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Review

Current state and next-generation CAR-T cells in multiple myeloma

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A B S T R A C T

Chimeric antigen receptor T cells (CAR-T cells) have emerged as a potentially transformative new approach to treating hematological malignancies. Ide-cel, an autologous B cell maturation antigen (BCMA) targeting CAR-T cells, has recently been approved to treat multiple myeloma (MM). Here, we review the main clinical trials of CAR-T cells in MM with the most advanced autologous BCMA-directed ide-cel and cilta-cel, the human CARs orva-cel and CT053, the alternative manufacturing process with P-BCMA-101 and bb21217, the dual CAR GC012F and the allogenic BCMA-directed CAR-T cells ALLO-715. In light of those clinical data, we provide an overview of CAR-T cells' main potential resistance mechanisms, including antigen loss, antigen spreading, anti-CAR antibodies, CAR-T cell exhaustion, and the emergence of a non-permissive microenvironment. Finally, we describe the principal area of research to build the next generation of CAR-T cells, with armored-, gated- or commuting-CARs, CARs associated with knock out of specific genes, and CAR-T cells made from γδT cells or NK cells.

1. Introduction

Multiple myeloma (MM) is the second most frequent hematological malignancy with an incidence of 6.5 per 100,000 persons per year. Despite considerable advances in treatment options, including new generations of proteasome inhibitors, immunomodulatory drugs, and immunotherapies with anti-CD38 antibodies, MM remains an incurable disease with a 5 years relative survival of 55.6% in 2011–2017 [1]. A substantial proportion of patients either do not respond to current therapies or acquire resistance to treatment highlighting an unmet need for improved therapeutic options for MM. Typically, patients with triple-class or penta-class refractory MM have an overall survival (OS) of 9.2 and 5.6 months, respectively [2]. The new treatment approaches have focused on immunotherapies to address this unmet need, including development of antibody-drug conjugates, bispecific antibodies, and chimeric antigen receptor (CAR) T cells. Their evaluation in phase 1 dose escalation studies have demonstrated high response rates in late-stage refractory MM.

CAR-T cells are T cells modified ex vivo to express a chimeric receptor with an antigen receptor containing a single chain variable fragment (scFv) and an intracellular T-cell receptor (TCR) signaling domain. The scFv is the recognition domain directed to target tumor cells. The intracellular domain of CAR contains various components with CD3-zeta (first generation), in addition to a costimulatory domain such as CD28 or 41bb (second generation) or both (third generation). A fourth-generation CAR-T cells is known as armored CAR-T cells and co-express key cytokines or suicide genes to enhance the efficacy and safety of CAR-T therapy. Notably, CAR-T target cell recognition does not require HLA presentation of antigens. To this end, the patient's own T cells are isolated and genetically modified to express the CAR, redirecting T cell specificity to the tumor-associated antigen. Adoptively transferred CAR-T cells are therefore equipped to induce and sustain remissions through a synergy of antibody-based target cell recognition and the memory and effector function of T cells. These results represent a substantial improvement compared to conventional therapies yielding complete and durable response rate.

B cell maturation antigen (BCMA) is currently the main target for CAR-T cells in MM as it is predominantly expressed on differentiated B cells including malignant plasma cells. BCMA, also known as TNF receptor superfamily 17 (TNFRSF17), delivers pro-survival signaling upon binding to its ligands - B cell activator of the TNF family (BAFF) and a proliferation inducing ligand (APRIL) - participating in the survival and proliferation of MM cells. BCMA is shed from the surface of MM cells by γ-secretase, releasing a soluble form BCMA (sBCMA), which serves as a biomarker of MM tumor burden. Additionally, soluble BCMA can limit therapeutic efficacy of membrane-bound BCMA-targeted therapies.

This review discusses the primary clinical data on anti-BCMA CAR-T cells in MM and known mechanisms of resistance and next generations

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CAR-T cells that are currently under research.

2. Clinical trials of CAR-T cells in myeloma

Since the development of CAR-T cells in MM, several clinical trials have been reported for this indication. So far, all CAR-T cells used in MM target BCMA. Most of them are autologous, but some allogenic products are emerging, they bear human or chimeric scFv and have various manufacturing processes. The main clinical trials data are reported here (Table 1).

2.1. Ide-cel

Idecabtagene vicleucel (ide-cel) is the first FDA and EMA approved autologous BCMA-directed CAR-T cell. It is a second-generation CAR, with CD3ζ as the T-cell activation domain and 4-1BB as a co-stimulatory domain. The phase 2 KarMMa trial evaluated the safety and efficacy at the dose of 150, 300 and 450 millions infused CAR-T cells [3]. A total of 140 patients were enrolled and 128 received ide-cel, with a median age of 61 (33–78) years old and a median of 6 (3–16) prior lines of treatment. All patients were refractory to the previous line of therapy and 84% were triple-class refractory (proteasome inhibitor, immunomodulatory drug and anti-CD38 immunotherapy). The overall response rate (ORR) was 73% for the whole cohort and 82% for the 450 M cohort, with 39% of complete remission (CR) and 28% of minimal residual disease (MRD) negativity at 10^{-5}. The median progression free survival (PFS) time was 8.8 months (95% CI 5.6–11.6) for the whole cohort and 12.1 months (95% CI 8.8–12.3) for the 450 M cohort. Prolonged PFS was observed in patients with more profound responses: 20.2 months in case of CR/sCR; 11.3 months in case of VGPR; 5.4 months in PR and 1.8 months in non-responders. The median overall survival was 24.8 months in the study. Regarding expansion and persistence of the CAR-T cells, the median peak of CAR-T cell expansion was observed at 11 days after infusion. Patients who responded had a higher peak exposure than non-responders. Ide-cel showed durable persistence in blood, with 36% of patients who could be evaluated having detectable CAR+ T cells at 12 months. The presence of these cells did not guard against recurrence.

Overall, 84% of the patients developed a cytokine release syndrome (CRS) with a median onset of 1 day (1−12) and a median duration of 5 days (1–63). CRS were grade 1 or 2 in 78% of the case and grade 3 or more in 6%. The incidence of CRS was dose-dependent and peak expansion-dependent. Patients grade 3 or more CRS had higher peaks of IL6 and IFNγ. Immune effector cell-associated neurotoxicity syndrome (ICANS) occurred in 18% of the patients with 14% of grade 1 or 2 and only 3% of grade 3. The median onset was 2 days, and the median duration was 3 days. In most cases, ICANS occurred during or after the onset of a CRS. All patients with grade 3 ICANS were older than 65 years old. Comparing patients who developed ICANS or not, no difference in efficacy was observed. Other toxicity was mainly hematologic with 91% neutropenia, 70% anemia, and 63% thrombocytopenia. The median times to recovery of grade 3 or more neutropenia and thrombocytopenia were 2 months and 3 months, respectively.

In the KarMMa study, out of 128 patients, 20 patients were older than 70 years old, with only 4 patients older than 75 years. The profile of efficacy (ORR and median PFS) and safety (CRS and ICANS) were similar to the overall cohort [4], demonstrating a potential for using CAR-T cells in a selected population of elderly fit patients.

Table 1
Main reported clinical trials of CAR-T cells in multiple myeloma. Data of efficacy and safety are shown.

	Ide-cel KARMMA[3] ($n = 128$)	Cilta-cel CARTITUDE-1[5] ($n = 97$)		bb21217 CRB-402[9] ($n = 69$)	P-BCMA-101 PRIME[8] ($n = 53$)	Orva-cel EVOLVE[6] ($n = 62$)	CT053[7] ($n = 20$)	ALLO-715 UNIVERSAL[11] ($n = 31$)
Phase	II	Ib/II		I	I/II	I/II	I	I
Target / Costim	BCMA / 4-1BB	BCMA / 4-1BB		BCMA / 4-1BB	BCMA / 4-1BB	BCMA / 4-1BB	BCMA / 4-1BB	BCMA / 4-1BB
scFv	Chimeric mouse	Chimeric llama		Chimeric mouse	Chimeric mouse	Human	Human	Human
Specificity	Autologous	Autologous		Autologous - PI3K inhibitor	Autologous - piggyBac	Autologous	Autologous	Allogenic - CD52 & TCR KOs
# of infused CAR-T cells	150-450 M	0.75 M/kg		150-450 M	51-1178 M	150-600 M	50–180	40-180 M
Population								
Age, median (range) years	61 (33–78)	61 (43–78)		62 (33–76)	60 (42–74)	61 (33–77)	55 (39–67)	65 (46–76)
# of prior lines, median (range)	6 (3–16)	6 (3–18)		6 (3–17)	8 (2–18)	6 (3–18)	4 (2−11)	5 (3−11)
Triple- / Penta-refractory	84% / 26%	86% / 28%		64% / NR	60% / NR	94% / 48%	NR	NR
Efficacy	@450 M:							
ORR	82%	98%		60%	50% - 75%	92%	100%	50% - 75%
CR, rate	39%	80%		28%	NR	36%	35%	NR
PFS, median months	12.1	66% @ 18 months		NR	NR	NR	NR	NR
CRS	@450 M:							
All grade / grade ≥ 3	96% / 6%	95% / 5%		70% / 4%	17% / 0%	89% / 3%	79% / 0%	45% / 0%
Median onset, days (range)	1 (1−10)	7 (1–12)		2 (1−20)	NR	2 (1–4)	2 (1–4); 1 (0–6)	NR
Median duration, days (range)	7 (1–63)	4 (1–97)		4 (1–28)	NR	4 (1–10)	4 (1–8); 3 (1–7)	NR
Tocilizumab / steroid use	67% / 22%	69% / 22%		45% / 15%	7% / 6%	76% / 52%	32% / 21%	19/10
ICANS	@450 M:	ICANS	Other*					
All grade/grade ≥ 3	20% / 6%	17% / 2%	12% / 9%	16% / 4%	4% / 4%	13% / 3%	NR	0% / 0%
Median onset, days (range)	2 (1–10)	8 (3−12)	27 (11–108)	7 (2–24)	NR	4 (1–6)	NR	NA
Median duration, days (range)	5 (1−22)	4 (1–12)	75 (2–160)	2 (1–188)	NR	4 (1–10)	NR	NA

Costim: costimulatory domain; scFv: single chain variable fragment; ORR: overall response rate; CR: complete remission; PFS: progression free survival; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; *Other neurotoxicity by means of nerve palsy and parkinson-like syndrome.

2.2. Cilta-cel

Ciltacabtagene autoleucel (cilta-cel) is another second generation autologous BCMA-directed CAR, with CD3ζ as T-cell activation domain, 4-1BB as a co-stimulatory domain and with 2 BCMA-targeting scFv to increase avidity. The CARTITUDE-1 [5] phase 1b/2 study has enrolled 113 patients from who 97 received cilta-cel with a median of 0.71×10^6 CAR+ viable T cells per kilogram. The median age was 61 (43–78) years old. Patients received a median of 6 (3–18) prior lines of treatment and 87% of them were triple-class refractory. The ORR was 98% with 80% of the patients achieving a sCR and 95% at least a VGPR. Among evaluable patients 92% had a negative MRD at 10^{-5}. The 24-months PFS rate at was 60.5% for the whole cohort (95% CI, 48.5–70.4).

Overall, 94% of the patients developed a CRS with a median onset of 7 days (1–12) and a median duration of 4 days (1–97). CRS were grade 1 or 2 in 94% of the case. Neurotoxicity occurred in 20% of the patients with 10% of grade 3. Among those, 16.5% had an ICANS with 2% of grade 3 or more, and 12.4% had a late onset neurotoxicity, with 9% of grade 3 or more. This late onset neurotoxicity occurred in a median of 27 days (11–108) with a median time to recovery of 75 days (2–160). It is characterized by either peripheral neuropathy such as facial nerve palsy or movement and/or neurocognitive changes described as Parkinson-like syndrome. Three cases of neurocognitive disorders following BCMA-targeting CAR-T cells have been reported recently – 2 following cilta-cel and one following ide-cel [6]. They demonstrate the expression of BCMA on neurons and astrocytes in the patient's basal ganglia as well as in the caudate of normal human brains, suggesting an on-target effect of anti-BCMA therapy.

Other grade 3 or 4 toxicity was mainly hematologic with 95% neutropenia, 68% anemia, and 60% thrombocytopenia. The median times to recovery of grade 3 or 4 neutropenia and thrombocytopenia were 2 weeks and 4 weeks, respectively.

The remarkable efficacy of cilta-cel will need to mature with time to assess whether a plateau of PFS will be reached, especially at earlier lines of treatments. Many have commented differences between cilta-cel and ide-cel. One of the main apparent difference is the lower number of infused cells with cilta-cel (target of 0.75×10^6/kg for cilta-cel and 450×10^6 for ide-cel). This translate into a longer time to peak expansion in cilta-cel than ide-cel, suggesting a more important in vivo expansion with cilta-cel.

2.3. BCMA-targeting CAR-T cells with human scFv

The potential immunogenicity of chimeric CARs (murine for ide-cel and llama for cilta-cel) represents a possible limiting factor of CAR-T cells therapy. To improve their efficacy, human CARs have been developed.

Orva-cel is a BCMA-directed CAR-T cells, with a fully humanized scFv binder to reduce the construct's immunogenicity. The EVOLVE phase 1b/2 dose escalation study has enrolled 44 patients receiving doses of orva-cel from 50 to 450 million cells. Patients had received a median of 7 (3–18) prior lines of treatment and 86% of them were penta-exposed. The ORR was 82%, with 48% of the patients achieving at least a VGPR. The follow up is too short to evaluate the duration of response [7]. However, the development of orva-cel may not be pursued due to the company decision.

CT053 is another BCMA-targeting CAR-T cell with a human anti-BCMA scFv and 4-1BB as costimulatory domain. In the LUMMICAR-2 dose escalation study, 20 patients received 150 to 300 millions CAR-T cells. The ORR was 100% with 50% of VGPR or better. The most common grade ≥ 3 adverse was hematological toxicity. CRS was observed in 86% of the patients, mainly grade 1 or 2 and only one patient experienced grade 2 neurotoxicity [8].

2.4. Alternative manufacturing process

The manufacturing consist in an in-vivo expansion of T-cell using IL-2 and a transduction of the chimeric receptor plasmid. This process can affect the T-cell fitness, thus novel manufacturing approaches could potentially improve the product quality. The majority of CAR-T cells are produced using retroviral or lentiviral vectors to deliver the CAR transgene. P-BCMA-101 is a BCMA-targeting CAR-T cell manufactured using a transposon-based system called piggyBac. This technology allows to introduce larger genomic material into the cells with potentially less immunogenicity than a virus-based vector. A total of 43 patients were enrolled in the PRIME phase 1/2 study, to receive 0.75 to 15 millions of P-BCMA-101 CAR-T cells per kg. The ORR was 57% for all subjects with a correlation to Cmax and AUC of cell expansion. CRS was seen in 17% of patients and only one case of neurotoxicity was observed [9].

Bb21217 uses the same CAR molecule as bb2121. Still, it is cultured with the PI3K inhibitor bb007 - during the manufacturing process - to enrich T cells with memory-like phenotype that has a superior proliferative capacity upon adoptive transfer. Overall, 69 patients with a median of 6 prior lines of treatment were included in the phase 1 CRB-402 dose escalation study. The ORR was 68% with 54% of the patients achieving a VGPR or better, with a median DoR of 17 months. CRS rate was 70% and neurotoxicity was observed in 16% of the cases, mainly grade 1 and 2. Responders had a higher peak of expansion of CAR-T cells, which was associated with an enrichment of CD4 and CD8 central memory T cells [10]. Moreover, CAR-T cells were persistent in 6 out of 8 patients at 24 months, suggesting a durable persistence of bb21217.

2.5. Dual targeting CAR-T cells

One of the limitation of targeted-immunotherapies is the potential heterogeneity of antigen expression among tumor cells and the loss of antigen after exposure. Targeting different antigens at the same time could overcome this issue. GC012F is a dual BCMA/CD19 targeted CAR-T manufactured in 24 to 36 h on the FASTCAR® platform. Sixteen patients with relapsed and refractory MM received 100.000 to 300.000 CAR-T cells per kg. The ORR was 94% with all patients achieving a VGPR or better. Median duration of response was not reached at 7.3 months of median follow up. CRS was observed in 87.5% of the patients, all cases were grade 1 or 2. No neurotoxicity was observed [11].

2.6. Allo-CAR-T cells

A current limitation of autologous CAR-T cells is represented by the time needed before administration of the product. The overall process requires a leukapheresis, the manufacturing itself and quality control of the product. During this time, bridging therapy is often administered; however, some patients have advanced diseases with limited treatment options. Allogenic CAR-T cells represent an off-the-shelf alternative. The potential immunogenicity and the short persistence of the product are the main challenges using allogenic-CAR-T cells.

ALLO-715 is an allogenic BCMA-targeting CAR-T cell with a human scFv and 41BB as a costimulatory domain. It is manufactured with a knockout (KO) of T-cell receptor α constant (TRAC) to minimize the risk of graft versus host disease (GvHD). It also contains a KO of CD52, which allows using an anti-CD52 antibody to deplete T cells and improve the engraftment of the CAR-T cells. The UNIVERSAL phase 1 study is the first allogenic BCMA CAR-T cells trial for MM. Ten patients were enrolled to receive 40 to 480 millions of cells after a lymphodepletion with fludarabine, cyclophosphamide and ALLO-647 (anti-CD52 Ab). The ORR was 60% with 40% of VGPR or better. CRS was seen in 45% of the cases and no patients developed neurotoxicity. The peak expansion was observed at 7 days with a persistence to up 120 days for higher doses. No GvHD was observed [12].

3. Mechanisms of resistance to CAR-T cells

The main mechanism of resistance to CAR-T cells are either antigen dependent (antigen escape, antigen shedding or anti-CAR antibodies) or T cell driven (CAR-T cell exhaustion and a non-permissive microenvironment), (Fig. 1). Their precise knowledge will help guide medical decisions [13] and improve the next generation of CAR-T cells.

3.1. BCMA antigen escape

BCMA represents a key target in MM as theoretically all patients at diagnosis or relapse express BCMA [14]. A first mechanism of resistance that can occur with BCMA-targeting immunotherapies is the antigen loss, as it is often observed with CD19-targeting immunotherapies in acute lymphoid leukemia. However, antigen escape does not appear to be as frequent in the context of anti-BCMA therapies, probably because it plays a role in the proliferation and survival of plasma cells [15]. In the KarMMa study, a loss of antigen at relapse was observed in only one patient out of 16 relapsing patients by immunohistochemistry assessment [16]. By measuring soluble BCMA in serum, only 3 patients out of 71 had undetectable or declining levels at the time of progression, suggesting an antigen loss in approximately 4% of the cases [16]. A few cases of biallelic deletion on chromosome 16 - encompassing the BCMA locus – are now reported at relapse after a BCMA-directed therapy [17,18]. This indicates that antigen escape is a rare event in the context of relapse after BCMA-targeting CAR-T cells. Moreover, the response rate to BCMA-targeting CAR-T cells is similar in patients with high or low BCMA expression [19].

3.2. BCMA shedding

BCMA can also undergo γ-secretase–mediated shedding from plasma cells, leading to circulation of soluble BCMA (sBCMA) [20]. This sBCMA can serve as a biomarker – especially when the disease is otherwise difficult to monitor - as it correlates to the MM tumor burden and the prognosis of patients [21,22]. Theoretically, high levels of soluble BCMA could interfere with CAR-T cells by coating BCMA-directed scFv and thereby function as an antigen-masking mechanism [23]. Drugs that inhibit γ-secretase could potentially enhance the efficacy of BCMA-targeting therapies by reducing shedding of BCMA from the cell surface and thereby increasing myeloma cell BCMA receptor density and decreasing potential interference by sBCMA [20]. However, to date, no clinical evidence exists suggesting that sBCMA levels can negatively impact BCMA-directed CAR-T cells.

3.3. Anti-scFv antibodies

Until recently, most of the BCMA-targeting CAR-T cells evaluated in clinic have derived their scFv from non-human species (mouse for idecel [19] and camelid for cilta-cel [24]). The use of non-human scFv can induce immunogenicity resulting from an adaptive immune response after CAR-T cell infusion, which may play a role in limiting the persistence of the CARs. A recent report of 17 patients with relapsed/refractory MM treated with the bi-epitopic BCMA-targeting CAR T LCAR-B38M (now named cilta-cel) revealed that seven of them had developed high-levels of anti-CAR antibodies [25]. Among them, six patients relapsed or progressed within 6 months after infusion. This incidence of relapse was significantly higher than that of patients without detectable humoral immunogenicity. Therefore, anti-CAR antibodies constitute a high-risk for relapse after CAR-T therapy. In this trial, 8 patients received cyclophosphamide and fludarabine as lymphodepletion, while 9 patients received cyclophosphamide only. Of note, 6 out of the 7 patients with anti-CAR antibodies had received cyclophosphamide only, suggesting that the lymphodepletion regimen has an essential impact on the incidence of this immunogenicity [25]. The observation of anti-scFv antibodies in patients relapsing following BCMA-targeting CAR-T cells also emphasizes the strategy of developing fully human or humanized scFv. It is unclear whether these anti-scFv Ab are limiting persistence of CAR-T cells and promoting relapse or if they have a role in blocking the efficacy of a second dose of CAR-T cell. Several evidences suggest that retreatment with a similar product is not efficient in case of anti-CAR antibodies, while changing anti-BCMA therapy can induce responses [13].

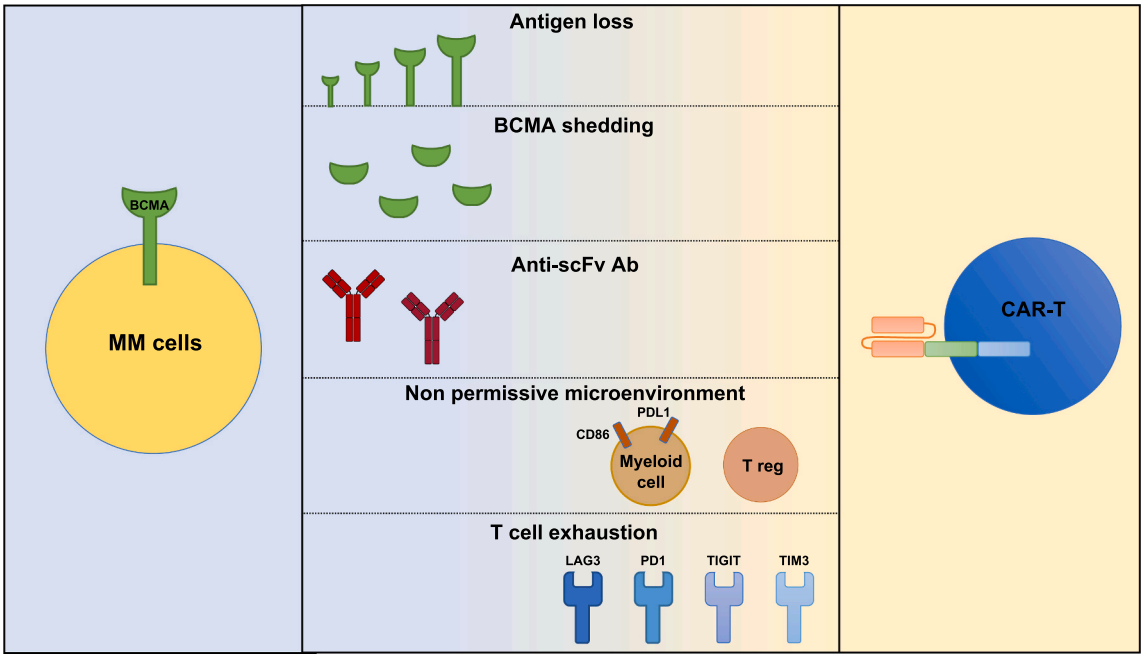

Fig. 1. Mechanism of resistance to BCMA-directed CAR-T cells. Antigen escape, loss of BCMA and the presence of anti-scFv antibodies are blocking the antigen-antibody interaction, while T-cell exhaustion and a non-permissive microenvironment are impairing CAR-T cell activation.

3.4. T-cell exhaustion and immunosuppressive tumor microenvironment

T cell exhaustion remains a potential major mechanism of relapse during BCMA-targeting therapies. In the context of CAR-T cells or bispecific antibodies, responses have been variably associated with a higher CD4:CD8 ratio and an increased frequency of $CD45RO^{-}CD27^{+}CD8^{+}$ T cells, reflective of stem memory T cells [26,27]. This T cell phenotype is more frequently observed in patients with MM at early stage of the disease [28]. In contrast, T cells with exhausted or senescent phenotypes are enriched in resistant patients to anti-BCMA BsAb or CAR T cells [27]. The fact that bispecific antibody activity depends on endogenous T-cell quality has also been illustrated in driving therapeutic efficacy of anti-CD19-CD3 blinatumomab treatment [29]. This indicates that the effectiveness of BCMA-targeting therapies may be more significant at the early stages of the disease when patients are less immuno-suppressed.

Moreover, little is known regarding the response of the tumor microenvironment to immunotherapies and its capacity to impact the response to subsequent immunotherapy. In vivo models demonstrate the synergistic activity of anti-PD-1 antibodies combined with BCMA/CS1-targeting CAR-T cells [30], suggesting that immune checkpoints may affect the efficacy of CAR-T cells or bispecific antibodies. The role of regulatory T cells (Treg) has also been demonstrated by an expansion of Treg clusters in resistant patients to anti-BCMA therapies [27]. Further studies with sequential sampling will help determine the role of the microenvironment on anti-BCMA failure.

4. Next generation CARs

A major limiting factor of adoptive CAR-T therapy is poor in vivo persistence, lineage stability of infused CAR-T cells, properties that are known to be critical for generating robust and durable Treg-mediated functional responses. Additionally, current methods of CAR-T cell therapy are limited for broader use by the risk of severe adverse events, including CRS and neurotoxicity. Hence, the next-generation CAR-T cell-based therapies are focused on designing CAR-T with improving efficacy while limiting toxicity. Here we want to briefly discuss the most recent pre-clinical development in CAR-T engineering that could apply to MM treatment (Fig. 2).

4.1. "Armored" CAR

Cytokines, including IL-2, IL-15, IL-21 critically control in vivo expansion, persistence and function of CAR-T cells. The current approved CAR-T products included TCR and co-stimulatory signaling domains. Efforts are undergoing to boost CAR-T therapeutic efficacy by cytokine modulation. The fourth generation (4G) CAR are engineered to secrete transgenic cytokinesupon CAR signaling. Several cytokines show interesting results in this setting [31]. One example is the use of IL-12 in 'T cells redirected for universal cytokine-mediated killing' TRUCKs [32]. The modified CAR-T cells activated effector cells in the tumor site, secreting IL-12 upon activation. Notably, IL-12 is a potent inflammatory cytokine with proven anti-cancer efficacy. Yet systemic delivery of IL-12 associated lethal dose-limiting toxicities that limits its therapeutic usage. Hence IL-12 secreting CAR-T cells not only could overcome the immunosuppression by the TME but also has the potential to safely deliver IL-12 to the target sites, reducing its systemic toxicity. Due to its ability to induce effector cells from adaptive and innate immunity, IL-12 secreting TRUCKs can augment the endogenous immune response against cancer. Other cytokines are able promote CAR-T cells functions, such as IL-18 [33] and IL-15 [34]. Another example is the co-expression of IL-15 and IL-21, which induces both the activation of T cells and the stimulation of the innate immunity [35]. IL-23, another member of IL-12 family, secreting CAR-T cells were also shown to mediate improved

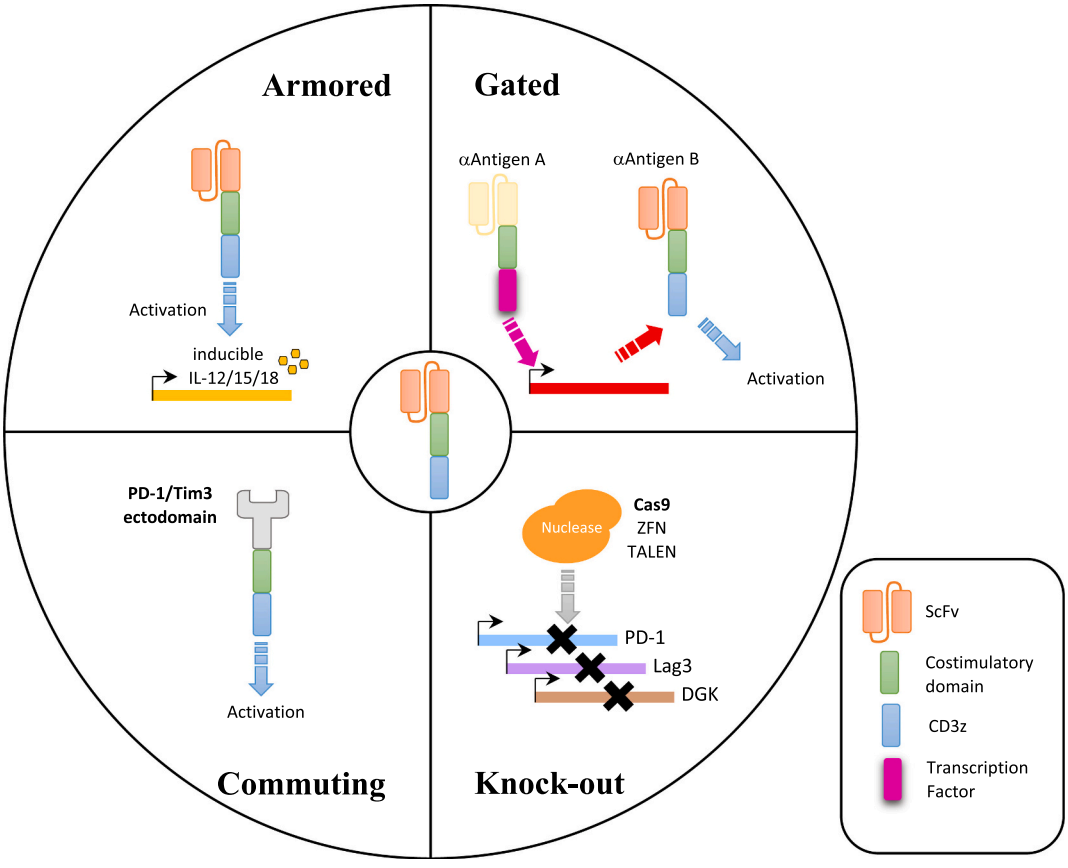

Fig. 2. Next generation CARs. Armored, gated, commuting and knock-out strategies are currently developed to improve CAR-T cells efficacy.

therapeutic efficacy in mice models. They exhibit diminished side-effects in comparison to IL-15 or IL-18 expressing CAR-T cells.

In the context of MM, pro-inflammatory signals in the bone marrow microenvironment are prone to tumor growth [36]. This implies that TRUCK strategies developed in MM will need to tune cytokines in order to reduce pro-inflammatory signals while maintaining durable anti-tumor responses.

4.2. "Gated" CAR

BMCA is the most efficient target in CAR constructs in a MM setting. However, BCMA shedding or BCMA loss are observed during CAR-T therapy in MM [16,20], making appealing the discovery of new targets. Many other antigens have been tempted (CD19, NKG2DL, CD38, CD138) [37] but none of these show robust results. One of the main problems is the broad expression of the targeted antigens, not exclusive for MM cells. Many different strategies have been developed to diminish the risk of either "on-target, off-tumor" toxicities or off-target recognition. Healthy cells that express the same antigen as tumor cells suffer collateral damages. These goals were pursued by linking the toxicity of CAR- T cells to a specific pattern of antigens by coupling multiple signals in a Boolean gates like mode.

AND-gate CAR-T cells harbor a dual targeting CAR, which requires the co-presence of both antigens to transduce the signal and activate the CAR-T cell. A first example of this approach is the use of a synthetic NOTCH receptor recognizing a first antigen, which will allow for the expression of the CAR recognizing a second antigen [38]. However, the delay between the first antigen recognition and the expression of the effective CAR can impair the specificity of the response. Another approach is to combine the expression of two different CARs targeting two different antigens, at the same time. Each of them being equipped with one part of the signaling domain (CD3z for one and CD28 for the other one). In this case, each CAR has a diminished ability to trigger the T cell response alone, but when they are both binding their antigens the signal behave like a single CAR. By equipping one of the two CAR with only the CD3z and the other with only a costimulatory signal (CD28), the authors achieve a safer and still efficient way to avoid the lack of a single tumor restricted antigen [39,40]. Another approach to limiting the off-target is the AND-NOT approach that relies on the addition of an inhibitor CAR (iCAR) that recognizes an antigen expressed only by healthy cells [41]. The most frequently used Boolean approach in clinical trials is the OR-gate. In this case, the CAR is bispecific with two scFv able to recognize two different antigens. The CAR-T cells will be activated if any of the two antigens are bound. OR-gates approaches are used with promising results [42,43].

4.3. "Commuting" CAR

Critical determinants of CAR-T therapeutic efficacy of CAR-T cells are the ability of CAR-T cells to home to the target site and their long-term persistence in a given patient. Yet non-permissive microenvironment, particularly due to stromal and myeloid cells, renders effector T cells less capable of tumor killing [44,45]. Although co-stimulatory signaling domains with the 2nd or 3rd generation CAR constructs were designed to promote CAR-T expansion and survival, these CARs may still be subject to adverse myeloma environments. The extent by which myeloma environments impede CAR-T functions remains a complex subject. CAR-T designing strategies such as "commuting CARs" that can transform an inhibitory signal into an activating signal could be utilized to improve response to therapy. Transforming growth factor-β (TGF-β) or PD-L1/2 are abundant within the tumor pro-inflammatory microenvironment. Accordingly, CAR able to bind TGFβ(antiTGFβ ScFV:CD28:CD3z) and produce Th1 cytokines in response to the TGFβ presence showed to be effective in modulating the TME [46,47]. Additionally, the ectodomain of IL-4Rα can be fused with the endodomain of IL-7R [48] or with the beta subunit of IL-2/15R [49] in order to reverse the inhibitory signal of IL-4 into a pro activation/proliferation signal. In the case of IL-6 CAR [50], the CAR can only neutralize IL-6, working as a sponge to avoid the inhibitory signal. Another possible use of the Switch receptor is to mitigate the immune-checkpoint functions such as CTLA4 and PD-1. Indeed, CTLA4:CD28 and PD1:CD28 CARs can increase the effectiveness of CAR-T cell [51,52].

4.4. Knock-out of immune checkpoint

MM creates a chronic inflammatory environment that promotes growth of the cancer cells at the expense of immune cell dysfunction [36]. CD8 + T cells from myeloma patients express several exhaustion and senescence markers including PD1, TIGIT, TIM3, CD57 [53]. Importantly, autologous stem cell transplantation (ASCT) in myeloma patients showed that T cells remains locked in dysfunctional state after transplantation [54]. Blockade of TIGIT receptor pathways significantly improves the function of CD8 + T cells upon ASCT in preclinical model of myeloma. Yet T cell dysfunction is likely involved multiple pathways including those that promotes senescence [53]. The advent of the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/ CRISPR-associated protein 9) technology in addition to the former TALEN (transcription activator-like effector nuclease) and ZFN (zinc-finger nuclease), opened a new frontier to genetically manipulate T cell cells including CAR-T cells. The extreme versatility of these technologies has led to an increasing number of applications.

In cell immunotherapy, the main application explored is the knockout of the immune checkpoints. PD-1 knockout has been shown to enhance the efficacy of CD8+ T cells in melanoma and polyclonal T cells in fibrosarcoma [55]. Targeting PD-1 with CRISPR/Cas9 also improves CD19 CAR-T cell cytotoxicity in vivo [56]. Yet a landmark paper recently showed that deletion of PD1 in CAR-T cells had a detrimental effect on their persistence in a phase 1 trial [57]. The deletion of lymphocyte-activation gene 3 (LAG3) with CRISPR/Cas9 does not improve the CD19 CAR-T cells functions [58]. In addition to the well-known immune-checkpoints, the deletion of other regulators such as REGNASE-1 and CD3-signaling regulator diacylglycerol kinase (DGK) improved T cell functions in preclinical model [59,60]. Efforts are underway to genetically delete multiple checkpoint inhibitors to improve efficacy of CAR-T cells in difficult to treat cancer models [61,62]. However, in depth studies are needed to establish the best target(s) that can be silenced or transiently blocked by antibodies to improve the CAR-T activity. For example, blockade of PD-1 by antibody shows improve CAR-T response, but its complete deletion in CAR-T cells via CRISPR/Cas9 led to diminished persistence of post adoptive transfer.

4.5. γδT cells

γδ T cells are non-MHC-restricted innate-like T-cells. Although the small number, they are fundamental due to the rapid response they show upon activation. For their activation, the γδ T cells rely on cytokine receptors, a broad number of stress associated receptors (NCR, NKG2D), and his invariant TCR repertoire (γ and δ). As a bridge between innate and adaptive immunity, many studies explored the possibility of using γδ T cells as an off-the-shelf resource for immunotherapies [63,64].

$V\delta1^+$ T cells are mainly found as a resident population in mucosal tissues. Hence, Vδ1+ T cells have adapted to a lower level of nutrients and oxygen, a condition often found in TME. Incubation in hypoxia ex vivo has been shown to enhance $\gamma\delta1^+$ T-cell cytotoxicity [65]. Of particular interest, *Knight et all* show that expanded $V\delta1^+$ T cells exert specific cytotoxicity against primary myeloma cells without the need of further genetic manipulation [66].

$V\delta2^+$ cells are the majority of circulating γδ T cells in humans. In particular, the $V\gamma9^+V\delta2^+$ clone is the most promising. This clone can do ADCC [67,68]; Recognize malignant cells either with innate receptors (NCR, NKG2D) and with TCR; They show somatic recombination of receptor genes, memory formation, and professional antigen

presentation [67]. Multiple reports demonstrate the ability of expanded Vδ2$^+$ T-cells to recognize and kill MM cells [69] through the mevalonate metabolites and cell adhesion molecule-1(ICAM-1) [70].

Due to the above mentioned characteristics, γδT cells have been successfully used for CAR therapy, producing sufficient cells from Vδ1+ and Vδ2+ subsets for clinical studies [65,71,72]. The specificity demonstrated for MM cells and the recent advances in the ex vivo expansion and manipulation of γδT cells depict a promising future for this approach to treat MM.

4.6. NK cells

Natural killer (NK) cells have the ability to mediate rapid anti-tumor responses against and thus offer an alternative to T cells in CAR therapies [73]. CAR-NK cells have already been evaluated in clinical trials [74].

Notably, CAR-NK cells retain the expression of their activating and inhibitory receptors. Thus, unlike CAR-T cells, CAR-NK cells can still exert their "natural" anti-leukemia effect if the tumor antigen targeted by CAR is downregulated [73]. In addition, given their different properties, CAR-NK cells may be safer concerning clinical complications, including CRS and neurotoxicity. Moreover, CAR-NK cells may potentially become an off-the-shelf tool, as they do not require a strict autologous HLA matching as T cells do. Accordingly, NK-based cell therapy, including anti-CS1 CAR modified NK cell-based therapy, has demonstrated treatment efficacy in the pre-clinical model of MM [75]. A further preclinical study shows that NKG2D-CAR NK cells and BCMA-CAR NK cells efficiently eradicate MM cells [76,77]. Recently, a BCMA CAR NK-92 cells, developed by Asclepius Technology Company Group (Suzhou) Co., Ltd., has been utilized for a trial of 20 patients with relapsed/refractory MM with BCMA expression in China in 2019 (NCT03940833). Bone marrow niches are one of the suggested mechanism of MM relapse, promising results came from a recent pre-clinical study that show the effectiveness of NK cells co-expressing an anti BCMA-CAR and the chemokine receptor CXCR4 to reach the tumor sites and control the MM progression [78]. Moreover, most of the current mAbs approved for the treatment of MM rely on the ADCC driven by NK cells, laying down the rational for combinatory treatment in relapsed/refractory MM. Thus, CAR-NK therapy may represent a complementary strategy to treat refractory MM patients.

5. Conclusion

The rapid development of BCMA-targeting CAR-T cells in MM since their first administration in 2014 to the FDA and EMA approvals of ide-cel in 2021 holds great expectation for the future of myeloma treatments. BCMA-targeting CAR-T cells have demonstrated remarkable efficacy in the context of relapsed and refractory MM. Current studies are evaluating CAR-T cells in earlier lines of treatment, including frontline, with the hope of achieving long-lasting remission in MM. Many different approaches are under investigation in order to improve CAR-T cells therapies. An optimal product would target different antigens on tumor cells to avoid antigen loss; it would be enriched in naïve memory phenotype to be resistant to exhaustion and increase their persistence; and it would also integrate an on−/off- system to limit toxicity in case of side effects. Further translational follow-up of patients treated with CAR-T cells will help understand biological mechanisms of efficacy and resistance in order to improve their efficacy.

Practice points

- Autologous BCMA-directed CAR-T cells used in multiple myeloma induce an overall response rate above 80% and median progression free-survivals from 12 to 22 months, in late stage refractory setting of the disease.
- New generation CAR-T cells with humanized scFv, alternative manufacturing or generated from allogenic T cells are effective.
- Loss of BCMA is observed in about 4% of the cases at relapse, with some bi-allelic deletions of BCMA on chromosome 16p13.13.

Research agenda

- Understanding the impact of the immune microenvironment in response and resistance to CAR-T cells.
- Determining the nature of resistant myeloma cells to CAR-T cells, despite high rates of MRD negativity.
- Improving CAR-T cells efficacy with newer approaches such as commuting CARs or knock-out of immune checkpoints.

Future considerations

- Future clinical trials will evaluate the efficacy of CAR-T cells in early line of treatments, including frontline and in combination with other treatment.
- Learning how to sequence anti-BCMA therapies will be important in clinical practice with future approvals of bispecific antibodies and antibody-drug conjugate.

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