractory multiple myeloma. Blood 2020;136:293.
146. Lonial S, Lee HC, Badros A, Trudel S, Nooka AK, Chari A, et al. Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. Lancet Oncol 2020;21:207–21.
147. Kumar S, Migkou M, Bhutani M, Spencer A, Ailawadhi S, Kalff A, et al. Phase 1 first-in-human study of MEDI2228, a BCMA-targeted ADC in patients with relapsed/refractory multiple myeloma. Blood 2020;136:26.
148. Lee HC, Raje NS, Landgren O, Upreti VV, Wang J, Avilion AA, et al. Phase 1 study of the anti-BCMA antibody-drug conjugate AMG 224 in patients with relapsed/refractory multiple myeloma. Leukemia 2021;35:255–8.
149. Poels R, Drent E, Lameris R, Katsarou A, Themeli M, van der Vliet HJ, et al. Preclinical evaluation of invariant natural killer T cells modified with CD38 or BCMA chimeric antigen receptors for multiple myeloma. Int J Mol Sci 2021;22:1096.
150. Chu J, Deng Y, Benson DM, He S, Hughes T, Zhang J, et al. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. Leukemia 2014;28:917–27.