REVIEW ARTICLE

BCMA loss in the epoch of novel immunotherapy for multiple myeloma: from biology to clinical practice

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Abstract

The treatment of multiple myeloma (MM) is evolving rapidly. In the past few years, chimeric antigen receptor modified T cells and bispecific antibodies are bringing new treatment options to patients with relapsed/refractory MM. Currently, B-cell maturation antigen (BCMA) has emerged as the most commonly used target of T-cell-based immunotherapies for relapsed/refractory MM. Clinical data have demonstrated promising efficacy and manageable safety profiles of both chimeric antigen receptor T-cell and bispecific antibody therapies in heavily pretreated relapsed/refractory MM. However, most patients suffer from relapses at later time points, and the mechanism of resistance remains largely unknown. Theoretically, loss of antigen is a potential tumor-intrinsic resistance mechanism against BCMA-targeted immunotherapies. Strategies to overcome this kind of drug resistance are, therefore, needed. In this review, we discuss the loss of BCMA in the new epoch of immunotherapy for MM.

Introduction

Multiple myeloma (MM), the second most common hematologic malignancy, is characterized by uncontrolled plasma cell proliferation, which typically causes destructive osseous bone lesions, acute kidney injury, anemia, and hypercalcemia.[1,2] In the past 20 years, integration of proteasome inhibitors and immunomodulatory drugs into the treatment of MM has significantly improved the survival outcomes of patients.[3] Although MM is currently considered a largely incurable disease, the evolution of MM therapy is ongoing.[4] In the mid-2010s, monoclonal antibodies targeting CD38 and signaling lymphocytic activation molecule F7 (SLAMF7), i.e. daratumumab and elotuzumab, were incorporated into the standard of care, bringing MM treatment into a new era of immunotherapy.[5] Unlike conventional chemotherapies, these novel agents should recognize specific surface antigens in order to locate MM cells and, in turn, kill them selectively. In principle, the presence of a target antigen is an essential prerequisite for successful treatment.

The next revolution of immunotherapy for MM started recently with B-cell maturation antigen (BCMA)-directed treatments, including antibody-drug conjugates (ADC), bispecific antibodies (BsAb), and chimeric antigen receptor (CAR) modified T-cell therapies.[6] Although these novel immunotherapies are highly effective even in heavily pretreated relapsed/refractory (RR) MM patients, most patients suffer from relapses at later time points. A recent meta-analysis showed a median progression-free survival of merely 12.2 months in RRMM patients who were treated with BCMA-targeted CAR T cells.[7] However, the underlying mechanism of resistance is currently not fully understood. To date, novel immunotherapies such as ADC, BsAb, and CAR T cells targeting other antigens have also been used in diverse hematologic malignancies including leukemia and lymphoma.[8-10] Antigen loss has already been described as a tumor-intrinsic mechanism of resistance against BsAb and CAR T-cell therapies for leukemia and lymphoma. For instance, CD19 loss was detected in approximately 40% of patients with B-cell acute lymphoblastic leukemia treated with anti-CD19 CAR T cells, and point mutations in the *CD19* gene were reported as a mechanism for CD19 loss in these patients.[11] Likewise, in B-cell non-Hodgkin lymphoma, CD20-negative relapses were observed in patients who received REGN1979, a CD20/CD3-targeted BsAb.[12,13] On the other hand, antigen loss following ADC treatments has been reported less frequently. Theoretically, antigen loss may also be a potential mechanism of resistance to anti-BCMA immunotherapies

for MM. Indeed, in MM patients, biallelic BCMA loss has been reported in three cases relapsing from BCMA-targeted CAR T-cell therapies.[14-16] In this review, we summarize the nature of BCMA loss based on the currently available data. Furthermore, strategies to overcome drug resistance caused by BCMA loss are discussed.

Biology of BCMA and anti-BCMA immunotherapies for multiple myeloma

The biology of BCMA as well as clinical data on BCMA-directed novel immunotherapies for MM have been summarized in previous review articles.[17-20] Since the current review does not focus on these issues, at this point, we provide just a brief overview for completeness of the subject.

Biology of BCMA

BCMA, also referred to as tumor necrosis factor receptor superfamily 17 (TNFRSF17) or CD269, is a transmembrane glycoprotein highly expressed in plasma cells and almost absent in other human tissues. BCMA can be cleaved from the cell membrane by γ-secretase, releasing soluble BCMA (sBCMA) into the blood stream.[21] The gene encoding BCMA is located on human chromosome band 16p13.1.[22] In normal plasma cells, BCMA binds to B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL), regulating the maturation and differentiation of B cells into plasma cells and supporting survival of long-lived plasma cells.[23-26] In MM, several survival and anti-apoptotic pathways could be activated by binding of BAFF or APRIL to BCMA, e.g. nuclear factor κ light chain enhancer of activated B cells (NF-κB), mitogen activated protein kinase (MAPK), and protein kinase B (AKT), resulting in MM cell proliferation and immunosuppression in the bone marrow microenvironment.[27] Importantly, the level of expression of BCMA is increased significantly on malignant cells compared to the level on healthy plasma cells.[26,28] Based on these biological features of BCMA, it is considered a target of therapy for MM. To date, three classes of BCMA-directed immunotherapies have been investigated in humans, including ADC, BsAb, and CAR T cells (Figure 1A). As BCMA acts as an important factor contributing to survival of malignant plasma cells, loss of BCMA could be expected to place plasma cells at a selective growth disadvantage.

Recent advances in anti-BCMA immunotherapies for multiple myeloma

In August 2020, the US Food and Drug Administration and the European Medicines Agency approved the first BCMA-targeted ADC, belantamab mafodotin, for patients with RRMM.[29] A few months later, the first anti-BCMA CAR T-cell therapy idecabtagene vicleucel (also referred to as ide-cel or bb2121) was approved for RRMM patients who have received four or more prior lines of therapy, including an immunomodulatory drug, a proteasome inhibitor, and an anti-CD38 monoclonal antibody.[30] Most recently, the second anti-BCMA CAR T-cell therapy, ciltacabtagene autoleucel (cilta-cel), has been granted Food and Drug Administration approval for the same indication as ide-cel.[31] Besides, multiple BCMA-directed T-cell engaging BsAb are under clinical investigation, and the early results have shown encouraging anti-MM efficacy.[32,33] Based on the outstanding anti-tumor effect and acceptable toxicity, these novel anti-BCMA immunotherapies may become a part of standard MM treatment in the near future.

BCMA-targeted antibody drug conjugates

An ADC is composed of a monoclonal antibody and a cytotoxic payload combined by a linker molecule. Belantamab mafodotin is the first-in-class ADC for MM patients. The pivotal randomized phase II DREAMM-2 trial demonstrated an overall response rate (ORR) of 31% and 34% in heavily pretreated RRMM patients receiving 2.5 mg/kg and 3.4 mg/kg of the drug every 3 weeks, respectively.[34] Keratopathy is the most common toxicity of belantamab mafodotin with an incidence of up to 100%, which may lead to treatment discontinuation.[35] Currently, belantamab mafodotin in combination with VRd (bortezomib, lenalidomide, and dexamethasone) is being evaluated in transplant-ineligible newly diagnosed MM.[36] Other anti-BCMA ADC, e.g. AMG224 and MEDI2228, are under clinical investigation.[37,38]

BCMA-targeted bispecific antibodies

BsAb bind to MM and T cells via CD3 and a tumor-specific antigen, e.g. BCMA, to build an immune synapse, which subsequently leads to T-cell activation and cytotoxic effects. The first-in-class BCMA-directed BsAb AMG420 showed an ORR of 70% at a dose of 400 μg/day.[33] However, further development of AMG420 has been stopped because of the product's short half-life and the need for continuous infusion.[17] For this reason, some other BsAb with extended half-lives have been developed, e.g. AMG701, teclistamab, REGN5458, TNB-383B, elranatamab, and CC-93269. Preliminary efficacy data for these novel agents demonstrated ORR of up to 90%, while some of the results were still immature.[39-44] The most common adverse event of BsAb is cytokine release syndrome, which occurs with an incidence of up to 90%.[45] Clinical trials evaluating BsAb in MM are ongoing.[46]

BCMA-targeted chimeric antigen receptor T cells

CAR T cells are another strategy to overcome tumor by utilizing the patient's own T cells. Genetically modified T cells with a CAR recognize tumor-specific antigens, e.g. BCMA, and activate T cells via the CD3ζ signaling domain. Additionally, some co-stimulatory domains such as CD28

and 4-1BB are incorporated to enhance T-cell activation and proliferation. So far, there are more than 20 different BCMA-directed CAR T-cell products investigated within clinical trials, mainly in the USA and in China, producing an ORR of up to 100% in some studies.[7,47-50] In the phase II KarMMa trial, the first-in-class BCMA-targeted CAR T-cell therapy ide-cel led to an ORR of 73%, and the median progression-free survival was only 8.8 months.[51] Notably, the updated results of the phase Ib/II CARTITUDE-1 study showed that cilta-cel not only produces a high ORR of 98% but also has encouraging long-term efficacy with a median duration of response of 21.8 months. Similarly to BsAb, BCMA-directed CAR T-cell therapy was associated with a very high rate of cytokine release syndrome of >80%, although this correlated with a good treatment response.[20] At present, various clinical trials evaluating BCMA-targeted CAR T-cell products, including allogeneic CAR T cells, are enrolling patients.[47]

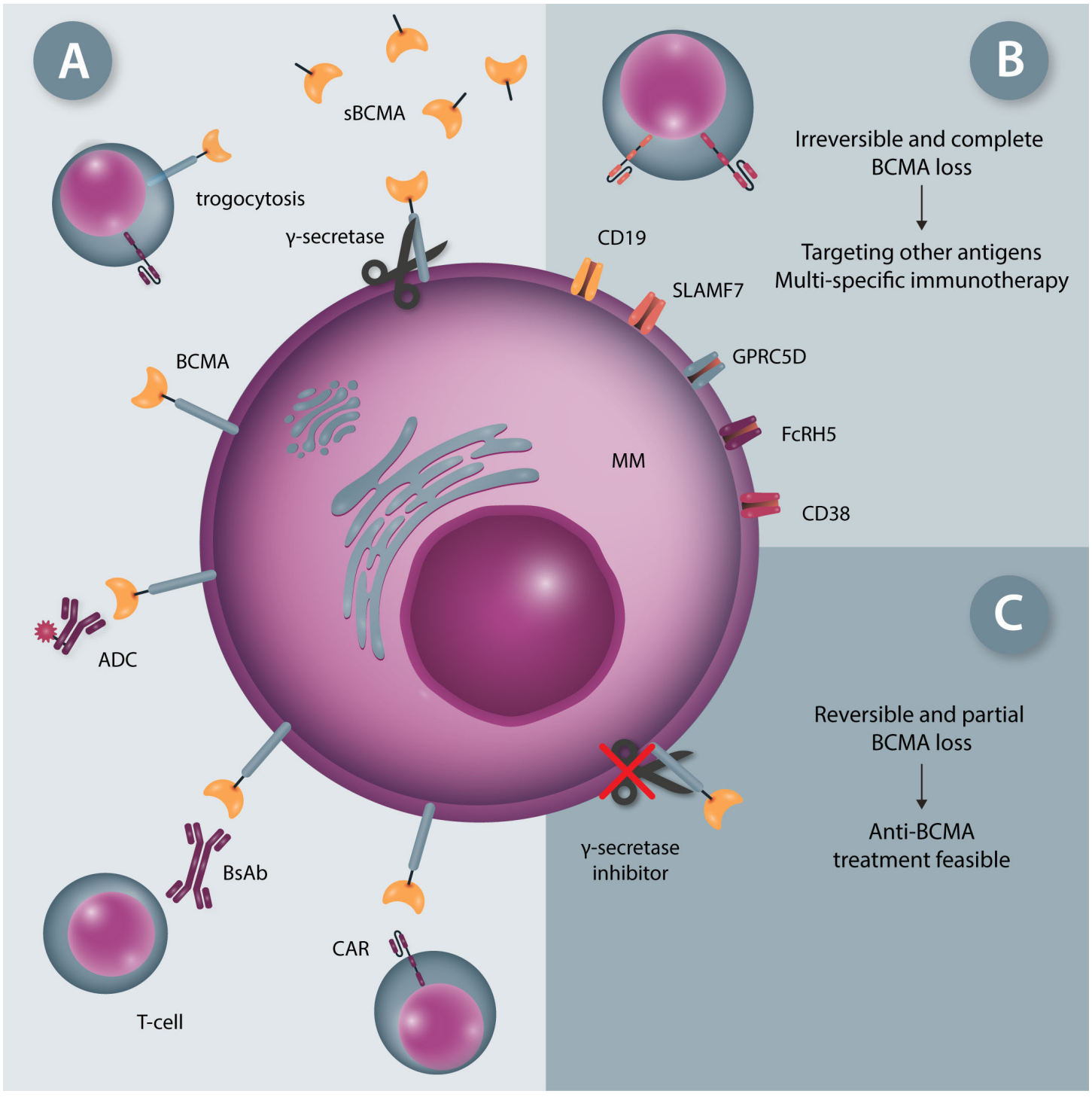

Figure 1. BCMA loss following targeted immunotherapies. (A) BCMA-directed immunotherapies. At present, anti-BCMA chimeric antigen receptor modified (CAR) T cells, bispecific antibodies, and antibody-drug conjugates are available for the treatment of relapsed/refractory multiple myeloma (MM). γ-secretase can shed BCMA from the membrane of MM cells and can subsequently release soluble BCMA into the blood stream. An increase of soluble BCMA level can lead to a decline of BCMA-binding capacity on MM cells. BCMA could be transferred to CAR T cells via trogocytosis, resulting in reversible partial BCMA loss. (B) Irreversible complete BCMA loss. In patients with irreversible complete BCMA loss, which is caused by homozygous *BCMA* gene deletion, other immunotargets could be considered for further treatments, e.g. CD38, FcRH5, GPRC5D, CD19, and SLAMF7. Multi-specific immunotherapies targeting more than one antigen seem to be a promising strategy to prevent drug resistance due to the loss of a single antigen. (C) Reversible partial BCMA loss. γ-secretase inhibition is one option to increase BCMA density on MM cells. When the BCMA expression level recovers at later time-points, anti-BCMA retreatment could be considered. ADC: antibody drug conjugate; BCMA: B-cell maturation antigen; BsAb; bispecific antibody; CAR T-cell; chimeric antigen receptor modified T cell; FcRH5: Fc receptor-homolog 5; GPRC5D; G protein coupled receptor class C group 5 member D; MM: multiple myeloma; RR: relapsed/refractory; sBCMA; soluble BCMA; SLAMF7: signaling lymphocytic activation molecule F7.

BCMA loss: biology and clinical implication

The novel anti-BCMA immunotherapies, especially BsAb and CAR T cells, are highly effective in RRMM. However, the currently available data have demonstrated that the majority of patients relapse at later time-points. As these novel agents are being integrated into standard care, elucidating the mechanisms of resistance would be the next step in the development of these drugs to improve their anti-MM efficacy and to plan a precise treatment strategy for each given patient. Currently, as these novel anti-BCMA agents are still in their "infancy", information on resistance mechanisms is still very limited. However, BCMA loss represents a potential tumor-intrinsic factor contributing to resistance against anti-BCMA immunotherapies. Here, we discuss the biology of BCMA loss and its clinical implications.

BCMA loss is not a common event

In general, the currently available data on anti-BCMA immunotherapies, mainly BsAb and CAR T cells, have demonstrated that BCMA loss after treatment is not a common event, with BCMA expression remaining positive in the majority of patients.[22] BCMA loss was mostly detected when patients suffered an unexpected relapse after immunotherapies. In Table 1 we provide an overview of clinical cases with BCMA loss after immunotherapies in RRMM.

The first case of BCMA loss was observed in a patient treated with BCMA-targeted CAR T cells. Ali *et al.* reported a patient who relapsed 2 months after CAR-BCMA treatment; flow cytometry of the patient's bone marrow showed a population of BCMA-negative malignant plasma cells, whereas some other MM cells remained positive for BCMA.[52] Similarly, Brudno *et al.* found a small number of MM cells that lacked BCMA expression, as determined by flow cytometry, in a patient 56 weeks after CAR-BCMA treatment. However, when resampling 8 weeks later, the MM cells of this patient presented mixed BCMA expression, suggesting a reversible BCMA loss.[53] In addition, Green *et al.* described a patient with BCMA-negative MM cells 60 days after anti-BCMA CAR T-cell therapy. Although some BCMA-positive MM cells still existed in this patient, the BCMA expression level and the BCMA antigen-binding capacity were strongly reduced.[54] Decreased BCMA expression levels were also observed by Cohen *et al.* in 12 out of 18 patients who received CAR-BCMA, including eight of nine responders and four of nine non-responders, while the BCMA expression "recovered" in later follow-up of these patients.[55] Furthermore, BCMA loss was found in three of 71 patients (4%) at progression in the KarMMa study investigating ide-cel.[51] With regard to BsAb treatment, Truger *et al.* reported a case of homozygous *BCMA* gene deletion after AMG420 treatment; this is the only patient with BCMA loss following BsAb therapies.[56] BCMA loss after ADC has not yet been reported. Collectively, findings based on flow cytometry of bone marrow demonstrated that complete or partial BCMA loss could occur in a small proportion of RRMM patients following anti-BCMA CAR T-cell therapy. However, at present, there is still limited experience with antigen loss following BCMA-targeted immunotherapies in MM patients, and determination of the level of BCMA expression is not part of routine tests at relapse. Therefore, the *de facto* incidence of BCMA loss is largely unknown. Theoretically, BCMA loss could appear at any time in the course of the disease.

Potential mechanisms of BCMA loss

Although BCMA loss has been reported in several studies, to date, the underlying mechanisms of this event are not fully understood. In this section, we summarize the potential mechanisms contributing to BCMA loss based on the currently available data.

With the rapid evolution of genomic diagnostics, such as whole-genome sequencing and single-cell RNA sequencing, the underlying biological mechanisms of BCMA loss after anti-BCMA immunotherapies began to be elucidated in the past few years. Da Vià *et al.* reported for the first time that homozygous *BCMA* gene deletion led to a complete and irreversible loss of BCMA expression in a RRMM patient who relapsed after ide-cel treatment. Furthermore, heterozygous *BCMA* gene deletion was present in 22% (37 out of 168) of MM patients (including a set of hyperhaploid MM cases) who had not received any anti-BCMA therapy, indicating a higher risk of homozygous *BCMA* gene alteration after anti-BCMA immunotherapies.[15] Likewise, Samur *et al.* and Leblay *et al.* found a similar bi-allelic BCMA loss (mutation + deletion or deletion + deletion) as a resistance mechanism in other RRMM patients treated with anti-BCMA CAR T cells.[14,16] A homozygous *BCMA* gene deletion was also confirmed in a RRMM patient who received AMG420 BsAb therapy.[56] The findings of these studies led to the hypothesis that the strong selection pressure exerted by these highly effective T-cell-based anti-BCMA immunotherapies might lead to selective expansion of a pre-existing minor population of BCMA-negative MM cells that could also appear in focal lesions and/or extramedullary manifestations due to spatial tumor heterogeneity. As a result of this, we observed permanent genetic and/or genomic changes after BCMA-targeted T-cell immunotherapies in these patients (Table 1). On the other hand, as anti-BCMA ADC are less effective than CAR T-cell or BsAb therapies in MM,[17] the selection pressure of ADC should also be lower. Thus, the incidence of BCMA loss in patients treated with ADC might be lower than that in patients treated with CAR T cells or BsAb. Interestingly, most of the patients with *BCMA* gene deletion also had *TP53* gene deletion, and *TP53* mutations were

Table 1. Selected published cases on BCMA loss following targeted immunotherapies for relapsed/refractory multiple myeloma.

Authors	Year of publication	Type of immunotherapy	Product	Clinical trial identifier	Time point of BCMA loss	Methods	Frequency of BCMA loss in the study#	Biological mechanism of BCMA loss	Major findings	Reference
Ali *et al.*	2016	CAR T-cell	CAR-BCMA	NCT02215967	2 months after treatment	Flow cytometry	1/12	NR	Partial loss of BCMA expression in MM cells at progression in one patient	52
Brudno *et al.*	2018	CAR T-cell	CAR-BCMA	NCT02215967	56 weeks after treatment	Flow cytometry	1/16	NR	Mixed BCMA expression in one patient, with some MM cells negative for BCMA	53
Green *et al.*	2018	CAR T-cell	NR	NR	60 days after treatment	Flow cytometry	1/7	NR	Presence of BCMA-negative MM cells in one patient. On MM cells retaining BCMA expression: 70% reduction of BCMA expression and 5-fold reduction in BCMA antigen binding capacity in this patient	54
Cohen *et al.*	2019	CAR T-cell	CART-BCMA	NCT02546167	1 month after treatment	Flow cytometry	12/18	NR	Reduction of BCMA expression intensity in 67% (n=12) of the patients, including 8 of 9 responders and 4 of 9 non-responders	55
Truger *et al.*	2021	BsAb	AMG420	NCT02514239	6 months after treatment	IHC, WGS and RNA-seq	Case report	Homozygous *BCMA* gene deletion	Complete BCMA loss caused by homozygous *BCMA* gene deletion in one patient	56
Da Vià *et al.*	2021	CAR T-cell	Idecabtagene-vicleucel	NCT03361748	5 months after treatment	IHC, WGS and RNA-seq	Case report	Homozygous *BCMA* gene deletion	Complete BCMA loss caused by homozygous *BCMA* gene deletion in one patient	15

Continued on following page.

Authors	Year of publication	Type of immunotherapy	Product	Clinical trial identifier	Time point of BCMA loss	Methods	Frequency of BCMA loss in the study#	Biological mechanism of BCMA loss	Major findings	Reference
Samur *et al.*	2021	CAR T-cell	Idecabtagene-vicleucel	NCT02658929	8 months after treatment	IHC, WGS and RNA-seq	Case report	*BCMA* gene deletion + mutation	Biallelic BCMA loss (mutation + deletion) in one patient	14
Leblay *et al.*	2020	CAR T-cell	NR	NR	NR	Cellular indexing of transcriptomes and epitopes by sequencing	Case report	Homozygous *BCMA* gene deletion	Complete BCMA loss caused by homozygous *BCMA* gene deletion in one patient	16
Munshi *et al.*	2021	CAR T-cell	Idecabtagene-vicleucel	NCT03361748	NR	NR	3/71	NR	Loss of tumor BCMA expression was suspected in 3 of 71 patients (4%) at progression	51
Wang *et al.*	2022	CAR T-cell	China	ChiCTR-OIC-17011272	NR	Flow cytometry	1/21	NR	One (5%) patient relapsed with BCMA-negative MM cells	76

BCMA: B-cell maturation antigen; BsAb: bispecific antibody; CAR T-cell: chimeric antigen receptor modified T-cell; IHC: immunohistochemistry; MM: multiple myeloma; NR: not reported; RNA-seq: single-cell RNA sequencing; WGS: whole-genome sequencing; #Among the patients with evaluable BCMA expression at baseline and relapse.

also more frequent in patients with both del16p and del17p than in those who had only del16p or del17p. However, it is still unknown whether the co-occurrence of BCMA and TP53 loss is only an accidental event or whether there is some correlation between the two. This observation has also raised the question of whether patients with *BCMA* gene deletion have more genomic changes than do those without *BCMA* gene alterations. Moreover, due to the so-called branching evolution and spatial genomic heterogeneity, BCMA expression might be heterogeneous in various focal lesions and/or at different time points in the disease course.[57-60] These issues have yet to be addressed in future studies.

As previously mentioned, γ-secretase can shed BCMA from the MM cell surface and release sBCMA into the blood.[21] This might explain the reversible partial loss of BCMA expression reported in previous studies.[53,55] Indeed, a preclinical study using cell lines and mouse models demonstrated that inhibition of γ-secretase activity could upregulate BCMA density on plasma cells and therefore increase the efficacy of anti-BCMA CAR T-cell therapy *in vivo*.[61] Thus, targeting γ-secretase might be a strategy to improve the anti-MM activity of BCMA-directed immunotherapies in patients without permanent BCMA loss.

Another potential mechanism related to BCMA loss is interference by sBCMA. In a recent study by Chen *et al.*, the authors showed that high levels of sBCMA in serum might lead to a consistent decrease in the binding of anti-BCMA antibody to tumor cells in patients with RRMM.[62] Since the majority of RRMM patients display elevated sBCMA, the functionally available BCMA on the surface of MM cells may be significantly reduced by sBCMA, meaning a functional BCMA downregulation in these patients.

Theoretically BCMA loss could also be caused by so-called antigen masking. In B-cell acute lymphoid leukemia relapsing after anti-CD19 CAR T-cell therapy, it was reported that an unintentional introduction of a CAR gene into a CD19-positive blast cell could lead to expression of CAR on leukemic cells. Subsequently, these CAR on the leukemic blasts bound *in cis* to the CD19 epitope on the cell surface, masking the tumor cells from recognition by the "true" CAR T cells, causing a functional loss of antigen.[63] However, this phenomenon has not yet been reported in CAR T-cell therapies for RRMM.

Further potential mechanisms that could be associated with BCMA loss include trogocytosis, and some epigenetic mechanisms, which have been described in other malignant hematologic diseases. Trogocytosis is a process in which the target antigen on tumor cells is transferred to CAR T cells. In mouse models of B-cell leukemia, trogocytosis could reduce the antigen (CD19) density on tumor cells, leading to fratricide killing, T-cell exhaustion, and a decreased anti-tumor effect by CAR T cells.[64] Moreover, after rituximab-containing therapy, downregulation of CD20 was observed in patients with diffuse large cell B-cell lymphoma. When the CD20-negative lymphoma cells were treated with 5-aza-2'-deoxycytidine *in vitro*, the expression of CD20 mRNA recovered within 3 days, suggesting that some epigenetic mechanisms might be involved in CD20 downregulation after rituximab.[65] Theoretically, these mechanisms may also be related to BCMA loss in RRMM patients. However, the role of these mechanisms in BCMA has not yet been extensively evaluated.

Strategies to overcome BCMA loss

Although BCMA-directed immunotherapies may no longer be effective in some patients because of loss of the target antigen, several other treatment options could still restore a response in such patients. In principle, the strategies to overcome BCMA loss are dependent on the underlying mechanisms. The reversibility of BCMA loss is the most crucial determinant for planning further treatments. Here, we discuss some alternatives that could be considered in RRMM patients with BCMA loss.

Homozygous *BCMA* gene deletion may lead to irreversible and complete loss of BCMA expression on the MM cell surface.[14,15] In these cases, BCMA-targeted therapies are irreversibly ineffective. One of the strategies to overcome this kind of BCMA loss is targeting other antigens such as CD38, G protein coupled receptor class C group 5 member D (GPRC5D), Fc receptor-homolog 5 (FcRH5), CD19, and SLAMF7[66] (Figure 1B). CAR T-cell and BsAb therapies targeting antigens other than BCMA have already been summarized in other review articles, which we would recommend for readers interested in this topic.[18,20,46,67,68] For instance, in a recent phase I study, the GPRC5D-directed BsAb talquetamab showed an encouraging ORR of 70% in RRMM patients, 30% of whom had been previously treated with anti-BCMA agents.[69] Moreover, cevostamab, an FcRH5-targeted BsAb, produced an ORR of 36.4% (8 out of 22) in RRMM patients previously exposed to anti-BCMA therapies.[70] These findings suggest that targeting antigens other than BCMA might be feasible in RRMM patients previously treated with anti-BCMA therapies, and BCMA expression status was irrelevant for these agents. However, in a recent study, heterozygous deletions in *GPRC5D* and *CD38* genes were found in, respectively, 15% and 10% of MM patients who were T-cell immunotherapy-naïve, suggesting an increased risk of antigen loss following highly effective immunotherapies. In contrast, gains of *FCRH5* and *SLAMF7* genes were significantly more frequent in RRMM than in newly diagnosed MM, indicating a low risk of antigen loss in the course of the disease. Importantly, heterozygous *BCMA* gene deletion was present in four out of 50 RRMM patients who were heavily pretreated with other drugs but had never received anti-BCMA immunotherapy.[56] In conclusion, the expression of immunotherapy targets should be evaluated during the treatment decision-making process.

Another option for patients with irreversible BCMA loss is so-called "multi-targeted" immunotherapy. For instance, a BCMA/CD200/CD16A trispecific antibody was developed to link NK cells and MM cells *in vitro*, and BCMA and CD200 double-positive MM cells were more effectively killed than cells positive for only one of the antigens.[71] For CAR T cells, this could be achieved by co-administration of different mono-targeted CAR T cells or by constructing a CAR T cell that could simultaneously recognize more than one antigen.[72] In preclinical settings with cell lines and mouse models, bispecific CAR T cells targeting BCMA and GPRC5D were able to enhance the interactions between MM and CAR T cells and were able to prevent relapse due to BCMA loss.[73] Feng *et al.* reported on a BCMA/CD38-targeted bispecific CAR T cell that could trigger robust cytotoxicity against MM cells expressing either BCMA or CD38 *in vitro* and was able to achieve complete tumor clearance in mice.[74] More recently, another bispecific CAR T cell targeting BCMA and CD24 has been developed by an US group, with a strong cytotoxic effect in xenograft mouse models. In a recently published phase I first-in-human trial, a bispecific CAR T-cell targeting BCMA and CD38 (BM38) produced an ORR of 87% and a median progression-free survival of 17.2 months. Interestingly, as demonstrated by flow cytometry, two patients had BCMA- or CD38-negative RRMM at baseline, and both patients responded to BM38 treatment. Unfortunately, the underlying mechanisms of CD38 and BCMA loss at baseline were not described in the report of the study.[75] These results suggested the feasibility of bispecific CAR T cells even in RRMM patients with expression of only one target antigen. A combination of two different mono-targeted CAR T cells is an alternative strategy to bispecific CAR T cells. Some clinical trials investigating combinations of anti-BCMA CAR T cells with CD19- or CD38-directed CAR T cells have shown similar antitumor efficacy in RRMM when compared with published data on mono-specific anti-BCMA CAR T-cell therapies.[76,77] Loss of one of the two antigens has already been reported after co-administration of two different mono-specific CAR T cells in RRMM.[76] Theoretically, because of the high immune selection pressure, loss of both antigens may be possible after multi-specific immunotherapies. However, studies addressing this issue are still lacking.

Compared with homozygous *BCMA* gene deletion, the more common type of BCMA loss is reversible downregulation of BCMA expression following anti-BCMA therapies.[53,55] If the BCMA status remains positive at relapse, retreatment with an anti-BCMA therapy could be considered in RRMM patients previously exposed to BCMA-targeted therapies (Figure 1C). Indeed, effective belantamab mafodotin treatment after relapse from anti-BCMA CAR T-cell therapy has been described in case reports.[78,79] The differences in the mechanisms of action between ADC and CAR T cells also support the rationale of anti-BCMA retreatment with belantamab mafodotin in these cases, as ADC react with MM cells primarily via T-cell independent mechanisms.[79] Another druggable target is γ-secretase, which can cleave BCMA from the MM cell membrane and lead to partial BCMA loss on the cell surface.[21] In a phase I, first-in-human trial of anti-BCMA CAR T cells in combination with JSMD194, a γ-secretase inhibitor, for RRMM patients, JSMD194 increased the level of BCMA expression and might augment the anti-MM activity of CAR T cells, with a comparable toxicity profile as that in other CAR T-cell trials. Moreover, teclistamab or belantamab mafodotin in combination with a γ-secretase inhibitor, i.e. LY-411575 or nirogacestat, is currently under clinical investigation in phase I trials.[80,81]

Conclusions

BCMA-targeted treatments such as CAR T cells, BsAb, and ADC have brought new hope for patients with RRMM, and will be administered in earlier lines of therapy. They help to improve tumor control and may cure MM. The persistence of target antigen on the MM cells is essential for these novel targeted immunotherapies. Irreversible complete BCMA loss, which is caused by homozygous *BCMA* gene deletion, seems to be relatively rare after BCMA-directed treatments, based on data from clinical trials investigating CAR T cells or BsAb. In contrast, reversible partial loss or downregulation of BCMA is a more common event following these targeted immunotherapies. As BCMA-directed treatments are becoming a part of standard care for RRMM, BCMA expression status should be considered in the selection of therapeutic agents for each patient. Monitoring of several biomarkers, such as sBCMA and tumor BCMA expression level, might be helpful when making therapeutic decisions. Analyses of BCMA status on different levels (whole-genome sequencing, single-cell RNA sequencing, flow cytometry and immunohistochemistry, etc.) will provide useful information to elucidate the underlying biological mechanisms of BCMA loss in each patient. These strategies are also aligned with the concepts of precision medicine. Data on BCMA loss are still very limited, as BCMA-directed immunotherapy is a young research field. In addition, resistance mechanisms are not fully understood, and BCMA loss is not the only cause of relapse after novel immunotherapies. Further studies are, therefore, needed.

Disclosures

No conflicts of interest to disclose.

Contributions

XZ, LR, and KMK performed the literature research, analyzed and interpreted the data, and drafted the work; JM and HE conceived the design of the work and substantially revised it. All the authors approved the submitted version.

References

1. van de Donk N, Pawlyn C, Yong KL. Multiple myeloma. Lancet. 2021;397(10272):410-427.
2. Cowan AJ, Green DJ, Kwok M, et al. Diagnosis and management of multiple myeloma: a review. JAMA. 2022;327(5):464-477.
3. Raza S, Safyan RA, Lentzsch S. Immunomodulatory drugs (IMiDs) in multiple myeloma. Curr Cancer Drug Targets. 2017;17(9):846-857.
4. Elnair RA, Holstein SA. Evolution of treatment paradigms in newly diagnosed multiple myeloma. Drugs. 2021;81(7):825-840.
5. Shah UA, Mailankody S. Emerging immunotherapies in multiple myeloma. BMJ. 2020;370:m3176.
6. Cho SF, Lin L, Xing L, et al. BCMA-targeting therapy: driving a new era of immunotherapy in multiple myeloma. Cancers (Basel). 2020;12(6):1473.
7. Roex G, Timmers M, Wouters K, et al. Safety and clinical efficacy of BCMA CAR-T-cell therapy in multiple myeloma. J Hematol Oncol. 2020;13(1):164.
8. Haslauer T, Greil R, Zaborsky N, Geisberger R. CAR T-cell therapy in hematological malignancies. Int J Mol Sci. 2021;22(16):8996.
9. Ansell SM, Lin Y. Immunotherapy of lymphomas. J Clin Invest. 2020;130(4):1576-1585.
10. Barsan V, Ramakrishna S, Davis KL. Immunotherapy for the treatment of acute lymphoblastic leukemia. Curr Oncol Rep. 2020;22(2):11.
11. Orlando EJ, Han X, Tribouley C, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. Nat Med. 2018;24(10):1504-1506.
12. Bannerji R, Allan JN, Arnason JE, et al. Clinical activity of REGN1979, a bispecific human, anti-CD20 x anti-CD3 antibody, in patients with relapsed/refractory (R/R) B-cell non-Hodgkin lymphoma (B-NHL). Blood. 2019;134(Suppl 1):762.
13. Bannerji R, Arnason JE, Advani R, et al. Emerging clinical activity of REGN1979, an anti-CD20 x anti-CD3 bispecific antibody, in patients with relapsed/refractory follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), and other B-cell non-Hodgkin lymphoma (B-NHL) subtypes. Blood. 2018;132(Suppl 1):1690.
14. Samur MK, Fulciniti M, Aktas Samur A, 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(1):868.
15. Da Via MC, Dietrich O, Truger M, et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. Nat Med. 2021;27(4):616-619.
16. Leblay N, Maity R, Barakat E, et al. Cite-seq profiling of T cells in multiple myeloma patients undergoing BCMA targeting CAR-T or Bites immunotherapy. Blood. 2020;136(Suppl 1):11-12.
17. Rasche L, Wasch R, Munder M, Goldschmidt H, Raab MS. Novel immunotherapies in multiple myeloma - chances and challenges. Haematologica. 2021;106(10):2555-2565.
18. Yu B, Jiang T, Liu D. BCMA-targeted immunotherapy for multiple myeloma. J Hematol Oncol. 2020;13(1):125.
19. Sanchez L, Dardac A, Madduri D, Richard S, Richter J. B-cell maturation antigen (BCMA) in multiple myeloma: the new frontier of targeted therapies. Ther Adv Hematol. 2021;12:2040620721989585.
20. Zhou X, Rasche L, Kortum KM, Danhof S, Hudecek M, Einsele H. Toxicities of chimeric antigen receptor T cell therapy in multiple myeloma: an overview of experience from clinical trials, pathophysiology, and management strategies. Front Immunol. 2020;11:620312.
21. Laurent SA, Hoffmann FS, Kuhn PH, et al. Gamma-secretase directly sheds the survival receptor BCMA from plasma cells. Nat Commun. 2015;6:7333.
22. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. Leukemia. 2020;34(4):985-1005.
23. Tai YT, Acharya C, An G, et al. APRIL and BCMA promote human multiple myeloma growth and immunosuppression in the bone marrow microenvironment. Blood. 2016;127(25):3225-3236.
24. Moreaux J, Legouffe E, Jourdan E, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. Blood. 2004;103(8):3148-3157.
25. Tai YT, Li XF, Breitkreutz I, et al. Role of B-cell-activating factor in adhesion and growth of human multiple myeloma cells in the bone marrow microenvironment. Cancer Res. 2006;66(13):6675-6682.
26. Cho SF, Anderson KC, Tai YT. Targeting B cell maturation antigen (BCMA) in multiple myeloma: potential uses of BCMA-based immunotherapy. Front Immunol. 2018;9:1821.
27. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM. Classical and/or alternative NF-kappaB pathway activation in multiple myeloma. Blood. 2010;115(17):3541-3552.
28. Lee L, Bounds D, Paterson J, et al. Evaluation of B cell maturation antigen as a target for antibody drug conjugate mediated cytotoxicity in multiple myeloma. Br J Haematol. 2016;174(6):911-922.
29. Wang B, Wu C, Zhong Q, et al. Belantamab mafodotin for the treatment of multiple myeloma. Drugs Today (Barc). 2021;57(11):653-663.
30. Mullard A. FDA approves first BCMA-targeted CAR-T cell therapy. Nat Rev Drug Discov. 2021;20(5):332.
31. Mullard A. FDA approves second BCMA-targeted CAR-T cell therapy. Nat Rev Drug Discov. 2022;21(4):249.
32. Usmani SZ, Garfall AL, van de Donk N, et al. Teclistamab, a B-cell maturation antigen x CD3 bispecific antibody, in patients with relapsed or refractory multiple myeloma (MajesTEC-1): a multicentre, open-label, single-arm, phase 1 study. Lancet. 2021;398(10301):665-674.
33. Topp MS, Duell J, Zugmaier G, et al. Anti-B-cell maturation antigen BiTE molecule AMG 420 induces responses in multiple myeloma. J Clin Oncol. 2020;38(8):775-783.
34. Lonial S, Lee HC, Badros 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(2):207-221.
35. Popat R, Nooka A, Stockerl-Goldstein K, et al. DREAMM-6: safety, tolerability and clinical activity of belantamab mafodotin (Belamaf) in combination with bortezomib/dexamethasone (BorDex) in relapsed/refractory multiple myeloma (RRMM). Blood. 2020;136(Suppl 1):19-20.
36. Usmani SZ, Alonso AA, Quach H, et al. DREAMM-9: phase I study of belantamab mafodotin plus standard of care in patients with transplant-ineligible newly diagnosed multiple myeloma. Blood. 2021;138(Suppl 1):2738.
37. Lee HC, Raje NS, Landgren O, et al. Phase 1 study of the anti-BCMA antibody-drug conjugate AMG 224 in patients with relapsed/refractory multiple myeloma. Leukemia. 2021;35(1):255-258.
38. Kumar SK, Migkou M, Bhutani M, et al. Phase 1, first-in-human study of MEDI2228, a BCMA-targeted ADC in patients with relapsed/refractory multiple myeloma. Blood. 2020;136(Suppl 1):26-27.

39. Harrison SJ, Minnema MC, Lee HC, 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(Suppl 1):28-29.
40. Moreau P, Usmani SZ, Garfall AL, et al. Updated results from MajesTEC-1: phase 1/2 study of teclistamab, a B-cell maturation antigen x CD3 bispecific antibody, in relapsed/refractory multiple myeloma. Blood. 2021;138(Suppl 1):896.
41. Zonder JA, Richter J, Bumma N, et al. Early, deep, and durable responses, and low rates of cytokine release syndrome with REGN5458, a BCMAxCD3 bispecific monoclonal antibody, in a phase 1/2 first-in-human study in patients with relapsed/refractory multiple myeloma (RRMM). Blood. 2021;138(Suppl 1):160.
42. Kumar S, D'Souza A, Shah N, et al. A phase 1 first-in-human study of Tnb-383B, a BCMA x CD3 bispecific T-cell redirecting antibody, in patients with relapsed/refractory multiple myeloma. Blood. 2021;138(Suppl 1):900.
43. Sebag M, Raje NS, Bahlis NJ, et al. Elranatamab (PF-06863135), a B-cell maturation antigen (BCMA) targeted CD3-engaging bispecific molecule, for patients with relapsed or refractory multiple myeloma: results from MagnetisMM-1. Blood. 2021;138(Suppl 1):895.
44. Costa LJ, Wong SW, Bermúdez A, et al. First 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): interim results of a phase 1 multicenter trial. Blood. 2019;134(Suppl_1):143.
45. Lancman G, Sastow DL, Cho HJ, et al. Bispecific antibodies in multiple myeloma: present and future. Blood Cancer Discov. 2021;2(5):423-433.
46. Zhou X, Einsele H, Danhof S. Bispecific antibodies: a new era of treatment for multiple myeloma. J Clin Med. 2020;9(7):2166.
47. Teoh PJ, Chng WJ. CAR T-cell therapy in multiple myeloma: more room for improvement. Blood Cancer J. 2021;11(4):84.
48. Raje N, Berdeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med. 2019;380(18):1726-1737.
49. Mailankody S, Htut M, Lee KP, et al. JCARH125, Anti-BCMA CAR T-cell therapy for relapsed/refractory multiple myeloma: initial proof of concept results from a phase 1/2 multicenter study (EVOLVE). Blood. 2018;132(Suppl 1):957.
50. Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet. 2021;398(10297):314-324.
51. Munshi NC, Anderson LD Jr, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med. 2021;384(8):705-716.
52. Ali SA, Shi V, Maric I, et al. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. Blood. 2016;128(13):1688-1700.
53. Brudno JN, Maric I, Hartman SD, 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(22):2267-2280.
54. Green DJ, Pont M, Sather BD, 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(Suppl 1):1011.
55. Cohen AD, Garfall AL, Stadtmauer EA, et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. J Clin Invest. 2019;129(6):2210-2221.
56. Truger MS, Duell J, Zhou X, et al. Single- and double-hit events in genes encoding immune targets before and after T cell–engaging antibody therapy in MM. Blood Adv. 2021;5(19):3794-3798.
57. Farswan A, Jena L, Kaur G, et al. Branching clonal evolution patterns predominate mutational landscape in multiple myeloma. Am J Cancer Res. 2021;11(11):5659-5679.
58. Rasche L, Chavan SS, Stephens OW, et al. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. Nat Commun. 2017;8(1):268.
59. Terragna C, Martello M, Santacroce B, et al. A branching evolution model at relapse characterizes multiple myeloma patients who responded to up-front combination therapy including new drugs. Blood. 2016;128(22):2080.
60. Rasche L, Schinke C, Maura F, et al. The spatio-temporal evolution of multiple myeloma from baseline to relapse-refractory states. Nat Commun. 2022;13(1):4517.
61. Pont MJ, Hill T, Cole GO, et al. Gamma-secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. Blood. 2019;134(19):1585-1597.
62. Chen H, Li M, Xu N, et al. Serum B-cell maturation antigen (BCMA) reduces binding of anti-BCMA antibody to multiple myeloma cells. Leuk Res. 2019;81:62-66.
63. Ruella M, Xu J, Barrett DM, et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nat Med. 2018;24(10):1499-1503.
64. Hamieh M, Dobrin A, Cabriolu A, et al. CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. Nature. 2019;568(7750):112-116.
65. Hiraga J, Tomita A, Sugimoto T, et al. Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance. Blood. 2009;113(20):4885-4893.
66. Bruno B, Wasch R, Engelhardt M, et al. European Myeloma Network perspective on CAR T-cell therapies for multiple myeloma. Haematologica. 2021;106(8):2054-2065.
67. Zhou X, Einsele H, Danhof S. [CAR T-cell therapy for multiple myeloma]. Internist (Berl). 2021;62(6):605-610.
68. Teoh PJ, Chng WJ. CAR T-cell therapy in multiple myeloma: more room for improvement. Blood Cancer J. 2021;11(4):84.
69. Krishnan AY, Minnema MC, Berdeja JG, et al. Updated phase 1 results from MonumenTAL-1: first-in-human study of talquetamab, a G protein-coupled receptor family C group 5 member D x CD3 bispecific antibody, in patients with relapsed/refractory multiple myeloma. Blood. 2021;138(Suppl 1):158.
70. Trudel S, Cohen AD, Krishnan AY, et al. Cevostamab monotherapy continues to show clinically meaningful activity and manageable safety in patients with heavily pre-treated relapsed/refractory multiple myeloma (RRMM): updated results from an ongoing phase I study. Blood. 2021;138(Suppl 1):157.
71. Gantke T, Weichel M, Herbrecht C, et al. Trispecific antibodies for CD16A-directed NK cell engagement and dual-targeting of tumor cells. Protein Eng Des Sel. 2017;30(9):673-684.
72. Simon S, Riddell SR. Dual targeting with CAR T cells to limit antigen escape in multiple myeloma. Blood Cancer Discov. 2020;1(2):130-133.
73. Fernandez de Larrea C, Staehr M, Lopez AV, 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(2):146-154.

74. Feng Y, Liu X, Li X, et al. Novel BCMA-OR-CD38 tandem-dual chimeric antigen receptor T cells robustly control multiple myeloma. Oncoimmunology. 2021;10(1):1959102.
75. Mei H, Li C, Jiang H, et al. A bispecific CAR-T cell therapy targeting BCMA and CD38 in relapsed or refractory multiple myeloma. J Hematol Oncol. 2021;14(1):161.
76. Wang Y, Cao J, Gu W, et al. Long-term follow-up of combination of B-cell maturation antigen and CD19 chimeric antigen receptor T cells in multiple myeloma. J Clin Oncol. 2022;40(20):2246-2256.
77. Zhang H, Liu M, Xiao X, et al. A combination of humanized anti-BCMA and murine anti-CD38 CAR-T cell therapy in patients with relapsed or refractory multiple myeloma. Leuk Lymphoma. 2022;63(6):1418-1427.
78. Gazeau N, Beauvais D, Yakoub-Agha I, et al. Effective anti-BCMA retreatment in multiple myeloma. Blood Adv. 2021;5(15):3016-3020.
79. Cohen AD, Garfall AL, Dogan A, et al. Serial treatment of relapsed/refractory multiple myeloma with different BCMA-targeting therapies. Blood Adv. 2019;3(16):2487-2490.
80. Pillarisetti K, Powers G, Luistro L, et al. Teclistamab is an active T cell-redirecting bispecific antibody against B-cell maturation antigen for multiple myeloma. Blood Adv. 2020;4(18):4538-4549.
81. Nooka AK, Weisel K, van de Donk NW, et al. Belantamab mafodotin in combination with novel agents in relapsed/refractory multiple myeloma: DREAMM-5 study design. Future Oncol. 2021;17(16):1987-2003.