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CAR T cell therapy for multiple myeloma: Where are we now and where are we headed?

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Abstract

While recent progress has been made in the management of multiple myeloma, it remains a highly fatal malignancy especially among patients with relapsed-refractory disease. Immunotherapy with adoptive T cells targeting myeloma-associated antigens are at various stages of development and have brought about a new hope for cure. This is a review on the emerging field of adoptively transferred engineered T cell based approaches, with an in-depth focus on chimeric antigen receptors (CAR) targeting multiple myeloma. The recent results from CAR T cells targeting B cell maturation antigen are encouraging but eventual resistance to the CAR T cell therapies remain problematic. With newer approaches in therapies for multiple myeloma, the role of transplantation is evolved to form a platform for T cell therapies.

Keywords

Multiple myeloma; chimeric antigen receptors; BCMA; Hematopoietic cell transplantation; CAR; immunotherapy; TCR

Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy in the United States and is associated with high rates of fatality [1]. Recent years have seen an emergence of newer drugs and regimens to treat MM with response rates approaching 100% [1-5]. These regimens provide a safer and more durable option for disease control in most patients with MM. In a small percentage of patients, conventional therapies might be curative by

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themselves [6,7]. However, in the majority these therapies are not curative and most patients with MM die from their disease. Thus, the quest for potentially curative but safer options continues. This is especially relevant for patients with relapsed or refractory MM.

Immunotherapy has revolutionized the landscape of cancer therapies in recent years [8]. This revolution has been powered by the parallel development of newer ways to reinvigorate endogenous tumor-reactive cells with immune check point blockades [9] and engineering of T cells ex vivo for adoptive transfer [10]. This has positively influenced the outlook for therapeutics of MM and heralded exploratory translational and clinical research focused to achieving improved responses in relapsed-refractory MM.

Allogeneic hematopoietic cell transplantation in MM

Allogeneic hematopoietic cell transplantation (alloHCT) is the first proven curative treatment for MM [11]. It is a viable option for select patients with relapsed-refractory MM in whom potential benefits of the procedure outweigh its risks and alternate options. The success of alloHCT in MM has been tempered by historical high rates of non-relapse mortality (NRM) which can reach 40% and adversely impacts overall survival [12]. NRM is primarily caused by infections due to prolonged immune suppression and graft versus host disease (GVHD), a broad immune attack of the donor T cells against normal host tissue.

Improvements in the process of alloHCT have, however, led to significant improvements in NRM. For example, using myeloablative conditioning and CD34+ selected allografts in 44 patients treated at MSKCC from 2007-2013 one-year NRM rates of 18% and grade II-IV acute and chronic GVHD rates of 2% and 0% respectively, were obtained [13]. In spite of improvements in NRM rates of MM, relapse remains a prominent problem in patients with relapsed-refractory disease. In our institutional experience, despite the use of high intensity conditioning to eradicate disease, in this heavily pre-treated, high risk population, progression free survival (PFS) in alloHCT recipients had a median duration 13.5 months and a PFS rate of 31% at 2 years, although a “tail of the curve” was seen with a significant minority of long term progression free survivors out to 6 years post-alloHCT.

This speaks to the curative potential of alloHCT, which is based on a combination of high intensity conditioning to eradicate disease, and in contrast to autologous HCT (autoHCT), reinfusion of an allogeneic hematopoietic cell source to induce anti-MM immunity. A graft-versus-myeloma effect is achieved where the T cells derived from the donor graft recognizes the recipient-derived malignant cells as foreign, and therefore targets, and eliminates them [14]. Thus alloHCT, despite its toxicities, remains the most established form of T cell based immunotherapy in MM, and serves as evidence for the curative potential mediated by the T cell recognition and lysis of MM cells. Multiple groups have been investigating enhancing anti-MM T cell responses, while simultaneously improving the safety of such approaches, through adoptive gene transfer of either a synthetic T cell receptor (TCR) or a chimeric antigen receptor (CAR) and re-infusion of autologous gene modified T cells.

Adoptive transfer of T cells for myeloma

Anti-myeloma immunity participates in controlling MM, and a compromised immune system is associated with more aggressive disease [15-17]. T cells reactive to myeloma-associated antigens are detectable and correlate with disease burden [18]. Prognostically significant T cell clones are also seen following autoHCT [19] and alloHCT [20]. These observations encourage the development of adoptively transferred T cell-based strategies to treat MM.

Endogenous T cell activation and reinfusion

It has been suggested that reduced intensity or non-myeloablative conditioning in combination with delayed adoptive transfer of donor T cells, in the form of donor lymphocyte infusion (DLI), can lead to retained graft-versus-myeloma effects while mitigating GVHD and overall NRM [21]. In an early attempt, four patients with advanced MM were treated with DLI leading to responses in three of the patients and a conversion of hematopoietic chimerism to donor-derived [22]. In spite of the response and full donor chimerism, the remission was not durable and relapse occurred. A subsequent dose escalation trial of DLI included 19 MM patients after alemtuzumab-related conditioning [23]. Of these patients, 12 had only modest response, which were associated with GVHD. With advent of newer agents, combination therapies with DLI have been attempted. Combination of bortezomib and dexamethasone prior to DLI showed only modest responses and co-occurrence of GVHD [24]. In another trial, lenalidomide was used as maintenance after DLI and showed mildly better responses and PFS compared to lenalidomide maintenance alone [25].

Given the difficulty of separating graft-versus-myeloma and GVHD with unmodified allogeneic T cells, adoptive transfer of ex vivo processed autologous T cells have been studied. Success of adoptively transferred T cell therapy is dependent on generation of myeloma-specific T cell clones that can be expanded ex vivo, proliferate and kill target cells in vivo in myeloma niches, and attain a long-lasting memory state for continued immune surveillance [26]. One strategy to obtain a large number of tumor specific T cells is to expand tumor-infiltrating lymphocytes (TILs) and adoptively transfer the expanded cells back to the patient. Indeed TILs have been used with measurable success in solid tumors like melanoma [27]. In MM, when compared to peripheral blood lymphocytes, bone marrow-infiltrating lymphocytes (MILs) have an increased likelihood of a precursor T-cell population with an enhanced tumor specificity [28]. Activated MILs (aMILs) were adoptively transferred after in vitro stimulation and expansion for 7 days in 22 patients with relapsed-refractory MM [29]. Patients were treated with high dose melphalan, autologous hematopoietic cell rescue, and infusion of aMILs. Among the treated patients, the overall clinical response was 54% with 27% complete response (CR, no paraprotein in urine or serum and <5% BM plasma cells) and 27% partial response (PR, >50% reduction in paraprotein). Remarkably, demonstrable myeloma-specific T cells responses were not uniformly observed in all the treated patients, but the presence of a myeloma-specific immune response was associated with clinical response.

TCR gene transfer for MM

To obtain a more uniform myeloma-specific response, myeloma-associated antigens have been targeted to generate redirected T cells for adoptive transfer. Several myeloma associated antigens have been identified. MAGE A3, which is a cancer-testis antigen (CTA), is expressed in myeloma cells. T cells have been genetically engineered to express an affinity enhanced synthetic TCR (sTCR) directed to a MAGE A3 antigen-derived peptide/human leukocyte antigen (HLA)–A*01 complex [30]. However its use has been restricted due to cross-reactivity against a cardiac myocyte associated antigen peptide titin, leading to fatal cardiovascular toxicity in the first two patients treated [31]. NY-ESO-1 is another CTA expressed in multiple cancers [32]. Advanced myelomas express NY-ESO-1 which is highly immunogenic [33]. NY-ESO^{c259} is an affinity-enhanced sTCR that recognizes the NY-ESO-1–derived peptide in the context of HLA-A*0201 [34]. 20 patients were treated on a trial with high dose melphalan, autologous hematopoietic cell rescue, and subsequent administration of adoptively transferred NY-ESO^{c259} T cells. Of note, for most of these patients it was their first autologous transplant. 14 had CR or near-CR, 2 patients had a very good partial response (VGPR; 90% reduction in paraprotein levels), and 2 had PR. The immune correlates of this adoptive cell therapy trial were promising. NY-ESO^{c259} T cells home to disease marrow, proliferated, and long-term persistence was seen that correlated with clinical activity. However the efficacy induced by the adoptive T cell therapy by itself in both these cases is confounded by the co-administration of high dose melphalan and autoHCT.

CAR T cell therapy for MM—Fundamental to specificity of naturally occurring and synthetic T cells is the interaction of a cognizant TCR complex with its corresponding peptide presented in HLA complex in the target cells (Figure 1). The requirement of TCR and HLA interaction for T cell effector function, which is unique to each individual, and can be down-regulated in neoplasms, can be overcome by the use of chimeric antigen receptors (CARs) [35]. Furthermore, CAR modified T cells do not rely on endogenous activation and co-stimulation but receive supra-physiologic stimulatory signals through the CAR. Chimeric antigen receptors are synthetic fusion proteins consisting of the variable portion of antibody, known as a single chain variable fragment (scFv) that can target an antigen displayed on the surface of a tumor cell [36]. The basic construct of the CAR gene encodes the scFv in frame with a transmembrane domain, and T cell activation and co-stimulatory signaling domains from the TCR CD3 zeta chain, and most commonly CD28 or 4-1BB. The synthetic gene is introduced in the T cell using retroviral or lentiviral vectors that ensure genetic integration and constitutive expression.

CAR T cell therapies targeting CD19 have been the most widely used and successful among all the chimeric antigen receptors to date [37]. CD19 is a surface antigen expressed on the membranes of precursor and mature B cells. It is also expressed on various B cell malignancies including B-acute lymphoblastic lymphoma, chronic lymphocytic leukemia and non-Hodgkin lymphoma [38]. Autologous CD19 CAR T cells have accordingly been used in CD19-expressing B cell malignancies with dramatic success [39–43].

CD19 CAR T cells for MM: MM, a neoplastic disorder of plasma cells, does not routinely express detectable CD19. There are however, several scenarios that can be imagined where patients with MM might benefit from CD19 targeted CAR T cell therapy. First, it may be possible that MM cells express low levels of CD19, below our limit of detection by routine flow cytometric and immunohistochemical approaches, which may be sufficient for CAR-mediated killing. Second, there may be MM progenitor cells, which are B cell in origin and presumably express CD19. Third, there may be regulatory CD19+ B cells that play a supportive/immunosuppressive role in the MM microenvironment, and eliminating this population may allow for an endogenous MM infiltrating lymphocyte anti-tumor effect.

The use of CD19 CAR T cells have been studied as a salvage therapy in MM patients with relapse-refractory myeloma who had previously received autoHCT [44,45]. In this study the patients were treated with high dose melphalan and a second autoHCT, followed two weeks later by CTL019, a lentivirally transduced CD19 CAR T cell product. The maximum target dose of CTL019 was 5×10^7 . The results of the first patient in this study were encouraging and published [44]. The day 100 bone marrow biopsy of the patient showed 1 to 2% overall cellularity with no plasma cells, and stringent complete response was seen. No evidence of cytokine release syndrome was observed. Twelve months after transplantation, the patient had no laboratory evidence, and no clinical signs or symptoms of MM. Surprisingly, at this time point, CTL019 was not detectable in blood and marrow and non-neoplastic CD19+ B cells had reconstituted suggesting that sustained CD19 CAR activity was likely lost or substantially reduced. Since the publication of the initial report, data from a total of 10 patients (inclusive of the patient above) were presented at the 2016 annual meeting of annual society of hematology [45]. Overall, 2 out of the 10 patients including the one presented in detail above, had “remission inversion” or a longer PFS after this second administration of high-dose melphalan. None had an ongoing clinical response. While, a favorable PFS correlated with peak frequency of CTL019 in the bone marrow, there was no association between PFS and peak frequency of CTL019 or duration of CTL019 persistence in peripheral blood.

κ.CAR T cells

One drawback of targeting antigens expressed on all B cells is the development of hypogammaglobinemia leading to profound immunosuppression. Since myeloma cells are monoclonally restricted for κ or λ immunoglobulin subtype, one strategy can be to target the monoclonal subtype of malignant B cells sparing the normal B cells. As a proof of principle, κ.CAR T cells were generated targeting κ light chain (referred to as κ.CAR T cells) [46]. Seven MM patients received conditioning with 12.5 mg/kg cyclophosphamide followed by κ.CAR T cell infusion (9.2×10^7 to 1.8×10^8 κ.CAR T cells/ m^2). The responses were modest and of the 7 patients, 4 had responses in the form of stable disease. The responses were substantially better with NHL and CLL. As with CD19 CAR T cells, one obvious drawback of using this approach is that, like healthy plasma cells, myeloma cells secrete and do not retain expression of immunoglobulin on their surface and the anti-myeloma effects would be dependent on targeting of MM precursors.

CD138 CAR T cells

CD138 (syndecan-1) is a surface molecule highly expressed on human plasma cells including myeloma cells [47]. A CD138 targeted CAR including a 4-1BB costimulatory domain was tested in five relapse-refractory MM patients in standard phase one dose-escalation trial with 3–6 planned CD3⁺ T-cell doses, averaging 7.56×10^6 cells/kg [48]. The best responses were SD in 3 of the patients at 3 months. No intolerable toxicities were observed in this process. This is surprising since CD138 is broadly expressed in human tissues including epithelial cells, vascular smooth muscle cells, and the endothelium [49]. It can be postulated in light of the modest response against myeloma seen in the above trial and the less-than-expected toxicities with CD138 CAR T cells was due to a less potent CAR activity and it is possible that a more potent CAR activity would result in vigorous toxicities.

BCMA CAR T cells

The ideal target antigen in MM for CAR T cell therapy would be a molecule that is highly expressed on the surface of all neoplastic plasma cells. B-cell maturation antigen (BCMA) is an excellent candidate due to its preferential expression in plasma cells [50].

BCMA is a TNF receptor superfamily 17 (TNFRSF17) that plays a central role in regulating B-cell maturation and differentiation into PC [51]. It is a type III transmembrane protein lacking a signal-peptide but containing cysteine-rich extracellular domains, and promotes B-cell survival and differentiation [52]. It is not present on naïve B cells or hematopoietic stem cells, but is upregulated during B-cell differentiation into plasmablasts [50]. It was initially believed that BCMA is not expressed in non-hematopoietic tissue. However recent data suggest that BCMA is abnormally expressed in non-small cell lung cancer cell (NSCLC) lines and may play a role in NSCLC tumors through the ERK1/2 signaling pathway [53]. Fortunately, there is no evidence of the expression of this molecule in normal essential non-hematopoietic tissue.

A first-in-human trial conducted at the NCI used autologous T cells transduced with γ -retroviral vector encoding a BCMA targeted CAR (NCI mBCMA CAR) [54] (Figure 2). The BCMA scFv of the NCI mBCMA CAR is derived from a murine hybridoma and includes a human CD28 co-stimulatory domain. Only patients with BCMA >50% by IHC or flow were treated, and 30% of the patients were excluded based on low expression of BCMA, ensuring more uniform BCMA expression. All patients received cyclophosphamide and fludarabine conditioning chemotherapy prior to CAR T cell infusion. The initial report on the first twelve patients treated in dose escalation cohorts with doses ranging from 0.3×10^6 to 9×10^6 CAR T cells/kg showed that two of the three patients treated at the highest dose level (9×10^6 CAR T cells/kg) showed dramatic responses, including a stringent CR. A dose of 9×10^6 cells/kg was also associated with grade 3–4 toxicities, most significantly, cytokine release syndrome in both the responding patients.

The trial utilizing NCI mBCMA-CAR T cells was the first demonstration of dramatic responses induced by non-CD19 targeted CAR T cell therapy. Subsequently three additional BCMA targeted CAR T cell trials for MM have opened in the US (Figure 2, Table 1). Bluebird mBCMA-CAR, known as bb2121, which grew out of the NCI program is being

tested in a multi-institutional trial. Initial data from this trial was presented at 2016 EORTC-NCI-AACR Molecular Targets and Cancer Therapeutics Symposium (**J.G. Berdeja 2016, unpublished**). The construct is a lentiviral vector encoding a mouse-derived scFv and includes a 4-1BB costimulatory domain. As above, this trial too includes patients with BCMA expression > 50% and all patients receive conditioning chemotherapy with fludarabine and cyclophosphamide. Initial reports of this dose escalation trial reveals that at the dose of 150×10^6 total T cells (second of three dose levels reported) 3 out of 3 patients had dramatic responses (1 VGPR, 2 sCR) lasting >20 weeks. Interestingly at the third dose level (450×10^6 total T cells), 3 of the 3 patients treated had only partial responses. Notably, no grade 3/4 toxicity was observed in any patient treated at the first 3 dose levels. The trial continues to enroll at higher dose levels.

University of Pennsylvania has also reported on a dose escalation trial with their BCMA targeted CAR T cell product. The UPenn huBCMA-CAR is a lentiviral construct encoding scFv against BCMA identified from a human library and includes the 4-1BB costimulation domain. Initial data from this trial was presented at the 2016 annual meeting of annual society of hematology [55]. Unlike the previous trials discussed, the patients included in this trial are not screened for BCMA expression, and the first cohort treated did not include any conditioning chemotherapy. From this cohort, nine patients have been reported on at a goal 500×10^6 total CAR T cells. This cohort is notable for a single patient with a sCR ongoing for greater than 12 months. In addition to the sCR, 2 other patients achieved VGPRs. Four of the nine patients reported required anti-IL6 receptor therapy with Tocilizumab for CRS. CAR T cell persistence has been measured up to 24 weeks. The trial is now enrolling patients to be treated with cyclophosphamide conditioning regimen.

It is worthwhile to highlight that the NCI and Blubird bio trials use a murine hybridoma-derived scFv, while the UPenn and the newly opened MSKCC studies utilized a human library screening approach to identify scFv's. Experience from mouse hybridoma-based CD19-CAR study from Fred Hutchinson Cancer center suggests potential advantages with a human library screening approach. It is observed that expansion after repeat administration of CAR T cells is limited in at least some patients by the development of host anti-murine scFv immune responses [56]. Repeated host anti-CAR immune responses may be less against human derived CARs. This may also mitigate the necessity of a strong conditioning regimen as seen in the UPenn huBCMA-CAR trial, where examples of impressive efficacy were noted without the use of prior conditioning chemotherapy. Additionally, a library provides investigators with an expansive selection of scFvs to select a superior lead candidate.

At MSKCC, we have recently opened the fourth phase I trial using human-library identified BCMA scFv (**ClinicalTrials.gov Identifier: NCT03070327**). The MKSCC huBCMA-CAR is a retroviral construct and includes a 4-1BB costimulatory domain. Patients with MM expressing any levels of BCMA positivity (>1%) are eligible. The conditioning regimen is cyclophosphamide for the first cohort, with patients treated in subsequent cohorts additionally receiving fludarabine.

Besides these trials in the United States, BCMA CAR trials are also open internationally. Investigators from Xi'an Jiaotong University reported exciting results from a phase I trial of LCAR-B38M anti-BCMA CAR T cells in 34 patients with relapsed/refractory multiple myeloma and two patients with plasma cell leukemia [57]. 100% of the patient reported an objective response with 15 complete responses. The exact nature of the scFv, manufactured by Nanjing Legend is not clear. Of note, the patients in the above trial received CAR T cells after a median of 3 lines of therapy, which is substantially earlier than the other trials described above.

In all the reported trials, resistance to BCMA CAR T cells among a substantial group of patients remains an area of active investigation. It is yet to be understood how BCMA expression (low vs high) impacts the efficacy of BCMA targeted CAR T cell therapy. Different thresholds for eligibility may have an impact on response rates and durability between trials. Once there is mature data, it will be important to consider this factor when choosing therapies for an individual patient. The mechanisms of resistance are still not well understood but the emergence of BCMA-negative myeloma cells and possible requirements of a higher threshold of surface BCMA expression for optimal killing are important clues to unraveling the mystery. In-depth immunological studies of the kinetics of T cells in the myeloma milieu and studies on the expression of BCMA on cell surface in the future trials will be of great importance in understanding the mechanism of resistance to BCMA targeted CAR T cells.

Non-BCMA targeted CAR T cells for MM

In addition to the above targets for CAR based therapies, there are additional candidate targets for CARs being explored in preclinical and very early clinical stages. CD38 (ADP-Ribosyl Cyclase) is type –II glycoprotein and a cell surface antigen structurally related to proteins such as transferrin receptor and the HLA-molecule [58]. It is consistently expressed on malignant plasma cells, making it an attractive target for CAR T cell therapy. It is also expressed on other B cell subtypes and other BM-derived progenitors including myeloid and lymphoid precursors, and subtypes of T cells [59]. Daratumumab is a CD38-targeting monoclonal antibody, approved for treating patient with relapsed-refractory MM [60]. The promising activity of daratumumab in MM has led to the evaluation of CD38-targeting CARs. In preclinical testing, retrovirus targeting CD38 has shown efficacy against patient-derived myeloma lines [61] and in xenograft models [62]. In spite of the promise, CD38 is a less than an ideal CAR target given that its expression in malignant plasma cells is known to decrease and is lost in the most aggressive forms, setting the stage for likelihood of resistance to CD38 CAR [63]. It is also expressed on activated T cells, which might lead to fratricide among CD38 CAR T cells [64]. Moreover, CD38 is also expressed on normal non-hematopoietic tissues including prostate epithelial cells, and while the antibody is safe for use in patients, the more potent potential of CAR T cells does not guarantee safety when targeting this widely expressed protein [65].

Other CAR approaches have included specific targeting of isoforms of widely expressed antigens. The isoform variant 6 (CD44v6) is expressed in a variety of malignant cells including myeloma cells and CAR T cells targeting CD44v6 mediated specific malignant

cell killing in xenogenic models while sparing normal CD44+ hematopoietic cells [66]. In another interesting approach, CAR T cells targeting CD70, which is expressed on various B cell malignancies including MM, killed CD70-positive tumor cell lines and primary tumors in vitro and in a murine SCID xenograft model [67]. CD70 is a natural ligand of CD27 and the CAR designed consisted of full-length CD27 as the antigen-recognition domain fused to the intracellular domain of the CD3- ζ chain. The CARs being tested in MM have not been limited to T cells alone. Natural Killer (NK) cells retrovirally transduced with CARs targeting SLAMF7 (CS1), a glycoprotein expressed on normal and malignant plasma cells [68], have shown capacity to lyse in vitro and xenogenic models of MM [69].

Future directions

AlloHCT as a platform for T cell therapy in myeloma

In light of the rapidly emerging cell therapy options in MM, the role of alloHCT is in the process of refinement. Most clinical trials with CAR T cells exclude patients who have undergone a prior alloHCT, thus potentially limiting treatment options for patients with high-risk MM who choose to undergo alloHCT early in their disease course and then relapse. Thus, not having the option to receive CAR T cells on clinical trials may influence the patients and their physicians to pursue experimental CAR T cell therapy prior to alloHCT. However, to date, the data is not clear if CAR T cells can replace alloHCT as a curative therapy. Further, the restricted availability of CAR T cell trials and the increasing possibilities of suitable donors of allograft, alloHCT remains relevant in therapies of MM, especially for high risk patients.

Fundamental to the relevance of alloHCT is its potential to cure difficult to treat MM delivered by a combination of high dose chemotherapy, tumor-free hematopoietic cell product, and graft versus myeloma effect. Preclinical studies with alloHCT have been focused on the development of engineered donor cell products that can enhance the antitumor effects across HLA barriers with minimal transplant-related mortality [70]. Studies in mouse models of alloHCT suggest that by optimizing the timing of adoptive transfer and specific targeting of effector functions of T cells, enhanced antitumor effects of donor T cells can be achieved without enhancing GVHD [71-73]. Thus alloHCT can be developed as a platform for therapy of relapse refractory myeloma for donor-derived CAR and non-CAR T based therapies. Preclinical [74] and clinical data [75,76] with donor-derived CD19 CAR T cells suggest that in an allogeneic setting, concurrent stimulation of CAR and alloreactive TCR can eliminate GVHD-causing donor T cells population while retaining non-alloreactive CAR expressing T cells that can mediate anti-tumor effects.

Recent understanding of T-cell development have fed forward into the prospects of using T-cell progenitor cells to enhance post-transplant immune reconstitution [77]. Since donor T cell progenitors undergo further education in the host thymus, host-directed tolerance develops minimizing the risk of GVHD. T cell progenitors can be further engineered to express CAR as an off-the-shelf T cell based therapy agent [78]. In humans, umbilical cord-derived T cell progenitors expressing CARs have shown promise in preclinical studies [79,80] and can be expected to be valuable in developing anti-myeloma cell therapeutics.

Overcoming immune resistance

Although MM is amenable to immunotherapy in the form of alloHCT and myeloma-directed adoptive T cell transfer, development of resistance and the lack of optimal responses remain problematic. In addition to newer target identification for CARs and sTCRs, efforts are also focused on identifying mechanisms of myeloma-intrinsic resistance to T cell mediated killing and engineering T cells and overcoming the resistance.

Combining CAR T cells and immune checkpoint blockades—T-cell exhaustion/senescence is a distinguishing feature of myeloma relapse [81]. As with endogenous T cells, PD1/PDL-1 axis can attenuate myeloma specific T cell activity. PD-L1 is expressed by myeloma cells [82,83] and the myeloma bone marrow induces expression of PD-L1 [84]. As a single agent, PD1 inhibition in relapsed-refractory MM has shown suboptimal responses in two small studies (4% observable response in 27 RRMM patients treated with nivolumab, [85] stable disease in 1RR MM patient treated with pidilizumab [86]). However, the observation of stable disease in 63% [85] in the heavily pretreated relapsed-refractory MM population suggests potential effectiveness in combinatorial settings. Clinical trials are underway to explore the effectiveness of PD-1 and PD-L1 inhibition in combination with proteasome inhibitors and immunomodulatory agents (IMiDs) in pretreated [87] and therapy-naïve MM patients [88]. Reports of early results show significant efficacy in the relapsed-refractory population when Pembrolizumab is combined with an IMiD (50% ORR with lenalidomide plus dexamethasone (KEYNOTE-023), 60% ORR with pomalidomide and dexamethasone (KEYNOTE-183). However due to unforeseen toxicities including deaths, the above trials have placed on hold, which have tempered the enthusiasm for the use of immune checkpoint blockades in MM.

The combination of immune checkpoint blockade with adoptive cellular therapy is nevertheless intriguing. In mouse models of sarcoma and breast cancer, in which the combination of PD-1 blockade with murine CAR T cells showed a significantly enhanced antitumor effects compared with either intervention alone [89]. CRISPR/CAS9 mediated disruption of the immune checkpoints rendering CAR T cells resistant to PD1 inhibition has also shown promise in preclinical models [90]. We predict that combination of adoptively transferred T cells and immune checkpoint blockade will prove to be synergistic in human studies.

Armored CAR—MM is known to have a complex immunosuppressive microenvironment consisting of recruited or co-opted immunosuppressive cell types and an immunosuppressive cytokine milieu which impairs activity of proimmune TH1 cells and restricts antitumor T cell immunity [91,92]. While the antigen-specific ability of CAR T cells are being exploited with discoveries of myeloma-specific targets, optimizing the signaling and cytotoxicity of CAR T cells are yet to be fully explored. Armored CAR T cells represent the next generation of optimization that focuses on reinforcing modified CAR T cells against the influences of an immunosuppressive tumor microenvironment [93,94]. Armored CAR strategies have spanned engineering endogenous cytokine production and enhancing costimulatory effects.

Effects of immune inhibitory TH2 cytokines can be overcome by modifying CAR T cells to secrete IL12 which enhances expression of IFN- γ and IL12 and leads to more effective clearance of tumors in murine models [80,95]. Enhanced effects of CAR T cells have also been shown with transgenic co-expression of IL15 [96] and IL21 [97]. Further development of IL15 expressing CARs were hampered out of concern for leukemogenic potential of IL15 [98]. Transgenic co-expression of IL12 and IL21 remain exciting and unexplored opportunities to enhance efficacy and persistence of myeloma specific CARs.

Thus far, the CAR T cells used in clinical trials and most preclinical studies utilize costimulatory-signaling domains such as CD28 or 4-1BB. The choice of costimulatory domain of the CARs may impact cellular activation, persistence [99,100], and development of immunologic exhaustion [101]. The immunosuppressive myeloma microenvironment can also be overcome using constitutive expression of CD40L, which facilitates cytolysis and proliferation of CAR T cells, thus augmenting tumor immunogenicity [102]. Additionally, improved antigen presentation mediated by constitutive expression of CD40L can recruit endogenous antitumor T cells and cause sustained responses.

Conclusion

Taken together, MM is a disease conducive to adoptive T cell-based therapies. Chimeric antigen receptors are currently being tested in clinical trials and initial results have been encouraging. Newer targets for T cell therapy are being investigated. However, emergence of resistance to cellular therapies due to intrinsic immunosuppressive networks remains a major barrier to achieving cure. Further research into the mechanisms of resistance is crucial to develop strategies to maximize durability of responses while minimizing toxicities associated with cell therapies. Developing appropriate platforms for myeloma targeted cell therapy and designing CAR T cell that are resistant to immunosuppressive microenvironment are exciting avenues for the future on myeloma targeted treatment.

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Figure 1. Activation of TCR and CAR

The TCR complex of a cytotoxic T cell binds to a peptide presented in the context of a HLA class- I molecule. This immunological synapse triggers a signaling cascade and in the presence of appropriate costimulatory signals cause activation of the cytotoxic T cells. A CAR is a synthetic fusion protein containing a single chain variable fragment (scFv) that can directly bind to target cell surface antigen. This engineered CAR synapse activates the immunoreceptor tyrosine-based activation motif (CD3e-ITAM) and, if present, costimulatory molecules causing activation of the CAR T cell.

Figure 2. The components of BCMA-CAR T cells

A schema depicting the components of the five BCMA targeted CARs currently being tested in clinical trials. NCI (National Cancer Institute); Bluebird (Bluebird Bio Multi-institutional trial of b2121); U Penn (University of Pennsylvania); MSKCC (Memorial Sloan Kettering Cancer Center)

Table 1

Summary of the trial design of clinical trials with BCMA-CAR T cells.

Institution/CAR	NCI mBCMA-CAR	Bluebird mBCMA-CAR (bb2121)	UPenn huBCMA-CAR	MSK huBCMA-CAR	Nanjing Legend LCAR-B38M
Clinicaltrials.gov Identifier	NCT02215967	NCT02658929	NCT02546167	NCT03070327	NCT03090659
BCMA Ag screening	>50%	>50%	None	>1%	Clear expression
Conditioning (Day 0 denotes day of CAR T cell infusion)	Cyclophosphamide and fludarabine on days -5, -4, and -3	Cyclophosphamide and fludarabine on days -5, -4, and -3	<i>Cohort 1</i> : none <i>Cohort 2, 3</i> : Cyclophosphamide on day-3	<i>Cohort 1</i> : Cyclophosphamide on day -2 <i>Cohort 2 onwards</i> : Cyclophosphamide and fludarabine on days -4, -3, and -2	Cyclophosphamide
Planned enrollment	38	50	27	24	100