TITLE: Latest Developments in Cellular Therapy for Multiple Myeloma

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DISCLOSURES
For all authors: Research Funding: all >10k
Tara K. Gregory's institution receives research funds in her name from the following: Abbvie, Amgen, Acetylon, Bluebird, BMS, Celgene, Celularity, Constellation, CRISP Therapeutics, CURIS, EMD Sorono, Genentech, Glenmark, Janssen, Kesios, Lilly, Novartis, Poseida, Sanofi, Takeda, Teva, Vivolux

For Dr. Berdeja: Consultancy: all <10k
Institution received consultancy payments from the following: Amgen, BioClinica, BMS, Celgene, CRISPR Therapeutics, Janssen, Karyopharm, Kite Pharma, Legend, Prothena, SecuraBio, Servier, Takeda

COMPLIANCE with ETHICS
This article involves a review of the MM CAR T cell therapy via a PUBMED literature review, recent presentations at medical conferences and ongoing research at ClinicalTrials.gov. It did not involve any studies with human or animal subjects performed by any of the authors.

Peer Review: Double-blind peer review.

Authorship: The named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Received: July 9, 2020

Accepted: December 21, 2020

Published Online: December 23, 2020

Citation: *Oncology & Hematology Review (US)*. 2020;16(2): Online ahead of journal publication

Support: No funding was received in the publication of this article.

ABSTRACT

Novel drugs and continuous therapy have improved outcomes in patients with multiple myeloma (MM), however current therapies are largely non-curative with diminishing durations of response with subsequent therapies. Cellular therapies including chimeric antigen receptor (CAR) T cells have started to illustrate deeper and longer durations of relapse in the relapsed/refractory (RR) population. Current research seeks to expand on the durability of response, novel combinations and targets as well as the timing of CAR T in the treatment schema. This review summaries the updates in cellular therapy for MM.

KEYWORDS

Multiple myeloma, chimeric antigen receptor T cells, BCMA

INTRODUCTION

MM is a malignant clonal disorder of plasma cells in the bone marrow with more than 32,000 new cases expected in the United States per year [1]. Many clinical advances including novel drugs, continuous therapy with treatment sequencing have improved survival statistics [2]. Despite this, MM remains largely incurable and the relapsed/refractory population remains vulnerable despite numerous new therapeutic options [3, 4]. CAR T cell therapy offers early impressive results in the relapsed/refractory setting providing hope that these therapies and others like it may be heralding in a new era in the treatment of MM that may continue to move us closer to that elusive cure. CAR T studies focus on heavily pretreated MM patient with future studies focusing on its placement in the treatment sequence as well as optimum targets.

BACKGROUND ON CAR T

CAR T cell therapy combines optimal antigen binding of a tumor cell surface molecule and direct T cell activation inciting a targeted immune response as a novel class of “living” drug in malignancies. T cells are collected via apheresis and then genetically engineered to express an artificial receptor or chimeric antigen receptor (CAR). The CAR consists of an extracellular antigen recognition domain or targeting domain, a transmembrane domain and an intracellular domain to activate effector functions in the T cell [5]. Second and third generation CARs combine additional costimulatory ligands such as CD28 or 4-1BB signaling domains to induce cytokine production and enhance function, differentiation, and persistence of the adoptive T cell [6]. (See figure 1)

Multiple investigators concurrently working on cell therapy described a murine B cell malignancy model in which T cells were transduced via a retroviral mechanism to target CD19 [7]. Treatment moved on to human subjects with acute lymphoblastic leukemia (ALL) and advanced B cell malignancies. A phase 2 study using the anti-CD19 CAR T product tisagenlecleucel in pediatric and young adult patients with CD19+ relapsed and refractory B-cell ALL resulted in an overall remission at 3 months of 81% leading to

the product's FDA approval in August of 2017 [8]. In advanced B cell malignancies, a lympho-depleting conditioning regimen with fludarabine and cyclophosphamide followed by adoptive T-cell transfer in 22 patients showed an overall response rate (ORR) of 75% including 55% complete remissions (CR) and 18% partial remissions (PR) [9]. This was quickly followed with the FDA approval of axicabtagene ciloleucel in October 2017 after a phase 2 trial in 111 patients showed a 99% manufacturing success rate and an ORR of 82% [10].

MM BCMA CAR T TARGET

Anti-CD19 CAR T has been explored in MM in addition to aggressive B cell malignancies. Although MM cells express CD19 infrequently, CTL019 cells are cytotoxic at low levels of CD19 expression. At the University of Pennsylvania (UPENN), a compassionate use pilot study of CTL019 cells were infused day +12 after high-dose melphalan and autologous stem cell infusion. The patient did not develop CRS and obtained a CR [10, 11]. Despite this proof of concept, the majority of MM cells do not express CD19.

Since then there has been an explosion of CAR T therapies in MM. B-cell maturation antigen (BCMA) has been the most important target to date [12]. BCMA, a type III transmembrane protein, is universally expressed on the surface of malignant plasma cells and a small fraction of mature B cells, yet is not expressed on normal human tissue including primary human CD34+ hematopoietic cell. As a member of the tumor necrosis factor superfamily, its overexpression augments MM cell growth and survival serving as a receptor for B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) [13, 14]. BCMA maintains surface and intracellular expression through relapse, extramedullary spread and residual disease after treatment making it an attractive therapeutic target [15]. Preclinical results of anti-BCMA CARs transduced with lentiviral vectors led to specific killing of 2/3 of autologous primary MM cells leading to the first in human studies [16]. Other targets such as GPRC5D, SLAMF7 and CD138 are being developed as well but are well behind BCMA.

FIRST WAVE CAR T for MM

Initial clinical data

The first clinical data of a BCMA CAR T included 12 patients with median of 7 prior treatments treated with an anti-BMCA CAR containing a murine single-chain variable fragment (scFv), a CD8α hinge and transmembrane region, a CD28 costimulatory domain and a CD3ζ signaling domain. Patients were dosed from 0.3×10^6 CAR T cells/kg up to 9×10^6 CAR + T cells/kg. This resulted in 7 patients who achieved stable disease (SD), 3 with PR, 1 with very good partial remission (VGPR), and 1 with sCR. The 2 patients treated with the highest dose experienced complete and rapid elimination of bone marrow involvement from MM [17]. In total, 26 patients were enrolled on this study [18] with 16 at the highest dose level of $9x10^6$/kg preceded by a conditioning regimen of cyclophosphamide and fludarabine. An impressive ORR of 81% was reported with high peak blood CAR T cell levels were reportedly associated with response. Importantly, all 11 patients who obtained a greater than PR achieved minimal residual disease (MRD) negative status by flow cytometry. Cytokine release syndrome (CRS) with hypotension, hypoxemia,

fevers and tachycardia as well as coagulopathy and neurotoxicity with delirium was described with the highest doses similar to what was has been seen in previous anti CD19 CAR T cell trials. Six of 16 patients required vasopressors, but CRS was reversible in all cases. These positive results provided the foundation of future BCMA directed CAR T cell trials. (See Figure 2)

Ide-cel (Idecabtagene vicleucel, bb2121)

Ide-cel is a second generation CAR T product based the initial product utilized by the Kochenderfer team. It is an autologous T cell product transduced with a lentiviral vector encoding an anti-BCMA single-chain variable fragment, a CD137 (4-1BB) costimulatory domain, and a CD3-ζ signaling domain. (See table 1) In the phase 1 CRB-401 study, ide-cel was infused using doses of 50×10^6 up to 800×10^6 CAR-positive (CAR+) T cells. Responses were only seen at the 150×10^6 dose and higher leading to 150×10^6 up to 450×10^6 CAR+ T cells in the expansion phase. The 33 enrolled patients were heavily pretreated with a median 7 lines of prior therapies. The most common ≥ grade 3 toxicities were neutropenia (85%), leukopenia (58%), anemia (45%), and thrombocytopenia (45%). CRS was reported in 76% of patients, but mostly grade 1 or 2 and only 6% ≥ grade 3. Neurotoxicity occurred in 42%, mostly grade 1 or 2 and only 1 patient (3%) with a reversible grade 4 neurotoxicity. The ORR was 85%, including 45% with complete responses 40% of whom subsequently relapsed. The median PFS was 11.8 months (95% confidence interval, 6.2 to 17.8). All patients who responded with ≥ PR and who were evaluated for MRD-negative status ($\leq10^{-4}$ nucleated cells). Ide-cel expansion correlated with responses and interestingly responses were not BCMA expression dependent. CAR T cells subsisted up to 1 year after treatment [19].

These findings lead to the confirmatory pivotal phase II KarMMa trial of ide-cel for patients with relapsed/refractory MM (RRMM) [20]. (See Table 2) Patients who had ≥3 prior regimens (including immunomodulatory drug (IMiD), proteasome inhibitor (PI), and CD38 monoclonal Ab) and who were refractory to their last regimen per IMWG criteria were enrolled. After conditioning with cyclophosphamide and fludarabine, $150–450\times10^6$ CAR+ T cells were given to each patient. A total of 128 patients with a median age of 61 years received a median of 6 prior regimens, 84% were triple-refractory and 26% were penta-refractory. At median follow up of 11.3 month the ORR was 73% including 33% CR/sCR, with a median progression free survival (PFS) of 8.6 months. The responses appear to be dose dependent; the 150×10^6 dose induced 50% ORR with each 25% CR and VGPR, the median duration of response (DOR) was not reached and the PFS was 2.8 months. The 300×10^6 dose appeared to be more efficient with ORR of 69% including 29% CR, 14% VGPR and 26% PR, the median DOR being 9.9, and the PFS was 5.8 months. The 450×10^6 dose appeared to be the most efficacious with ORR of 82% including 39% with CR/sCR, 26% with VGPR and 17% with PR, the median DOR was 11.3 months and the PFS was 12.1 months. The median overall survival (OS) was impressive with 19.4 months for all patients and all subgroups, including older and high-risk patients, appeared to benefit equally. In 33 patients who achieved CR/sCR, 79% were MRD negative @10^{-5} by NGS. Cytopenias (97%) and CRS (84%) were common though CRS was mainly grade 1-2. The median onset to CRS was 1 day and only 7 patients had grade 3-5 CRS. Neurotoxicity developed in 18%, but only 3% had grade 3 and no grade ≥4 were reported. A median peak CAR+ T cell expansion occurred at 11 days and the expansion of CAR T cells was higher in responders. CAR T cells were detected in 59% of patients at 6

months and in 36% at 12 months post treatment. An additional cohort enrolled 3 patients at an 800× 10^6 dose with and ORR of 100% including 100% with CR and MRD negative [21].

Cilta-cel (ciltacabtagene autoleucel, JNJ-4528, LCAR-B38M/JNJ-68284528)

LCAR-B38M is an autologous CAR T product that also contains a 4-1BB co-stimulatory domain and a CD3-ζ T cell signaling domain but contains 2 BCMA-targeting single chain antibodies. (see Table 1) LEGEND-2 is a first-in-human, phase 1, single arm clinical trial conducted in RRMM performed at 4 centers in China using the LCAR-B38M construct [22]. The study enrolled 74 patients at 4 sites with 57 pts enrolling from one study site. These 57 patients had a median of 3 prior lines of therapy, 68% were PI exposed, 86% IMiD exposed, and 60% had both prior PI and IMiD exposure. Few were daratumumab exposed. Patients received single agent cyclophosphamide followed by 3 sequential infusions with LCAR-B38M at 20%, 30% and 50% of the total dose. Common adverse events (AEs) reported were fever (91%), CRS (90%), thrombocytopenia (49%), and leukopenia (47%). Grade ≥3 AEs were reported in 2/3 of pts, specifically leukopenia (30%), thrombocytopenia (23%), and increased aspartate aminotransferase (21%). CRS occurred in 90% of patients and was primarily grade 1-2 (82%); only 4 pts (7%) had grade 3 events and no grade 4/5 CRS was observed. Neurotoxicity was observed in only 1 patient (grade 1 aphasia, agitation, seizure-like activity). The median time to onset of CRS was 9 days and all but 1 CRS events resolved. The overall response rate was 88%, CR was 74%. Impressively median PFS was 19.9 months and 28.2 months for patients who achieved CR [23].

To confirm these results from LEGEND-2 in a more homogenous relapsed/refractory population, the phase 1B/2 dose confirming study CARTITUTE-1 was initiated. (see Table 2) Cilta-cel (JNJ-68284528, JNJ-4528) is identical to the CAR construct used in the LEGEND-2 study [24]. In the phase 1b portion of the study, 29 patients received cyclophosphamide and fludarabine lymphodepletion and then a single infusion at a target dose of $0.75x10^6$/kg. Patients were heavily pretreated with a median of 5 prior lines of therapy. Median time to CRS was 7 days which is different when compared with median onset of CRS from Ide-cel which is only 1 day. Only 10% of patients developed neurotoxicity, and only 3% ≥ grade 3. The ORR was 100%, 86% sCR, 97% ≥VGPR and 3% PR. The median time to ≥CR was 3 months and the 9 months PFS was 86%. Of 16 pts in CR, 13 were MRD negative by NGS at 10^{-5} and 11 at 10^{-6}. Interestingly, at 6 months follow up, 22/28 of patients had CAR+ T cells below the level of quantification in peripheral blood, suggesting CAR-T persistence may not correlate with depth of response to Cilta-cel [25]. Updated phase 1b/2 follow up data in 97 patients with a 12.4 month follow up showed an ORR of 96.9% and a CR rate of 67%. Median PFS has not been reached and low grade CRS occurred in 92% of patients still with a later onset of 7 days. New neurotoxicity was presented with 20.6% any grade neurotoxicity and grade 3 or greater at 10.3%. This was further divided into immune effector cell-associate neurotoxicity syndrome (ICANS) or other neurotoxicities such as neurocognitive changes, nerve palsy, and peripheral motor neuropathy. ICANS all grades was 16.5% and ≥3 2.1% with a median time to onset of 8 days with median duration of 4 days. Other neurotoxicities occurred any grade in 12.4% of patients with ≥ 3 at 9.3% with a late median time to onset of 27 days with a duration of 75 days [26]. Further phase 2 outcomes are eagerly awaited.

MECHANISMS OF RESISTANCE TO BCMA CAR T

Despite impressive response rates and durability in RRMM patients, the majority will eventually relapse. The mechanisms of resistance to BCMA-directed CAR T therapy are not well understood [27]. Unlike CD19 where up to 75% of relapsed patients had loss of expression of the CD19 antigen on target cells [10], BCMA loss appears to be relatively uncommon [17]. In the study of Ali et al [17], a partial loss of BCMA expression by malignant plasma cells was detected only in 1 of 12 patients and in the KarMMa trial only 2 patients who progressed were found to have lost BCMA expression on myeloma cells. This suggests that patients who progressed after BCMA targeted therapy may very well respond to alternate BCMA targeting therapy [28] and it is imperative these patients are not excluded from clinical trials directed at BCMA as is the current practice. Relapse appears to be occurring both in the setting of loss of CAR T cells and in the setting of persistence of CAR T cells. Thus various mechanisms appear to be at play including anti-BMCA CAR directed antibodies and off-target binding of BCMA CARs on soluble BCMA (sBCMA). Additionally reversible antigen loss through trogocytosis can occur by transferring the target antigen to T cells. This antigen loss leads to a decrease of target density on tumor cells and inhibits T cell activity via T cell exhaustion or even T cell killing. Furthermore, T cell exhaustion with chronic antigen exposure, overexpression of PD-1 post infusion leading to functional BCMA CAR T cell inhibition via PD-1/PD-L1 axis, and upregulation of tumor inhibitory signals have been postulated [17, 29-32]. As with prior therapies in MM, a combination approach seems to be necessary to best control the disease. Could the intrinsic heterogeneity of MM require this same paradigm be followed with CART therapy? Perhaps combinations with various immune-modulating therapies such as IMiDs and anti CD38 antibodies may ultimately be required. Perhaps this may be an opportunity where dual targeting CART products that may prove advantageous.

SECOND WAVE CAR T for MM

Several new generation products are now being tested clinically trying to build on the impressive results of the earlier products but in efforts to overcome some of the possible resistance mechanisms. The efficacy of gene transfer makes it feasible to express CARs in specific subsets of T cells. Central memory (Tscm) cells have superior proliferative capacity, longer telomeres, and improved survival after adoptive transfer compared to effector memory (TEM) and effector (TE) T cells [33]. Thus efforts to select for this T cell phenotype has been a goal and priority of the next generation of products.

Orva-cel (Orvacabtagene autoleucel, JCHARH125)

Orva-cel is an anti-BMCA CAR containing a lentiviral construct with a fully human scFv, optimized spacer, 4-1BB costimulatory and CD3-ζ activation domains. (see Table 1) It avoids off target binding with a low affinity for sBCMA, is active on target cells that express low BCMA density, minimizes tonic signaling to reduce antigen-independent exhaustion and the modified spacer increases engagement of the antigen binding to the scFv thus increasing cell kill [34]. It is manufactured to produce an equal CD4:CD8 ratio enriched for a central memory phenotype thought to increase persistence and durability. The phase 1/2 EVOLVE trial enrolled 62 RRMM patients with a median of 6 prior therapies at dose levels of $50x10^6$, $150x10^6$, $300x10^6$, $450x10^6$, and $600x10^6$. (see Table 2) At the 3 higher dose levels, cytopenias were common at all dose levels and 2 deaths occurred due to infection and grade 5 mast cell activation syndrome (MAS)/hemophagocytic lymphohistiocytosis (HLH). Overall CRS occurred in 89% of patients

with only 2 patients experiencing ≥ grade 3 and had a median time to onset of 2 days and a median time to resolution of 4 days. At a median follow up of 6.9 months, the ORR is 92% with 36% CR, 32% VGPR and 24% PR. At the $600x10^6$ dose level, 100% of patients are MRD negative @10^{-5} by NGS at 3 months. It has a 100% manufacturing success rate with robust expansion at all dose levels through day 29 with a trend towards increased expansion at greater dose levels and 69% CAR persistence at 6 months. High baseline levels of sBCMA did not impact orva-cel activity. Based on this data, the phase 2 study plans to move forward at the $600x10^6$ dose level [35].

bb21217

bb21217 is identical to ide-cel (bb2121) utilizing the same scFv, 4-1BB costimulatory domain and CD3-ζ activation domains with the exception of co-culturing with a phosphoinositide 3 kinase inhibitor (PI3K) bb007 during ex vivo culture to enrich the drug product for T cells displaying a memory-like phenotype. In preclinical models, both CARs eliminated MM tumors in mouse models, but the bb21217 product was able to prevent tumor growth when re-challenged with a second tumor without reinfusion of cells. The bb21217 CAR showed higher levels of CCR7 and CD27 suggesting a higher level of memory-like T cells and lower levels of CD57 which is a marker of T cell exhaustion [36, 37]. In the phase I study, dose levels include 150×10^6 (n = 12), 300×10^6 (n = 14), and 450×10^6 (n = 43). At a 23 month follow up of the first dosing cohort, the ORR is 83% and median duration of response is 11.1 [38]. A proprietary manufacturing change took place and at dose level 3, the original ORR was 60% with a CR rate of 32% and in the modified dose level 3 cohort, the ORR was 84% with a CR rate of 32%. All patients in CR were also MRD negative and duration of response in this cohort at 7 month follow up has not been reached. Increased Tscm expansion correlated with increase level and duration of response [39]. It will be interesting in subsequent follow up if apparent longer persistence translates to more durable responses.

P-BCMA-101

P-BCMA-101 utilizes an anti-BCMA Centyrin™ fused to a CD3ζ/4-1BB signaling domain with a larger range of binding affinities and smaller, more stable and potentially less immunogenic. The piggyBac™ (PB) DNA Modification System, as opposed to a viral vector, requires only plasmid DNA and mRNA producing an increased memory like phenotype population. The higher cargo capacity allows for incorporation of other genes, a safety switch that allows for rapid depletion of product in vivo if indicated by adverse events, and a selection gene that allows for enrichment of CAR+ cells [40]. A phase 1 dose escalation study from 0.75 to 15×10^6 P-BCMA-101 CAR-T cells/kg was conducted in patients with RRMM with only 1 patient experiencing low grade CRS in the first cohort. Longer term outcomes are still pending [41]. The PRIME phase 2 study of the P-BMCA-101 product evaluates outpatient infusion as well as novel design features [42]. A new manufacturing process utilizing a Nanoplasmid improved ORR at dose 0.75 x 106 from 66.7% to 50% and will be utilized as the manufacturing process moving forward.

CT053

CT053 is a second-generation CAR utilizing a fully human BCMA-specific single chain fragment variant (25C2) with high binding affinity. The CAR was initially studied in a single arm phase I investigator initiated program in Eastern China. A median 17.7 month follow up reported on 21 patients receiving the 1.5 x 10^8/kg dose level after standard LDC. CRS was low grade at 62.5% with a median onset of 3 days. Response showed an ORR of 87.5% with an impressive 79.3% CR rate. Median PFS was 18.8 months with improved DOR in patients without extramedullary disease (21.8 months vs. 10.3 months). CAR T persistence was noted up to 161 days and persistence correlated with response [44] . To confirm these results, Lummicar-2: a phase Ib/2 study is enrolling subjects who have received 3 or more prior lines of therapy including a PI, IMiD and an anti-CD38 antibody. Patients receive standard LDC with a single infusion of CT053 a dose level 0 targeted at 1.5 – 1.8 x 10^8/kg (N=8) and an escalated dose level 1 targeted at 2.5 – 3 x 10^8/kg (N=6). Manufacturing time was 8-10 days. 10 patients were evaluable at a median follow up of 4.5 months. CRS rates were low grade at 77% to 83% with a median onset of 2 to 4 days respectively. An ORR of 94% with CAR T expansion and persistence noted for up to 6 months. The DOR and depth of response continues to deepen over time.

FUTURE OF CART FOR MM

Incorporating CARs into Earlier Lines of Therapy

While the second wave of CAR T therapy in RRMM has focused mainly on product persistence to address durability of response, future directions are multifaceted. (See Table 3) Both ide-cel and cilta-cel are in the process of conducting studies in the earlier relapsed setting 1-3 priors. High risk MM is an attractive population in which to offer earlier CAR therapy given their significantly lower ORR, shorter median PS and OS compared to standard risk patients.

Bispecific CARs, CARs in Tandem, Novel Targets

MM is a disease of high clonal competition and as previously mentioned, antigen loss in a forthcoming clone can account for BCMA CAR T relapse. Therefore another approach to increase ORR and PFS in CAR T therapy is dual antigen targeting to mitigate antigen loss with treatment of different clones. Partnering BCMA targeting with anti-CD19 can trigger elimination of malignant cells as CD19 is expressed on both myeloma cells and their progenitors. GC012F, a first in human BCMA/CD19 dual CAR, was constructed by linking BCMA and CD19 scFv joined by a CD8 hinge, transmembrane domain, costimulatory domain and CD3 ζ. Patients have been enrolled at 3 dose levels with a primary end point of dose limiting toxicity of GC012F and secondary endpoints of MRD at 3 and 6 month infusion, ORR, PFS, OS and DOR. 16 patients have been enrolled including 6 patients at the third dose level of 3 x 10^5/kg cells. The ORR of the study is 93.8% at 6 months, 100% MRD negative at 3 and 6 months at dose level 3 and no DLTs. Low CRS grades is present at 87.5% with a median onset of 6 days [46].

Others have looked at using unique antigen targeting CARTs in tandem dosing to address higher risk populations. A single-center, single-arm phase 2 feasibility study enrolled 21 patients received

lymphodepleting chemotherapy followed by humanized anti-CD19 CAR T cells on day 0 and split dose murine anti-BCMA CAR T cells on days 1 and 2. At a median follow up of 179 days, the 20 of 21 patients showed a response to treatment [47]. Subsequently, a combined infusion has been escalated to 10 patients with R-ISS III disease with tandem autologous transplantation and combined infusion of CART-19 and CART-BCMA cells post-transplant as consolidation treatment. CRS occurred in all patients, limited to grade 1 and 2 and 100% of patients achieved VGRP at 100 days post ASCT [48]. Another trial is treating high risk patients to consolidate their first or second line of therapy with BCMA-CART plus or minus huCART19 [21]. Non-BCMA targets, other than CD19 are also being investigated including products directed at CD38, CD138, CS1/SLAMF7, APRIL and GPRC5D are being assessed (See Table 3) [49-54].

Gamma secretase inhibitors (GSI)

A novel CAR product seeks to prevent MM relapse post CAR T infusion utilizing GSI drug infusions with an anti-BCMA CAR. GSIs increase BCMA surface density and decrease sBMCA thus theoretically increasing the efficacy of the anti-BMCA CARs. Patients received GSI (JSMD194) monotherapy administered every 48 hours over 5 days times three doses prior to lymphodepleting chemotherapy and then infusion of a fully humanized BCMA CAR in combination with JSMD194 dosed three times a week for three weeks, starting on the day of CAR infusion. The run-in dosing of JSMD194 increased plasma cell BCMA expression from 75% to 99% and soluble BCMA decreased by 2.0 fold. BCMA antigen binding capacity increased a median of 20-fold and best overall response rate was 100% in 6 evaluable patients [51].

Allogeneic CAR-T cells

Until now, the majority of CAR T products have been autologous. There are several issues that arise as a result including: insufficient collection of T cells, failure of engineering BCMA CAR T cells, suboptimal T cell substrate, and prolonged time period between collection and infusion of cells. Allogeneic CAR-T cells may be able to overcome these issues by providing readily available off-the-shelf CAR T Cells without need of prior autologous collection. New issues introduced by the use of an allogeneic product is the potential for GVHD and lack of persistence. There are several products currently in development and in early clinical trial. First studies are now open to enrollment evaluating safety of the allogeneic CAR T cell products targeting Myeloma cells. UCARTCS1 targets the antigen CS1 whereas the products BCMA-UCART, ALLO-715, CTX120 and PBCAR269A are all allogeneic BCMA CAR T cell products that are currently being tested in phase 1 trials [49, 50, 55-57]. Anti-BCMA ALLO-715 is an off the shelf CAR T with a 4-1BB costimulatory and CD3-ζ activation domains. The TCR alpha constant gene is disrupted to decrease GVHD risk and the CD52 gene is disrupted to use ALLO-647 (an anti CD52 mAb) for selective and prolonged host lymphodepletion. The primary endpoint of tolerability with 31 patients with an average of 5 prior lines of therapy with all refractory to last line of therapy and 94% penta refractory patients showed low CRS rates (45%). Response and durability of the product were dose dependent and at the third dose level (3.2×10^6 cells), the ORR was 60% with 40% in VGPR or better [58].

NK CAR-T cells

Natural killer (NK) cells can identify and kill malignant cells in the absence of antibodies and major histocompatibility complex molecules. CAR NK cells are commonly engineered from donated umbilical cord blood NK cell lines such as NK-92, or induced pluripotent stem cells [59]. One of the possible concerns is the short in vivo persistence, though it is unclear if persistence of a CAR cell product is really necessary to induce a sufficient response as seen in the CARTITUDE-1 study where loss of persistence of CAR T cells did not correlate with lack of response, thus it is quite possible that a brief and rapid onset of killing might be sufficient to induce a durable response. Compared to CAR T-cell therapy, the potential benefits of CAR NK cell therapy include “off-the-shelf” availability and a potentially low toxicity profile, thus it might be advantageous to perform repeated and frequent dosing with these cell products similar to bispecific antibodies [60]. Preclinical data show that genetic modification of NK-92MI cells with an anti-CD138 CAR enhances the cytotoxicity and potentiates the anti-myeloma effect with NK cell-based therapy. Both NKG2D-CAR NK cells and BCMA-CAR NK cells also appear to be equally efficient to eradicate MM cells [61]. A phase 1 CAR NK cell trial targeting BCMA for relapsed/refractory Multiple Myeloma using adoptive BCMA CAR-NK 92 product is now open for enrollment [62].

CONCLUSION

CAR T cell therapy in the vulnerable relapsed refractory MM patient population is showing unprecedented responses with single treatments of a biological agent as opposed to continuous therapy. Future directions are multifocal with increasing the quality of CAR products in terms of T cell persistence with memory-like phenotypes, optimum CD4:CD8 ratios and multi-antigen products as well as timing of therapy in the treatment sequence. As these patients progress on various treatments, they do decrease their T cell populations. This begs the question of off the shelf CAR Ts from optimal donors

or more provocatively placing CAR T cell therapy earlier in the sequence of treatment take advantage of a more robust host T cell population for the potential of cure. Additional modifications to the CAR T product such as bispecific targeting or novel combinations with IMIDs, monoclonal antibodies and other anti-MM drugs. Moving CARs to the outpatient setting is also part of the next frontier of cellular therapy demanding outpatient management and prevention of CRS. Despite advances in treatment, there are still socioeconomic barriers to treatment for advanced therapies for RR MM. Future treatment will not only depend on the CAR T product manufacturing success and treatment response, but patient access as well.

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Figure 1: Chimeric Antigen Receptor Structure

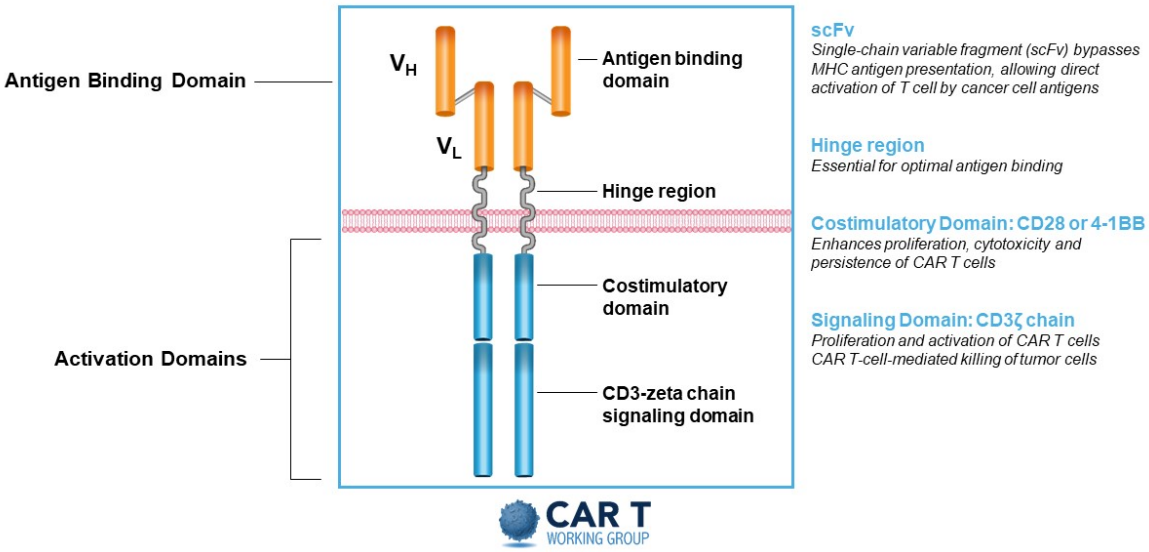

Figure 2: MM CAR T Studies and Timeline

Timeline of Earliest and Most Advanced Products

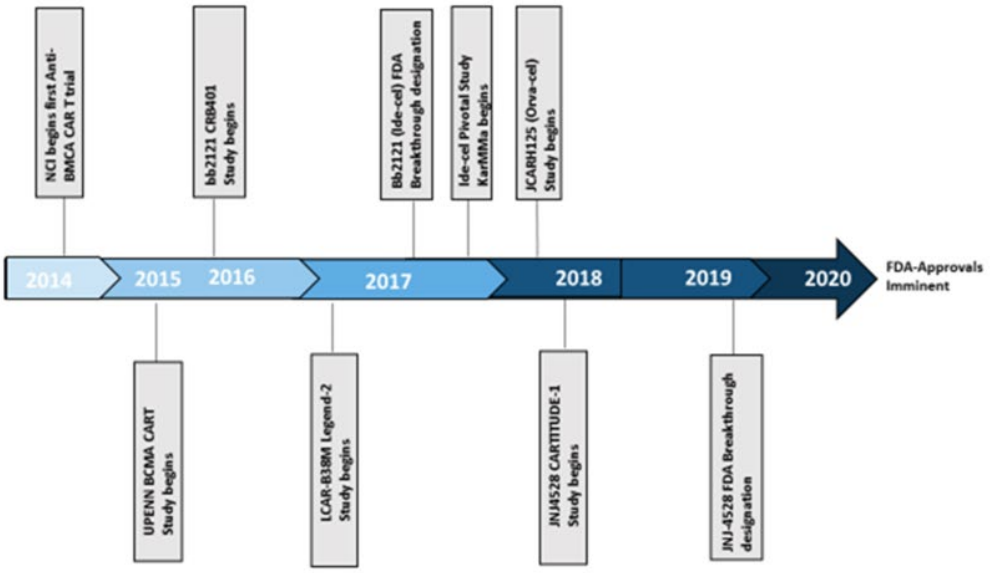

Table 1: Characteristics of Leading BCMA-DIRECTED CAR T Cells in MM

	Ide-cel[20]	Orva-cel[34]	Cilta-cel[26]
Nomenclature	**Bb2121** **Idecabtagene vicleucel**	**JCARH125** **Orvocabtagene autoleucel**	**LCAR-B38M** **JNJ-68284528**
Target	BCMA	BCMA	BCMA
Ag-binding domain	scFv (M)	scFv (H)	2-VHH (C)
Vector	Lentiviral	Lentiviral	Lentiviral
Costimularoty Domain	CD3/41BB	CD3/41BB	CD3/41BB
Special Qualities		-Modified spacer to enhance binding -Designed to deliver purified CD4+ and CD8+ CAR T product enriched for central memory phenotype	2 BCMA binding domains to enhance avidity

Abbreviations: BCMA, B cell maturation antigen; scFv, single chain variable fragment; M, murine; H, human; VHH, variable heavy chain; C, camelid

Table 2: Summary of completed/ongoing pivotal trials for the 3 lead products

	Ide-cel[20]	Orva-cel[34]	Cilta-cel[26]
Study	**KarMMa**	**EVOLVE**	**CARTITUDE-1**
# of patients treated	**128**	**62**	**97**
Population	**Relapsed/Refractory**	**Relapsed/Refractory**	**Relapsed/Refractory**
# Prior Treatments	**6**	**6**	**6**
CAR+ T Cell Dose	**150-450 $x10^6$**	**300-600 $x10^6$**	**0.75 $x10^6$ /kg**
ORR	**73% (82%@ 450$x10^6$)**	**92%**	**94.8%**
CR	**33% (39% @ 450$x10^6$)**	**36%**	**67%**
MRD negative (≥10-5 by NGS)	**79% (33 pts in ≥ CR)**	**84% (≥PR at month 3)**	**94% (of 52 patients evaluable)**
CRS All Grades (Grade 3/4)	**84% (5%)**	**89% (3%)**	**92% (5%)**
Neurotoxicity All Grades (Grade 3/4)	**18% (3%)**	**13% (3%)**	**20.6% (2.1%)**
Med Time to CRS	**1 day**	**2 days**	**7 days**
Med PFS	**8.8m (12.1m @ 450$x10^6$)**	**Not reported**	**76.6% @ 12mos**
Med OS	**19.4m**	**Not reported**	**Not reported**
CAR T Persistence	**59% at 6 months 36% at 12 months**	**69% at 6 months**	**67% at 6 months**

Abbreviations: ORR, overall response rate; CR, complete response; MRD, minimal residual disease; CRS, cytokine release syndrome; PFS, progression-free survival; OS, overall survival

Table 3: Future directions

TIMING	Earlier lines of therapy Front-line in high risk disease or as consolidation/maintenance
NOVEL CONSTRUCTS	Bispecific CARs, suicide genes, safety signals, PD-1 knockouts
COMBINATIONS	GSI, IMiDs, check point blockade, vaccines, monoclonal antibodies, cytokines, multiple(tandem) CARs
ALTERNATE TARGETS	CD38, CD138, CS1/SLAMF7, APRIL, GPRC5D
ALLOGENEIC DONOR	"Universal" or "off-the-shelf" CAR T cells
ALTERNATE CELLS	Natural killer cells

Abbreviations: CAR, chimeric antigen recetor; GSI, gamma secretase inhibitor; IMiDs, immunomodulating drugs