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The Promise of Chimeric Antigen Receptor (CAR) T Cell Therapy in Multiple Myeloma

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Abstract

A cure for multiple myeloma (MM), a malignancy of plasma cells, remains elusive. Nearly all myeloma patients will eventually relapse and develop resistance to currently available treatments. There is an unmet medical need to develop novel and effective therapies that can induce sustained responses. Early phase clinical trials using chimeric antigen receptor (CAR) T cell therapy have shown great promise in the treatment of relapsed and/or refractory MM. In this review article, we provide an overview of the CAR constructs, the gene transfer vector systems, and approaches for T cell activation and expansion. We then summarize the outcomes of several early phase clinical trials of CAR T cell therapy in MM and the novel CAR T targets that are under development. Finally, we explore the potential mechanisms that result in disease relapse after CAR T therapy and propose future directions in CAR T therapy in MM.

Keywords

Chimeric antigen receptor; multiple myeloma; treatment; efficacy; toxicities

I. Multiple myeloma

Multiple Myeloma (MM) is a malignancy of terminally differentiated plasma cells typically characterized by clonal proliferation of these plasma cells in the bone marrow. MM represents 1% of all malignancies and 18% of hematologic malignancies in the United States; accounting for an estimated 32,110 new diagnoses and 12,960 deaths in 2019 alone [1]. The diagnosis of is made based on the presence of ≥ 10% clonal plasma cells in the bone marrow, and evidence of end organ damage (typically manifested as hypercalcemia, renal insufficiency, anemia, and/or bone lytic lesions-the so-called CRAB criteria), or in the

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Competing interests

The authors declare no competing conflicts of interest.

absence of end-organ damage if a patient has any ultra-high risk features: ≥ 60% clonal bone marrow plasma cells, the ratio of involved serum free light/uninvolved serum free light of ≥100, or ≥ 2 focal lesions on MRI [2–5].

Symptomatic MM requires treatment with chemotherapy typically consisting of a 2 to 3 drug combination that incorporates an immunomodulatory agent, along with a proteasome inhibitor and/or monoclonal antibody. The immunomodulatory drugs (IMiDs) (i.e., thalidomide, lenalidomide, and pomalidomide) and the proteasome inhibitors (PIs) (i.e., bortezomib, carfilzomib, and ixazomib) are the mainstay in the treatment of MM and have shown significant efficacy in the management of both newly diagnosed as well as relapsed and refractory MM patients [6–9]. Two monoclonal antibodies (daratumumab and elotuzumab) were approved by the FDA in 2015 and provide additional options for the treatment of MM [10–15]. Treatment of MM patients with combination regimens, followed by consolidation with high-dose chemotherapy and autologous hematopoietic stem cell transplant (ASCT) and maintenance has significantly improved the survival and outcomes of patients with MM [16–21]. The median survival of patients with newly diagnosed MM now reaches 7–9 years, and a small proportion of myeloma patients survive longer than 10 years.

However, despite these improvements, MM remains an incurable disease and nearly all MM patients will eventually relapse and develop resistance to currently available agents. Treatment options for these patients are particularly limited. Adoptive transfer of immune effector cells such as chimeric antigen receptor (CAR) T cells has shown encouraging results in early phase clinical studies. This review provides an overall summary of this approach in the treatment of MM.

II. Chimeric antigen receptor (CAR) T cells

1. Chimeric antigen receptor constructs

Cancer patients have profound defects in anti-tumor immunity, resulting in the failure to suppress tumor growth [22, 23]. The objective of CAR T therapy is to activate the anti-tumor immunity by adoptive infusion of a sufficient number of immune effector cells. Most of the immune effector cells used for the adoptive transfer are T cells, although NK cells have also been used. Autologous immune effector cells are typically used but allogeneic immune effector cells have been reported [24–26].

To achieve the specific and effective adoptive anti-tumor immunity, the T cells are transduced with a construct that consists of four major elements (Figure 1): a single-chain variable fragment (scFv), a transmembrane domain, costimulatory molecule(s), and a stimulatory molecule. The scFv fragment serves to direct T cells to the tumor cells and is commonly derived from the variable region of an antibody specific for tumor surface antigen. It is a fusion protein of the variable regions of the heavy and light chains of immunoglobulin connected with a short linker peptide. This scFv fragment plays an essential role in CAR T therapy and determines the specificity and effectiveness of CAR T treatment [27]. The amino acid composition, the position, and the number of scFv fragments have significant impact on the effectiveness of CAR T therapy [28]. The scFv fragment is typically cloned from hybridoma cells that produce the antibody. Phage display library

screening has been demonstrated to be an effective approach to identify scFv fragment [29, 30]. In addition to using the antibody variable region against tumor antigens for generation of the scFv fragment, some groups have used the sequence of specific ligands that bind to unique tumor cell receptors to generate the antigen-binding domain [31, 32].

The transmembrane domain consists of a hydrophobic α-helix derived from CD8, CD28 or immunoglobulin that is inserted into the membrane lipid bilayer spanning the cell membrane [33, 34]. Although the main function of the transmembrane domain is to anchor the CAR in the T cell membrane, and increase the strength of the bond between CAR T cells and targeted tumor cells, there is some evidence that it also plays a role in T cell activation [35, 36].

The stimulatory and costimulatory molecules are required for complete T-cell activation as defined by the classical two-signal hypothesis [37]. Signal 1, is provided by the interaction between the T-cell receptor (TCR) and the peptide-MHC complex on the antigen-presenting cells (APCs). Signal 2, is a costimulatory signal mediated by the interaction between the costimulatory molecule, CD28 on the T cell, and CD80 (B7.1) or CD86 (B7.2) on the APC [38, 39]. Therefore, the current version of CAR T constructs encode both the costimulatory molecule(s) and stimulatory molecule. Examples of co-stimulatory molecule(s) include one or more of CD27, CD28, ICOS, 4–1BB, OX40 [40]. It is unclear if these different molecules confer differential levels of costimulation. Most commonly used stimulatory molecules derive from CD3-zeta chain, although the FcεRIγ has been used in previous generations [41, 42].

2. Gene transfer technologies

The development of gene transfer technologies has permitted the efficient gene transfer of the chimeric antigen receptor to T cells. As summarized in Table 1, both viral and non-viral approaches have been used for gene transfer and have advantages and disadvantages. For CAR T gene transfer, retroviral and lentiviral vectors are commonly used. Retroviral vectors stably integrate into the dividing target cell genome, allowing for the transgene to be passed on and expressed on all daughter cells. The limitations of retroviral vector include inability to transduce non-dividing cells, and random integration with the risk of insertional mutagenesis [43, 44].

In contrast, lentiviral vectors transduce both dividing and non-dividing cells and stably integrate into the target cell genome. Transduction with lentiviral vectors typically produces higher titer viruses with more stable virions, and a significantly lower risk for insertional mutagenesis owning to their lack of viral genes that cause other disease pathologies in third generation lentiviral vectors [45, 46].

Once virus is produced, viral preparation undergoes several processes to remove contamination such as endotoxin or mycoplasma and undergo stringent testing to ensure compliance with good clinical practice manufacturer guidelines. These specific processes and standards are beyond the scope of this article but are available for review [47, 48].

3. T cell activation and expansion *ex vivo*

Before transduction, the T cells need to be activated and expanded *ex vivo*, typically in bioreactor. Typical activation is done using a synthetic molecule mimicking endogenous dendritic cell (DC) activation of the T cell. CD3/CD28 dynabeads are a commonly used commercially available method for activating T cells *ex vivo* [49–52]. Beads are used at varying ratios to T cells, but typically in the range of 1:1 to 1:5. This activation phase commonly lasts about 24–48 hours, before cells are subsequently harvested and plated for growth. T cells will then be transduced and subsequently expanded again before infusion. Exposure to fetal bovine serum and even human serum can increase odds of pathogen transmission upon reinfusion. Both Xeno-free serum, as well as other serum free methods are being explored to limit this exposure and comply with GMP [49, 53].

III. Early phase multiple myeloma CAR T clinical trials targeting BCMA

B-cell maturation antigen (BCMA), also referred to as tumor necrosis factor receptor superfamily member 17 (TNFRSF17) or CD269, is the receptor for BAFF and APRIL and is expressed consistently on myeloma cells and normal plasma cells at varying intensities [54–56]. BCMA has been shown to promote multiple myeloma pathogenesis, and targeting BCMA has been shown to have potent anti-myeloma activity [56–59]. BCMA antigen can be cleaved by gamma-secretase and released into blood circulation, and soluble levels of BCMA are often elevated in MM patients and seem to correlate with disease burden [60–62]. Several clinical trials have recently reported efficacy data using CAR T cells targeting BCMA and they are reviewed below and summarized in Table 2.

1. NIH study

An anti-BCMA CAR was first developed at the National Institutes of Health (NIH) and consisted of an anti-BCMA scFv, a CD28 costimulatory domain, and a CD3-zeta T-cell activation domain [55]. A gamma-retroviral vector was utilized to transduce CAR-BCMA to autologous T cells. A phase I, dose escalation study was conducted (ClinicalTrials.gov:) and tested 4 dose levels: 0.3×10^6, 1×10^6, 3×10^6, and 9×10^6 CAR+ T cells/kg of bodyweight. Patients received a conditioning chemotherapy regimen of 300 mg/m2 of cyclophosphamide (Cy) and 30 mg/m2 of fludarabine (Flu) on days −5 to −3 followed by an infusion of CAR-BCMA T cells on day 0. A total of 26 patients were enrolled and 13 patients received the highest CAR T cell dose level of 9.0×10^6 cells/kg [63, 64]. The median prior lines of treatment were 11.

CAR-BCMA T cells exhibited clear anti-myeloma activity. At the highest CAR T cell dose, 9 of the 11 evaluable patients obtained objective anti-myeloma responses with 2 patients (18%) achieving a stringent complete response (sCR), 5 patients (45%) achieving a very good partial response (VGPR), and an additional 2 patients (18%) obtaining a partial response (PR). Eight of 10 evaluable patients obtained minimal residual disease (MRD) negative status by bone marrow flow cytometry.

Eleven of 12 evaluable patients (92%) developed cytokine-release syndrome (CRS) after infusions of CAR T cells with 5 patients having ≥ grade 3 CRS. The most prominent signs

of CRS were fever, tachycardia, hypotension, and reduced cardiac function. Of thirteen patients, 4 received the interleukin (IL)-6-receptor antagonist tocilizumab to treat CRS; 2 of these 4 patients also received corticosteroids. Two patients experienced delayed neutropenia and thrombocytopenia. Toxicity was significant but limited in duration and controllable. This study provides the proof of concept of CAR-BCMA T therapy in the treatment of relapsed/refractory, poor-prognosis MM.

2. MCARH171 – Memorial Sloan Kettering Cancer Center

Memorial Sloan Kettering reported the results of their phase I clinical trial using a retrovirally transfected CAR T called MCARH171 as well as pre-clinical data with JCARH125. JCARH125 is created via lentiviral transfection (1:1 CD4 to CD8 ratio pre transduction and expansion) and contains 4–1BB and CD3ζ stimulatory domains, CD28 transmembrane domains and an alphaBCMA/125 scFv (ClinicalTrials.gov:) [65].

MCARH171 is composed of a CD3ζ and 4–1BB stimulatory domains with a CD8a hinge/transmembrane and an α-BCMA/171 scFv. A dose escalation study was recently reported that was designed to evaluate 4 doses: 1×10^6 cells/kg, 150×10^6 cells, 450×10^6 cells, and 800×10^6 cells [66]. Patients received conditioning chemotherapy with Cy 3 gm/m2 as a single dose or Flu 30 mg/m2 daily and Cy 300 mg/m2 daily for 3 days followed by MCARH171 infusion in 1–2 divided doses. Eleven patients had been enrolled at time of data reporting with a median of 6 prior lines of therapy including a PI, an IMiD, a CD38 antibody, and ASCT. All patients had detectable BCMA expression with the vast majority (91%) having > 80% expression. Patients were grouped into those dosed with $\leq 150 \times 10^6$ or less cells and $\geq 450 \times 10^6$ cells for analysis. 50% of those in the low dose group had CRS, none were above grade 2, but two dosed at 150×10^6 required tocilizumab. Three of the four evaluable patients in the high dose group had CRS, two at grade 3. Overall 100% of evaluable patients had grade 4 neutropenia, all but one had grade 4 lymphopenia, and all patients had grade 2 or higher thrombocytopenia.

The trial showed limited efficacy at low dose. Overall response rate was 64% with a median PFS of 106 days. Patients in the high dose cohort ($\geq 450 \times 10^6$ cells) reportedly had deeper and more durable responses including 3 VGPRs although the PFS of the high dose cohort was not independently reported. CRS was seen in 6 patients (60%) with only two patients having ≥ grade 3 and tocilizumab administered to 3 patients. No dose limiting toxicities (DLTs) were reported.

3. UPENN/Novartis Study

The University of Pennsylvania reported a phase I study using an anti-CD3/anti-CD28 beads *ex vivo*-expanded, autologous T cell product engineered by lentiviral transduction to express a fully human BCMA-specific CAR with CD3ζ and 4–1BB signaling domains (ClinicalTrials.gov:) [67]. A total of 25 patients were enrolled into 3 cohorts: 1) $1–5 \times 10^8$ CAR T cells alone; 2) $1–5 \times 10^7$ CAR T cells with 1.5 g/m^2 + Cy; and 3) $1–5 \times 10^8$ CAR T cells with 1.5 g/m^2 + Cy. CAR T-BCMA cells were given as split-dose infusions over 3 days (10% on day 0, 30% on day 1, and 60% on day 2), with Cy given on day −3. The median

prior lines of therapy were 7 (range 3–13), and all patients had previously been exposed to a PI and IMiD.

Grade 3 or higher toxicities were seen in 96% of patients; most commonly cytopenias. CRS was observed in 88% of subjects and was ≥ grade 3 in 32% (all of whom were treated at the highest dose). Seven patients (28%) were treated with IL-6 blockade. Neurotoxicity was seen in 8 patients (32%) most commonly manifesting as confusion and/or aphasia. Three patients (12%) had ≥ grade 3 neurotoxicity including 1 patient with PRES which was determined to be a DLT. Additional DLTs were cardiomyopathy and hemothorax each of which occurred in 1 patient. One patient died during the trial after developing grade 4 CRS complicated by candidemia. Notably, his labs after his 3 infusions showed disease progression including 13% circulating plasma cells.

Overall response rate was 48%, and was 44% in cohort 1, 20% in cohort 2, and 64% in cohort 3. Across all cohorts there were 1 sCR, 1 CR, and 5 VGPRs. These data demonstrated that CAR T-BCMA infusions following Cy lymphodepletion is feasible and has significant clinical activity in highly-refractory MM patients who had limited treatment options. CRS remains a common but manageable toxicity.

4. Bluebird studies

Bb2121 is a second-generation CAR T therapy using autologous T cells transduced with a lentiviral vector encoding an anti-BCMA scFv, a 4–1BB costimulatory motif and a CD3-ζ T cell activation domain. This was a multicenter, 2-part, phase I study of patients with relapsed refractory MM (ClinicalTrials.gov:). The first part of the study (dose escalation) enrolled patients who had received ≥ 3 prior lines of therapy including a PI and an IMiD, and had ≥ 50% BCMA expression on their plasma cells [68, 69]. Twenty-one patients were enrolled in the dose escalation part and received CAR T cells at doses of 50×10^6, 150×10^6, 450×10^6 or 800×10^6. In the dose-expansion phase, patients had to have received daratumumab and been refractory to their last line of therapy, BCMA expression was required, but no specific cutoff was defined. Twelve patients were enrolled in the dose expansion phase and received CAR T cells in the range of $150 – 450 \times 10^6$. Patient received lymphodepletion with Flu (30 mg/m^2)/Cy (300 mg/m^2) given daily for 3 days (−5, −4, and −3) followed by a single infusion of bb2121 on day 0. The median number of prior lines of treatment was 7 and 8 for dose escalation phase and dose expansion phase, respectively.

At the median follow-up of 11.3 months, CRS, primarily grade 1–2, was reported in 25 of 33 (76%) patients; 2 (6%) patients had grade ≥ 3 CRS that resolved in 24 hours. Grade ≥ 3 neutropenia was observed in 28 patients (85%); grade ≥ 3 anemia was seen in 15 (45%) patients, and grade ≥ 3 thrombocytopenia in 15 (45%) patients. One patient (3%) had grade 4 neurotoxicity. There were no grade 4 or 5 CRS events. One patient died during the trial due to cardiopulmonary arrest which was considered to be unrelated to the study treatment.

At the dose of 50×10^6 (N=3), ORR was 33% with the only response being a PR. At the cell dose of 150×10^6 (N=8), ORR was 75% with: 5 (63%) patients achieving a sCR, and 1 (12%) patient achieving a PR. At doses $>150 \times 10^6$ (n=30), ORR was 90%, with 12 (40%) sCR, 3 (10%) CR, 9 (30%) VGPR, and 3 (10%) PR. Of the 16 patients with a disease

response tested for minimal residual disease (MRD) all 16 (100%) were MRD negative, albeit with a relatively low threshold of 10^{-4} with only 3 patients achieving MRD negativity at a level of 10^{-6}. With a median follow-up of 11.3 months median progression-free survival was 11.8 months in patients infused with ≥ 150×10^6 CAR T cells compared to 2.6 months in the patients in the 50×10^6 cohort. Median progression-free survival in the 16 responders who were MRD negative was 17 months. Bb2121 shows promising efficacy at dose levels ≥ 150×10^6 CAR T cells with deep and durable ongoing responses and manageable CRS and neurotoxicity [70]. These data support the potential of bb2121 anti-BCMA CAR T cell therapy as a new treatment for RRMM.

BB21217 is a second generation anti-BCMA CAR T developed by Bluebird Bio which uses the scFv, 4–1BB costimulatory motif and CD3-ζ T cell activation domain as bb2121 but adds the PI3K inhibitor bb007 during the *ex vivo* T cell expansion phase. By limiting PI3K signaling and upregulating AKT, the population of CAR T cells is enriched for long-lived memory-like T cells displaying CD62L+ and CD27+ [71]. Mouse studies which re-challenged animals with tumor implantation at day 30 on the opposite flank from prior showed no tumor growth at day 90, in contrast to bb2121 which showed marked growth. Currently a phase 1 dose escalation trial is enrolling patients with RRMM who have previously been treated with ≥ 3 regimens including a PI and IMiD (ClinicalTrials.gov:). Planned doses are 150×10^6 cells and escalating to 300×10^6, 450×10^6, and 800×10^6 with 3 days of Flu and Cy at days −5, −4 and −3.

As of June 2018 (the most recent report) 8 patients had been treated all at the 150×10^6 dose with plans for a total enrollment of 50 patients [72]. Median number of prior lines of therapy was 9. CRS was seen in 5 (63%) of patients including one patient who had DLTs of grade 3 and grade 4 encephalopathy. This patient was noted to have high tumor burden which was thought to play a role in these toxicities. At time of data cut-off 7 patients were evaluable for response with an ORR of 86%. One (14%) patient had a sCR, 3 (43%) achieved a VGPR, and 2 (29%) had a PR.

Interestingly, most responses appear to deepen over time with CR achieved as late as 10 months. Examination of T cell populations (n=6) in these patients showed an increase of CD62L+/CD45RA− cells, and a trend towards increased CD27+/CD45RA− cells. On this note, of 7 examined patients, 6 still had detectable CAR vector copies at 3 months, and 3 out of 3 patients had detectable CAR vector copies at 6 months. Finally, no change in vector copy number, serum M protein, serum free light chain, or sBCMA seemed discernable when patients were stratified into high tumor burden and low tumor burden groups. Bb21217 opens the door for a new wave of myeloma CAR-T trials examining how enriching for memory-like sub-populations of T cells may prolong disease remission by increasing the capability of controlling myeloma relapse.

5. Nanjing Legend/Janssen LCAR-B38M study

Nanjing Legend Biotech reported the safety and efficacy of LCAR-B38M, a dual epitope-binding CAR T cell therapy, in patients with relapsed/refractory MM. At data cutoff, this phase I, single-arm, open-label, multicenter study enrolled a total of 57 patients (ClinicalTrials.gov:). The median number of prior lines of therapy was 3 (range, 1 to 9),

including prior PI (68%), IMiDs (86%) and both PI and IMiDs (60%) in the majority of patients. Ten (18%) patients previously underwent ASCT. Autologous T cells were engineered with lentiviral vector to express a BCMA targeting domain against 2 distinct BCMA epitopes connected by GGGGS linker, a CD8α hinge and transmembrane domain, a 4–1BB/CD137 cytoplasmic domain and a human CD3zeta domain. Patient received 3 doses of Cy 300mg/m2 on day −5, −4, and −3 for lymphodepletion and median CAR T cell dose of 0.5×10^6 cells/kg (range, 0.07 to 2.1×10^6) that was split into 3 infusions (20, 30, and 50% of total dose administered over 7 days [73].

All patients experienced ≥ 1 adverse events. 37/57 patients (65%) had grade ≥ 3 AEs that include leukopenia (17/57; 30%), thrombocytopenia (13/57; 23%), and aspartate aminotransferase increased (12/57; 21%). CRS occurred in 51/57 patients (90%). However only 4 patients (7%) had grade ≥ 3 cases and only one patient had neurotoxicity manifesting as grade 1 aphasia, agitation, and seizure-like activity.

The overall response rate was 88% with 39/57 patients (68%) achieving a CR, 3/57 (5%) achieving a VGPR, and 8/57 (14%) a PR. MRD at the sensitivity of 10^{-4} was negative for 36/57 (63%) patients. The median time to response was 1 month (range, 0.4 to 3.5). At a median follow-up of 8 months, median progression-free survival was 15 months (95% CI, 11 to not estimable). Median overall survival for all patients was not reached. These data demonstrated that LCAR-B38M CAR T cell therapy has a manageable safety profile and can achieve deep and durable responses in patients with RRMM.

6. Correlation between BCMA expression level and CAR T responses

Whether the level of BCMA expression correlates with the response of BCMA-CAR T therapy is still an area of active debate. In the bb2121 BCMA-CAR T trial, tumor response was independent of plasma cell BCMA expression: the objective response rate for myeloma patients with low BCMA expression, (i.e., <50% bone marrow plasma cells expressing BCMA) was 100%. This response rate was not significantly different from the 91% response rate seen in myeloma patients with high BCMA expression (≥50% bone marrow plasma cell expression) treated with the same dose of CAR T cells. Despite this, the bb21217 trial is only enrolling patients with ≥50% BCMA expression. Similarly, in the Nanjing Legend CAR T trial, BCMA expression did not appear to correlate with clinical response: An ORR of 92% was observed in patients with < 40% BCMA expression compared with an ORR of 82% in patients with ≥ 40% BCMA expression. There was no correlation between BCMA expression and median PFS or OS: Median PFS was 15 months for patients who had < 40% BCMA expression and 11 months for patients who had ≥ 40% BCMA expression. Median OS was not reached for both the < 40 and ≥ 40% BCMA expression groups. Notably, baseline levels of soluble BCMA did not correlate with response to the UPENN/Novartis BCMA CAR T. However, patients that had a PR or better to the treatment had a decrease in sBCMA that peaked at 60 days ($p<.001$) [67].

One concern is that the current IHC assay for measuring BCMA expression may not accurately represent BCMA expression level and that a flow based assay would be more accurate. However, use of a flow-based assay would require fresh, un-fixed tumor cells for measurement which can be difficult to coordinate.

BCMA levels from bone marrow aspirate measured by flow cytometry mean fluorescence intensity (MFI) were also examined in the UPENN/Novartis study. Patients achieving a PR or better showed a decrease of BCMA MFI at day 28 (p=.02) compared to pre-treatment (from 4000 to 944), whereas non-responders did not display a decrease at day 28 (2704 to 2104). However, this response was not durable and may have be skewed by the small number of patients with very high BCMA MFI [67].

IV. Early phase clinical trials targeting other myeloma antigens

1. Phase I clinical trial with CAR T therapy targeting the κ light chain

Ramos et al. at the Baylor College of Medicine and Houston Methodist Hospital conducted a phase 1 clinical trial in patients with κ^+ non-Hodgkin lymphoma/chronic lymphocytic leukemia or MM (ClinicalTrials.gov:) (Table 3) [74]. Autologous T cells were genetically engineered by retroviral vector to express a murine scFv fragment targeting the κ light chain of human immunoglobulin, a CD28 costimulatory domain and a CD3 ζ domain. A spacer region derived from the human IgG1-CH2CH3 domains was cloned in-frame between the scFv and the signaling domain. Eight myeloma patients were enrolled in the study. Seven of 8 patients had prior ASCT. Patients received no or limited lymphodepletion chemotherapy (12.5mg/kg Cy). Cell dose was in the range of 0.92 to 1.9×10^8 cells.

All infusions were well tolerated. Only 1 MM patient experienced a grade 3 lymphopenia that was deemed possibly related to therapy. None of the adverse events reported were considered to be related to κ CAR T infusion. No patients experienced symptoms consistent with severe CRS.

No objective responses were observed in the MM patients. 5 of 8 patients had stable disease. One patient had a progressive decline in their paraprotein levels (2,240 to 1730 mf/dl IgG) and improvement in anemia (11.6 to 14.4 g/dl hemoglobin), which was sustained for 2 years. Overall, the response with κ CAR T in patients with κ-light chain restricted myeloma has been modest.

2. Clinical trial with CAR T therapy targeting CD19

Typically, malignant plasma cells isolated from MM patients lack CD19 expression [75–77]. However, recent reports suggest that there may be CD19+ population of plasma cells in MM patients with very refractory disease [78–80]. Additionally, patients with monoclonal gammopathy of undetermined significance (MGUS) have be shown to have high expression of CD19 [81]. These data suggest that these cells may represent myeloma-initiating cells (i.e. myeloma stem cells). These cells contribute to the relapse of myeloma and are thought to be derived from post-germinal center B cells, which exhibit B cell phenotypes such as the expression of CD19 or CD20. To eliminate these myeloma-initiating cells, Garfall et al. at the University of Pennsylvania performed an early phase clinical trial in which relapsed/refractory myeloma patients received CAR T cells against CD19 (CTL019) following salvage high dose melphalan and ASCT (ClinicalTrials.gov:) (Table 3) [82]. All subjects had previously undergone ASCT with less than 1 year progression-free survival. Twelve

patients enrolled in this clinical trial with 10 subjects received CTL019 CAR T therapy. Median total lines of prior therapy were 6 (range 2–10).

Most adverse events were attributed to high-dose melphalan conditioning. CRS was observed only in one subject and was grade 1 in severity. At day 100 after ASCT, 8 of 10 subjects exhibited a partial response or better. Because this overall response rate could also be due to ASCT alone, to assess the effectiveness of CTL019 the authors compared progress-free survival after ASCT+CTL019 (PFS2) to each subject's PFS after prior ASCT (PFS1). In 8 of 10 subjects, PFS2 was shorter than PFS1. However, in 2 subjects, PFS2 was substantially longer than PFS1 (479 vs. 181 days for one subject; 249 vs. 127 days for the other subject). Moreover, in these two subjects, the presentation of the disease progression after ASCT+CTL019 treatment was different from their disease progression prior to ASCT +CTL019 therapy: the rise in monoclonal immunoglobulin production was more gradual and there was no evidence of multiple myeloma by standard anatomic pathology assessment at time of progression. Expression of Sox2, a transcription factor that governs self-renewal and pluripotency, has been linked to myeloma-propagating capacity in myeloma cell lines [83, 84]. In the two subjects with longer PFS2, there was increase in anti-Sox2 antibody response and T cell response after CTL019 treatment. These results suggest that CAR T therapy targeting $CD19^{+}$ myeloma-propagating cells following ASCT may be beneficial and could potentially lead to longer progression-free survival.

3. Clinical trial with CAR T therapy targeting CD138

CD138 is highly expressed on MM cells and is involved in their development and proliferation. Guo et al. describes the outcomes of 5 MM patients treated with a CD138 CAR T (ClinicalTrials.gov:) (Table 3) [85]. A lentiviral vector was used to transduce autologous T cells to express the fusion gene, anti-CD138-ScFV-CD8α−4–1BB-CD3ζ. Patients received an average of 0.756×10^{7} cytokine-induced killer cells per kilogram. Four of the 5 patients developed fever, chills, and nausea within 40 minutes to 4 hours after cell infusion which lasted up to 2 hours. The best response was stable disease seen in 4 patients which lasted up to 7 months.

V. CAR T therapy under preclinical development (Table 4)

1. CAR T therapy targeting SLAMF7

SLAMF7 (CD319, CS-1) is a member of the signaling lymphocytic activation molecule family of trans-membrane receptors. SLAMF7 is expressed on NK cells and a proportion of $CD8^{+}$ T cells. SLAMF7 is uniformly expressed on malignant plasma cells in newly diagnosed multiple myeloma and in relapsed myeloma after intensive chemotherapy [86–88]. Furthermore, SLAMF7-CAR T cells were effective against myeloma cells *in vitro* and *in vivo* in a murine xenograft model [89]. Importantly, SLAMF7-CAR T cells induce selective fratricide of $SLAMF7^{+/high}$ NK cells, $CD4^{+}$ and $CD8^{+}$ T cells, and B cells, but spare $SLAMF7^{-/low}$ cell subsets, which may be sufficient to preserve normal lymphocyte function including anti-viral immunity [88]. These data provide strong preclinical data for the potential use of SLAMF7-CAR T cell therapy in multiple myeloma. SLAMF7-CAR T clinical trial was recently initiated in the National Cancer Institute (NCI).

2. CAR T therapy targeting BCMA with APRIL

As previously discussed, several early phase clinical trials have demonstrated efficacy of BCMA CAR T therapy in the treatment of MM. All these studies used a scFV fragment derived from anti-BCMA antibody to direct CAR T cells to target BCMA expressing myeloma cells. BCMA expression could vary and the expression could be down-regulated after BCMA targeted CAR T therapy. Therefore, a CAR T therapy that targets both $BCMA^+$ and $BCMA^-$myeloma cells is highly desirable. Lee L et al. recently reported an "A proliferating-inducing ligand" (APRIL)-based CAR T cells [90]. APRIL is a natural high-affinity ligand for both BCMA and transmembrane activator and calcium-modulator and cyclophilin ligand (TACI). TACI was expressed in 39 out of 50 primary MM cells (78%). An oncoretroviral vector was constructed that encoded a truncated APRIL fused to a spacer domain, a CD28 transmembrane and the CD28-OX40-CD3ζ endodomain. The APRIL CAR T cells induced cytolysis of MM cells at low antigen densities, at low E:T ratio, and in the presence of soluble APRIL, BCMA, and TACI *in vitro* and *in vivo*. More importantly, APRIL CAR T therapy resulted in disease control in an *in vivo* escape model, suggesting that the dual-antigen targeting of BCMA and TACI enhances continued disease suppression in the event of BCMA downregulation or loss in patients who have co-expression of both antigens on their tumor cells.

3. CAR T therapy targeting A2/NY-ESO-1_{157}

NY-ESO-1 is a well-known cancer-testis antigen and is highly expressed in poor-prognosis multiple myeloma patients especially those with relapsed disease or high risk cytogenetics [91]. NY-ESO-1 is highly immunogenic and induces spontaneous humoral and cellular immune responses. Cytotoxic T cells specific for NY-ESO-$1_{157–165}$ peptide presented by an HLA-A*02:01 molecule (A2/NY-ESO-1_{157}) on myeloma cell surfaces are functionally active and can kill primary MM cells. Maruta et al. generated second-generation CAR T cells with A2/NY-ESO-1_{157}-specific scFv linked with CD28 and CD3ζ [92]. Two different A2/NY-ESO-1_{157}-specific single chain fragment variable (scFv) genes were tested: one encodes variable regions of a light (L) and a heavy (H) chain in order of LH, and the other one encodes HL. For comparison, a bispecific antibody (BsAb) composed of each A2/NY-ESO-1_{157}-specific scFv and a CD3ε-reactive scFv was also generated. It was found that five out of five A2/NY-ESO-1_{157}- CAR-transduced T cells showed A2/NY-ESO-1_{157}-specific reactivity, resulting in anti-myeloma reactivity to $A2^+$NY-ESO-1^+U266 myeloma cells. Newly generated BsAbs successfully linked A2/NY-ESO-1_{157} expressing targets and $CD3^+$ T cells. Interestingly, compared with CAR-T cells, BsAb-stimulated T cells showed superior A2/NY-ESO-1_{157}-reactive cytokine production capacity. These data suggest that A2/NY-ESO-1_{157} is an important target in CAR T therapy or BsAB therapy for MM.

4. CAR T therapy targeting integrin β7

Most of the current targets used in CAR T therapy are also expressed on normal cells/tissues, resulting in unwanted toxicities and side effects. Therefore, identifying cancer-specific cell-surface antigens is critical for future CAR T development. Hosen et al. recently screened >10,000 anti-MM mAb hybridomas and identified MMG49 as an MM-specific mAb [93]. The MMG49 epitope is located in the N-terminal region of the integrin β7 chain

and is hidden in the resting integrin conformer but exposed in the active conformation. Interestingly, there was high MMG49 reactivity on MM cells due to elevated expression and constitutive activation of integrin β7. This is in contrast to other cell types including normal lymphocytes where the MMG49 binding is minimal, making MMG49 a very attractive, specific target for MM CAR T therapy. Indeed, CAR T cells derived from MMG49 recognize and kill MM cells *in vitro* and *in vivo* without damaging normal hematopoietic cells. This study opens a new avenue to target cancer specific cell-surface proteins that undergo conformational changes in cancer cells.

VI. Mechanisms of resistance/relapse after CAR T therapy

Despite the promise of CAR T therapy in the treatment of MM, patients still relapse with CAR T therapy even after achieving MRD negativity. The progression-free survival in bb2121 and LCAR-B38M trial was 17.7 and 15 months, respectively. The mechanisms for the relapse remain to be characterized but may include the following.

1. Down-regulation or loss of target antigen

The most commonly cited reason for relapse to CAR T therapy is the development of cancer cells which no longer express the target antigen. Loss of target antigen has been reported in several CAR T trials to date [94–97]. In the UPENN/Novartis trial, the investigators noted significantly diminished levels of BCMA antigen on MM cells following CAR T therapy, but did not comment if this correlated with relapse. A solution to this issue is to create CAR T cells that target multiple tumor antigens either by using multiple CAR T cells in combination or using bi/tri-specific CAR T cells which have multiple unique scFVs on a single CAR T cell. Yan et al. tested a CD19/BCMA CAR T in patients with RRMM. As of last data report 8 patients had been treated with 5 evaluable for response. The overall response rate was 100% including 1 sCR, 1 VGPR, 2 PR, and 1 SD [98].

2. Inaccessibility of myeloma cells by CAR T cells.

Myeloma cells reside in the bone marrow microenvironment consisting of wide spectrum of cell types and extracellular matrix proteins, including fibronectin, collagen, laminin, and osteopontin [99]. This microenvironment could shield MM cells from CAR T therapy. A similar phenomenon was recently illustrated by Cazaux et al. Using intravital imaging they tracked CD19 CAR T cells in B cell lymphoma-bearing mice and found that many of these cells were trapped in the lungs in the form of large cell aggregates and never reached their target destination [100]. Myeloma cells in particular have developed specific abilities to evade immune detection, such as upregulation of immune suppressive molecules (reviewed in [101]), increased Tregs and myeloid derived suppressor cells (MDSCs) [102, 103], and secretion of VEGF, HGF, fibroblast growth factor (FGF), and stromal-cell-derived factor (SDF)-1α by bone marrow stromal cells [104] to avoid the killing of CAR T cells.

3. Insufficient persistence of CAR T cells

Insufficient persistence of the CAR T cells has been postulated as a probable cause of loss of efficacy, and ultimately relapse of myeloma in response to CAR T therapy. In CD19-CAR T cell trials for chronic lymphocytic leukemia and for acute B lymphoblastic leukemia, some

studies showed that the degree of *in vivo* expansion and persistence of CAR T correlated with response [105, 106], although other trials have noted durable responses with absence of detectable CAR T cells [107]. In the bb2121 study CAR T cell expansion was correlated with response, and CAR T cells persisted up to 1 year after infusion in 20% of patients. Notably, CAR T levels were significantly higher in patients who achieved a response to the therapy at 28 days after infusion [68]. It will be interesting to see if this data is replicated as more MM patients are treated with CAR T approach.

4. Enrichment of immunosuppressive cells such as MDSCs or pDCs.

MDSCs have been shown to be elevated in peripheral blood of MM patients [108]. In the context of MM, MDSCs have been shown to increase tumor burden, reduce immune penetrance into the tumor microenvironment, and induce MM-related angiogenesis [109]. The coadministration of NK cells capable of targeting NKG2D, and thus eliminating MDSCs, was able to increase survival in solid tumor bearing mice infused with CAR T cells versus those treated with CAR T cells alone [110]. There is also a long history of this receptor's therapeutic potential in myeloma [111]. A clinical trial testing the efficacy of CAR T cells expressing NKG2D in 7 AML and 5 MM was safe with only mild (< 3) toxicities, however no objective responses were seen [112]. It remains to be seen if combination of this CAR T targeting MDSCs with an anti-myeloma CAR T therapy would have increased efficacy and/or toxicity.

Plasmacytoid dendritic cells (pDCs) in the bone marrow offer similar immunosuppressive support to myeloma, increasing its survival, drug resistance, and growth [113]. The targeted therapy SL-401 is a recombinant CD123 genetically fused to a truncated diphtheria toxin payload, which specifically targets pDCs. Use of SL-401 showed antimyeloma activity in preclinical models as both a single agent and synergism with bortezomib, dexamethasone, lenalidomide, and pomalidomide [114–116] and in clinical trials with pomalidomide and dexamethasone [117]. Unfortunately, the drug was associated with increased risk of death due to capillary leak syndrome in a trial of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN). A trial investigating an allogenic CAR designed to target CD123 (UCART 123) was in clinical trials for AML and BPDCN but was placed on hold on September 5, 2017 by the FDA due to a death of a patient. The hold was subsequently lifted on November 7, 2017 [118] and the trial evaluating safety and efficacy of UCART123 in patients with AML is currently ongoing (ClinicalTrials.gov:).

VII. Second CAR T therapy

At the 2019 ASCO annual meeting, Li et al. presented 4 cases who received human BCMA-targeting CAR T therapy (CT103A) after the patients' myeloma relapsed from a murine BCMA-targeting CAR T therapy. The CT103A CAR construct is a lentiviral vector encoding a fully human scFV, CD8a hinger and transmembrane, 4–1BB costimulatory and CD3-zeta activation domain. Patients received 1–6 × 10^6 CAR T cells/kg. All 4 patients had objective responses: 3 patients achieved CR and 1 patient had VGPR. Larger patient size with longer follow up are needed, but this study demonstrated the potential of a second CAR T therapy in patients who relapses from previous CAR T therapy [119].

VIII. Summary and future direction

CAR T therapy has shown promising results and safety profiles. It is highly likely that in the near future CAR T therapy will become an important modality of treatment especially for relapsed/refractory MM. It remains to be determined when will be the best time to incorporate CAR T therapy in MM: as part of induction therapy, or in the relapse setting, as an alternative to ASCT, or an adjunctive to ASCT? Finally, the mechanisms of resistance/relapse with CAR T therapy remains to be characterized and the approaches to overcome resistance/relapse will be crucial for the ultimate success of CAR T therapy in MM. Overall, it is still unclear whether CAR T therapy will become another option in an increasingly complex arsenal of multiple myeloma treatment options, or bring about a new standard of care to a disease that currently remains incurable.

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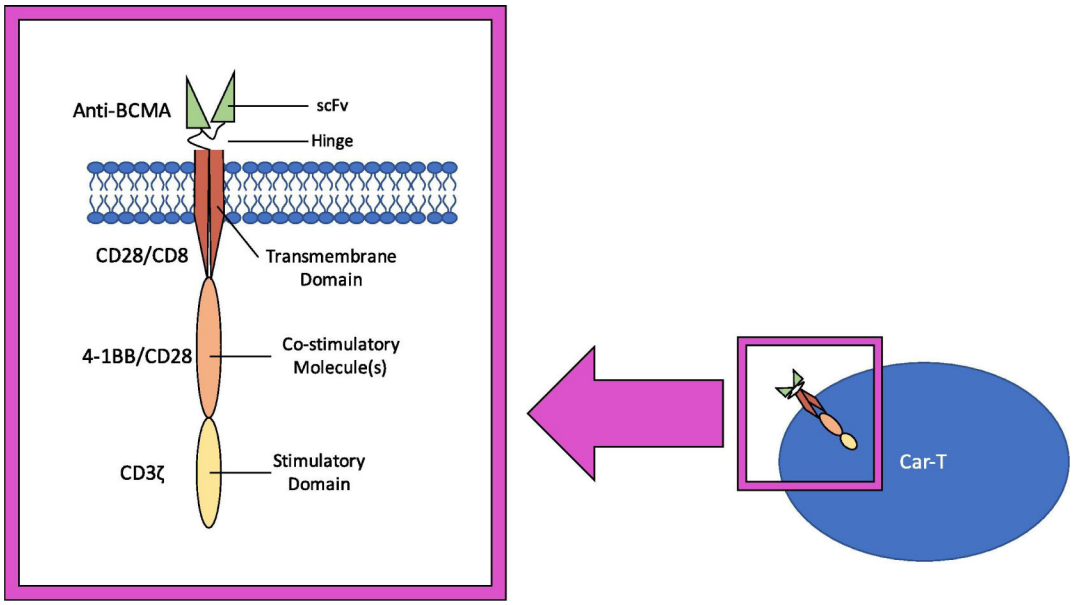

Figure 1:
CAR T construct

Table 1:

Various gene transfer approaches.

Approach	Notes
Adenoviral vector	High efficiency, no integration, no long-term expression
Adeno associated vector	Integration at chromosome 19, difficulty in production, low capacity (small amount of DNA), host immunity against AAV
Retroviral vector	Only infecting proliferating cells (no quiescent cells), random integration, insertional mutagenesis
Lentiviral vector (HIV)	Infecting proliferating and quiescent cells, no insertional mutagenesis reported
Feline FIV vector	Similar to HIV lentiviral vector, no concerns for HIV recombination
Nonviral approaches	Non-viral, low efficiency

Table 2:

BCMA-CAR T therapy trials.

	NCI	MSK	University of Pennsylvania	Bluebird 2121	Nanjing Legend
Authors/ presentation	Ali et al, Blood 2016. 128:1688 Brudno et al, ASH 2017	Mailankody et al, ASH 2018.	Cohen et al, ASH 2016 Cohen et al, ASH 2017 Cohen et al, JCI 2019	Berdeja et al. ASH 2017 Raje et al. ASCO 2018 Raje et al. NEJM, 2019.	Fan et al, ASCO 2017 Zhao et al. J Hem Onc 2018
n	26	11	24	43	57
vector	Retroviral vector	Retrovial Vector	Lentiviral vector	Lentiviral vector	Lentiviral vector
Costimulatory domain	CD28	4–1BB	4–1BB	4–1BB	4–1BB
Activation domain	CD3-zeta	CD3-zeta	CD3-zeta	CD3-zeta	CD3-zeta
conditioning	Cyclophospham ide +fludarabine	3 day Cy+Flu (300 + 30mg/m^2) or 1 day Cy (3000mg/m^2)	With or without Cy	Cy+Flu (day −5, −4 and −3)	Cy
Dosing	4 dose levels: 0.3 ×; 1 ×; 3 ×; and 9 × 10^6 CAR T cells/kg	3+3 Dose Escalation: 3 at 1 × 10^6 3 at 150 × 10^6 4 at 450 × 10^6 1 at 800 × 10^6	3 cohorts: 1–5 × 10^8 CAR T; Cy 1.5g/m2 + 1–5 × 10^7 CAR T; Cy 1.5g/m2 + 1–5 × 10^8 CAR T (Cy: day −3; CAR split: 10% day 0; 30% day 1; 60% day 2	1 infusion; Dose escalation: 50×, 150×, 450×, and 800× 10^6	0.07 to 2.1 × 10^6 CAR T cells/kg
Prior lines Of treatment (median)	11	6	7. 100% PI and IMiD refractory; 67% dara-refractory; 95% high risk cytogenetics.	7–8	More than 2
CRS	On the highest dose, 92% had CRS (grade 1–4)	6/10 And 3/4 at ≥450×10^6	Cohort 1: 8/9; Cohort 2 and 3: 9/12	Manageable; 27/43 (63%): 2: >grade 3, no DLTs, No neurotoxicities	Grade 3 AE: 37/57 (65%); CRS: 51/57 (90%); grade 3 CRS 4/57 (7%)
Response	On the highest dose, 9/11 (82%) ORR: 2 sCR, 5 VGPR, 2 PR	At ≥450×10^6 2/5 still at VGPR and 3/5 have progressed. 5/11 VGPR	Cohort 1: 6/9 (1 sCR, 2 VGPR, 1PR, 1MR) Cohort 2: 2/5 (1 PR, 1 MR) Cohort 3: 5/6 (1 CR, 3 PR, 1 MR)Cohort 1: 6/9 (1 sCR, 2 VGPR, 1PR, 1MR) Cohort 2: 2/5 (1 PR, 1 MR) Cohort 3: 5/6 (1 CR, 3 PR, 1 MR)	On the highest dose, ORR: 95.5%; CR 50%; VGPR 36.4%, PR 9.1%	ORR: 88%: 39/57 (68%) CR; 3/57 (5%) VGPR; 8/57 (14%) PR
MRD	8/10 MRD negative by flow	Not Assessed		16/16 MRD negative: PFS: 17.7 Months	36/57 (63%) MRD negative at 1 × 10^4 PFS: 15 months

Table 3:

Other CAR T therapy trials in MM

	Baylor	**U Penn**	**China**
Authors/presentation	Ramos et al, JCI 2016; 126:2588	Garfall et al, JCI Insight, 2018; 3:e120505	Guo et al, J Cell Immunol 2016, 2:28
Target	Kappa light chain (CRL-1758 hybridoma)	ASCT + CTL019	CD138
n	8	10	5
vector	Retroviral vector	Lentiviral vector	Lentiviral vector
Costimulatory domain	CD28	4–1BB	4–1BB (CD137)
Activation domain	CD3-zeta	CD3-zeta	CD3-zeta
conditioning	3 patients without Cy; 5 with Cy	ASCT followed by CTL019	various
Dosing	9.2 to 19 × 10^7 CAR T cells/M^2	4.4 × 10^8 (range 1.1 to 6 × 10^8)	0.756 × 10^7 cytokine-induced killer cells/kg
Prior lines of treatment (median)	1–5	2–10	5–18
CRS	None	None	fever
Response	5: SD; 3: NR	2 patients significantly longer PFS (479 vs 181; 249 vs 127)	4/5: stable disease
MRD	Not done	Not done	Not done

Table 4:

CAR T therapy under development

	Germany	**UK**	**Japan**	**Japan**
Authors/presentation	Gogishvili et al. Blood, 2017	Lee et al, Blood 2018;131:746	ASH 2018	Hosen et al, Nat Med 2017
Target	SLAMF7	APRIL (ligand for BCMA and TACI)	A2/NY-ESO-1 (cancer testis antigen)	Integrin β_7
scFv	huLuc63 (elotuzumab)	Note: no scFV; ligand-based antigen binding domain	Clone: 3M4E5	Screening >10,000 hybridomas
vector	Lentiviral vector	Oncoretroviral vector	Lentiviral vector	Retroviral vector
Costimulatory domain	CD28	CD28 and OX40	CD28	CD28
Activation domain	CD3-zeta	CD3-zeta	CD3-zeta	CD3-zeta
MM cell lines	+++	+++	+++	+++
Primary myeloma cells	+++	+++		
Xenograft mouse model	+++	+++		+++
Toxicities	Preserves lymphocytes including virus-specific T cells	Works in $BCMA^{+}TACI^{-}$ or $BCMA^{-}TACI^{+}$ Dual antigen targeting		Without damaging normal hematopoietic cells
Clinical trial	started		Ongoing	