Recent advances of FDA-approved CAR-T therapies in multiple myeloma

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Abstract. For a long time, malignant blood cancers faced great difficulty in development of successful treatments due to their mobility and evasive nature. Of these conditions, multiple myeloma (MM) is an untreatable cancer due to its highly relapsing and refractory nature, which will eventually dissipate all efforts in controlling the disease. Previous treatments only control the progression of myeloma to an extent and prolong patients' lives shortly. Thus, multiple myeloma patients are in dire need of new treatment options to prevent or postpone the eventual relapse. The discovery and development of CAR-T therapy show promising results for MM treatment. Recently approved therapies by the FDA, Abecma and Carvykti, displayed high response rates with low relapses in patients who underwent the drug trials. However, therapeutic applications of CAR-T have encountered various obstacles. The treatment is largely associated with cytokine release syndrome and other adverse events, ranging from systematic to organ toxicities. In addition, specificity and cost are pressing issues that seek solutions. Despite difficulties, many CAR-T options targeting MM are under active research and investigation. With further development and optimization in additional drug trials, the application of CAR-T therapy can offer a new approach to controlling multiple myeloma for those suffering from drug resistance.

Keywords: CAR-T, Clinical, Treatment, myeloma.

1. INTRODUCTION

In recent years, advancements in immunotherapy have provided new treatment options for cancer patients who were previously untreatable with the available technologies. Of these malignant conditions, one disease with high mortality remained incurable; that is, multiple myeloma [1]. The condition accounts for 25% of all lymphohematopoietic cancer (LHC) deaths, and 2% of all cancer cases in the US [2]. Multiple myeloma is a disorder derived from the abnormalities in the maturation of B-cells. Following an adaptive immune response, some of the B-cells will differentiate into plasma cells, which are responsible for the production of antibodies for pathogen neutralization [3]. However, mutations in various oncogenic loci can result in myeloma cell development [4]. The condition stems from the uncontrolled proliferation of plasma cells, particularly in the bone marrow where B-cell maturation occurs [2,4]. Excessive cell growth results in cell tropism in the marrow and hinders normal B-cell development. Furthermore, the antibody-secreting character of plasma cells can lead to increased M protein production of myeloma cells after they have undergone pathogenesis [5]. This can lead to increased immunoglobulin levels circulating in the bloodstream, resulting in chronic stimulation of the immune system.

Like other patients with malignant blood cancers, the first treatment option for MM often involves chemotherapy. These high-dose and cytotoxic chemotherapies rely on the cancer cells’ inability to conduct normal cellular repair mechanisms, such as DNA double-strand repairs [6]. However, the chemicals often exert a toll on healthy cells, as their actions are non-specific. Thus, various methods with higher MM specificity and less cytotoxicity were developed. These options include inhibitors to the 20S proteasome subunit, which is crucial for myeloma cell proliferation [7]. Antibody-drug conjugates allowed improvements in the targeting of MM cells [8]. Furthermore, hematopoietic stem cell (HSC) replacement therapy also offers a non-cytotoxic treatment method, as its efficacy lies in inducing differentiation of healthy B cells lacking the oncogenic mutations [8]. While these methods are rapidly evolving, most MM patients suffer from the refractory and relapsing effects of the
condition. However, the development of CAR-T has provided a new and effective option for those suffering from blood cancers.

First designed in 1993, the emergence of CAR-T therapy marked a milestone for cancer immunotherapies. The original CAR-T has undergone multiple development cycles, leading to the current 5th generation chimeric antigen design [9]. The method requires the isolation of T-cells from the patient’s blood, upon which the CAR transgene will be transduced via a retroviral vector [10]. The genetically engineered T cell will thus express the CAR receptor designed to have specificity against its target. While effective, CAR-T therapy harbours many risks to the host once transfused back to the patient’s body. Being live cells, they are able to produce various signalling molecules and secrete them into the bloodstream for circulation [11]. Very often, CAR-T cells disrupt normal immune system functions by secreting pro-inflammatory cytokines. This phenomenon is known as the cytokine-release syndrome, and it constitutes one of the most dangerous side effects of CAR-T therapy [12]. Overproduction of pro-inflammatory cytokines can initiate a prolonged, hyperreactive innate immune response involving many different cell types [13]. For example, the pro-inflammatory cytokine IL-1 and TNF-α polarize macrophages into a pro-inflammatory phenotype, whose overactivation can lead to tissue damage [14]. In addition, while displaying promising results in targeting blood cancers, CAR-T treatments show reduced efficacy against solid tumours [15]. Therefore, CAR-T therapies are still under active research and optimization to minimize side effects.

In 2022, the US FDA issued approval for Ciltacabtagene autoleucel (cilta-cel) under the trademark name CARVYKTI. The success of a second CAR-T therapy option for MM following Abecma is encouraging to the field of CAR-T research. The approval of Carvykti represents the feasibility and great potential for CAR-T based therapies for MM. This review focuses on the research and development of Carvykti, as well as the prospects and limitations of CAR-T for malignant blood cancers.

2. The immune system and CAR-T

The human immune system is an intricate and powerful defence against pathogens and malfunctioning cells. The immune system is divided into two sub-categories: the innate immune system and theadaptive immune system, where the former offers rapid clearing of pathogens, and the latter develops a systematic defence that results in the formation of memory cells [16]. Both systems have distinct characteristics yet are connected in various pathways where the innate immune response can lead to the activation of the adaptive response.

T cells are derived from the lymphatic precursors and matured in the thymus. Once matured, the naïve T cells circulate in the body [17]. The circulation promotes contact with antigen-presenting cells (APC) such as dendritic cells, which could in turn activate the T cell [18]. T cells exist in two forms based on their T cell receptor (TCR) type: the αβ T cells and the γδ T cells [17]. The αβ T cells have two subtypes: the CD4+ T cells which differentiate into helper T cells and Treg cells, and the CD8+ T cells which execute cytotoxic abilities [18]. The activation of T cells requires three signals. First, the TCR needs to interact with the major histocompatibility complex (MHC) on APCs. Second, the co-stimulatory signal through the engagement of CD28 on the T cell and B7 on dendritic cells. Without the co-stimulatory signal, the T cell enters a stage of anergy. The third signal is subset-dependent, where different cytokines provided by the dendritic cell polarize the CD4+ T cells into a variety of subtypes, some of which can produce signals such as interleukin-2 (IL-2) to activate the CD8+ T cells [18].

In order to mount an effective immune response against an antigenic particle, the immune cells need to initiate a sequence of signalling processes. These characteristic signal-dependent responses are therefore utilized in the design of CAR-T cells. These T cells are engineered from the somatic T cells of the patient, in which the gene encoding for the CAR construct is transduced using a lentiviral vector [19]. Since the T cells are autologous, the effect of graft responses is lower. Laboratory engineered CAR protein usually consists of multiple domains for effective signal transduction. The
A construct has its recognition domain, usually a single chain fragment of variable region (scFv) linked to the transmembrane and cytosolic region. The cytosolic domains consist of a direct signalling domain, such as CD3ζ, and a co-stimulatory domain for activation of other TCR-related pathways [19]. This provides sufficient stimulatory signals in the process of T cell activation, which allows effective signal amplification and induction of their cytotoxic abilities upon receptor binding. Once the extracellular domain of a CAR construct is bound to the recognized epitope, the intracellular domain would elicit a cellular response to initiate the T cell’s cytotoxic effects (Figure 1) [19], [20].

**Figure 1.** CAR-T exerts its cytotoxic abilities upon CAR-BCMA engagement when targeting myeloma cells.

CAR-T therapies have undergone rounds of development, where the 4th and 5th generation CAR is being studied [9]. The first generation includes the scFv domain for recognition and a single CD3ζ domain for intracellular signalling. Second-generation CAR is optimized through the incorporation of a co-stimulatory domain, most commonly CD28 and 4-1BB (CD137). This allows more efficient T cell activation, as the co-stimulatory signal is crucial in native T cell activation during defence against pathogens. Later generations of CAR are largely designed based on the core structure of a second-generation construction. The third-generation CAR consists of two co-stimulatory domains compared to the single one in the second generation [21]. Fourth-generation CAR-T (TRUCKs, T cells redirected for antigen-unrestricted cytokine-initiated killing) cells require two transgenes: a CAR construct and an inducible gene, named the nuclear factor of the activated T cell (NFAT)-responsive cassette [10]. Upon CAR-activation engagement, signalling through CD3ζ activates the NFAT promoter, inducing the expression of the cytokine transgene. Studies have reported that co-transduction with an inducible cytokine such as IL-12 enhances T cell cytotoxicity in treating solid tumours. In addition, the approach reduces the instances of systematic toxicity, as the release of IL-12 is temporally and spatially restricted (Figure 2) [22].

**Figure 2.** 1st to 4th generation CAR designs.

Additional CAR designs are currently being explored. For example, the 5th or next-generation CAR includes the incorporation of cytokine receptors such as IL-2R. This facilitates the activation of additional cellular signalling pathways (e.g., JAK-STAT pathway), allowing control of CAR-T activation in an antigen-dependent manner [9].
3. Current FDA-approved CAR-T therapies for multiple myeloma

3.1. Abecma

The success of CAR-T usage in treating acute lymphoblastic leukemia has shed light on CAR-T based therapies for other malignant blood cancers [19]. Since 2013, BCMA-targeting CAR-T cells for multiple myeloma treatment have been undergoing active research and clinical trials. In 2017, the drug named Idecatagene Vicleucel (ide-cel; bb2121) designed by Bluebird Bio was granted the breakthrough-therapy designation by the US FDA [23]. The treatment then received approval from the FDA in March 2021. Ide-cel, now sold under the trademarked name Abecma, represents the hallmark in CAR-T therapy development for refractory and relapsing multiple myeloma (R/R MM). The CAR construct of ide-cel utilizes a second-generation CAR, with a murine anti-BCMA scFv domain for recognition. The intracellular domain contains a 4-1BB co-stimulatory domain and a CD3ζ activating domain for cellular signalling [23]. A variant of ide-cel (bb21217) was also developed by Bluebird bio based on the same construct. However, this CAR design consists of a phosphoinositide 3-kinase (PI3K) inhibitor. The purpose of this construct was to enrich a memory-like phenotype in T cells, which could increase in potency and persistence in the body [24].

3.2. Carvykti

A second treatment against MM using CAR-T technology was approved by the FDA in February 2022. Citlacatagene Autoleucel (cilta-cel), with the product name of Carvykti, provided a new option for R/R MM patients. The CAR construct was a product based on LCAR-B38M first trialled under the name LEGEND-2 in China. After preliminary efficacy evaluation, LCAR-B38M was produced by Johnson & Johnson, which subsequently underwent a phase 1b/2 trial named CARTITUDE-1 and a phase 2 trial named CARTITUDE-2 in the U.S. to further assess its safety [25]. Different from Abecma, Carvykti consists of a bispecific CAR construct in the extracellular domain [23]. The CAR possesses two llama-derived antibody domain that allows targeting of two different BCMA epitopes. This enhances the recognition of the CAR in both specificity and binding affinity, allowing the CAR-T cells to function with greater efficiency [23].

3.3. Comparison

Despite the differences in the epitope-recognition domains, the CAR for both Abecma and Carvykti consists of a TCR subunit CD3ζ signalling domain and a 4-1BB co-stimulatory domain instead of the native. In T cells, CD3ζ serves as the signalling domain of TCRs, owing to its long immunoreceptor tyrosine-based activation motifs (ITAMs) [26]. A co-stimulatory domain brings the co-receptor-associated kinase Lck in proximity, which double phosphorylates the ITAMs [26]. Phosphorylated CD3ζ ITAM now provides a docking site for the zeta-chain-associated protein kinase 70 (ZAP70). Upon binding ITAM with its tandem SH2 domains, ZAP70 activates the linker for activation of T cells (LAT), which promotes the activation of multiple signalling pathways [27]. The incorporation of the 4-1BB domain allows T cell activation to exhibit a memory cell-like phenotype, prolonging CAR-T effectiveness [28]. Studies have shown CAR-T cells exhibit an enhanced survival and expansion when co-stimulated with 4-1BB compared to CD28. This allows prolonged effects of CAR-T when transfused into the patient, which offers lengthened protection against myeloma proliferation [28], [29]. Therefore, the design allows effective and prolonged T cell activation upon engagement of CAR with BCMA.

The overall response rate (ORR) of Abecma was reported to be 73% (94 out of 128) after 13.3 months, where 33% (42 out of 128) showed a complete response (Table 1) [30]. The minimal residual disease (MRD) negative status, represented by the presence of less than 10-5 nucleated disease cells was assessed, and 26% of all patients were confirmed to satisfy this status. Of the 42 patients who had shown a complete response, 79% were confirmed to be MRD negative. The overall survival rate was 78% at 12 months [30]. The ide-cel variant consisting of a PI3K inhibitor domain does not show enhanced response rate, where the ORR is 55%. However, peripheral blood sampling of the patients
showed T cells with a more proliferative, less senescent phenotype, suggesting a potentially prolonged response [24].

Carvykti, under evaluation of the study CARTITUDE-1 showed a 97% ORR 12 months post-infusion, and the stringent complete response was 67%. Using the same criteria of MRD assessment as Abecma, 93% of the patient were MRD negative [25]. The survival rate also showed promising results, where 89% of the patients survived with 77% not experiencing any relapsing myeloma in the course of the study. The response was found to deepen over time, and at time of completion of CARTITUDE-1, the median response time was not reached, suggesting a prolonged, strong CAR-T reaction [25].

Table 1. Overview of clinical trial results of Abecma and Carvykti

| Drug name | Clinical data | Main side effects (Any grade) | Main side effects (Grade 3-4) |
|-----------|---------------|------------------------------|------------------------------|
| Abecma    | ORR 73%       | Any 100%                     | Any                          |
|           | 33% CR 91%    | Hematological Neutropenia    | CRS                          |
|           | 26% MRD 26%   | Anemia 70%                   | Hematological Neutropenia    |
|           |               | Gastrointestinal Diarrhea 35%| Diarrhea                     |
|           |               | Nausea 29%                   | Crasiteanization 89%         |
|           |               | Hypokalemia 35%             | Anemia 60%                  |
|           |               | Fatigue 34%                 | Hypokalemia 2%              |
|           |               | Hypophosphatemia 30%        | Hypophosphatemia 16%        |
|           |               | Hypocalcemia 27%            | Hypocalcemia 8%             |
|           |               | Pyrexia I 25%               | Pyrexia 2%                  |
|           |               | Neurotoxicities 18%         | Neurotoxicities 3%          |
| Carvykti  | ORR 97%       | Any 100%                     | Any                          |
|           | 67% CR 95%    | CRS                          | CRS                          |
|           | 93% MRD 93%   | Hematological Metabolic I.   | Hematological                |
|           |               | Gastrointestinal Fatigue 37%| Metabolic 16%               |
|           |               | Cough 35%                   | Gastrointestinal 8%          |
|           |               | Aspartate AT increase 29%   | Cough 5%                    |
|           |               | Alanine AT increase 25%     | Fatigue 4%                  |
|           |               | Neurotoxicities 21%         | Cough 0%                    |
Both treatments elicit the common side effects of CAR-T therapy. All patients treated with Abecma experienced adverse events, with 99% of the patients experiencing that of grade 3 or 4. 91% of the patients had neutropenia, with 89% being grade 3 or 4. Cytokine release syndrome (CRS) was also observed in 84% of the patients, albeit mostly grades 1 and 2 [25], [30]. A similar phenomenon was present in Carvykti treatments, where 100% of the patients experienced adverse effects. 92% of the patients had neutropenia, with 95% being grade 3 or 4. In addition, 95% of the patients (92/97) experienced cytokine release syndrome, but only 4 out of the 92 were grade 3 or 4 [25], [30]. Other common side effects for both treatments include additional hematological, metabolic, gastrointestinal events, etc.

Various other anti-BCMA CAR-T therapies are undergoing phase 1 or phase 1/2 clinical trials. For example, JCARH125 developed by Juno Therapeutics, CT053 by CARsgen Therapeutics, and MCARH171 by Poseida Therapeutics show promising results in their initial trials. The majority of new CAR-T options use the second-generation CAR design, while some are based on third- or fourth-generation CARs [23].

4. Other treatments for multiple myeloma and comparison with CAR-T

There have been multiple treatments developed for multiple myeloma, of which the majority involve proteasome inhibitors and immunomodulators. Proteasome inhibitors mainly target the 20S subunit of MM proteasome, leading to a hindrance of NF-KB activation by growth factors and cytokines [7]. The inhibition could also exert oxidative stress and ER stress, which induces the production of pro-apoptotic proteins, leading to tumour cell death. Common proteasome inhibitors include Bortezomib, Carfilzomib, Ixazomib, etc. [7]. While proteasome inhibitors are effective, specificity is a common issue that can be attributed to many side effects such as cardiotoxicity and a decrease in blood cell counts [7].

Immunomodulators are also important in the MM treatment regimen, as MM is often associated with compromised immune systems. The aggregation of myeloma cells in the bone marrow impairs B cell differentiation, which weakens the adaptive immune responses [31]. Commonly used immunomodulators include Lenalidomide and Pomalidomide. These drugs promote natural killer (NK) cell-mediated cytotoxicity on plasma cells [7]. Pomalidomide can also decrease the osteoclast activity in bone, which relieves the lytic bone disease associated with MM. Other treatments such as glucocorticoids (Dexamethasone) and histone deacetylase inhibitors (Panobinostat), are often used in combination with other treatments [7]. Anti-CD38 antibody treatments are also promising in MM treatments [32].

MM patients are usually assessed for eligibility for autologous stem cell transplantation (ASCT) once they are diagnosed. This eligibility will determine the composition of the therapeutic regimen [33]. MM patients often receive combinational therapy consisting of many cycles of treatment, particularly due to the refractory and relapsing properties of MM. If the patient is eligible for ASCT, they will receive 3-4 rounds of induction treatment using common initial therapy combinations [33]. These usually include Lenalidomide, Dexamethasone, and Bortezomib [33]. Following treatment, hematopoietic stem cells will be harvested and transplanted. As the transplantation occurs post initial treatments, the tumour burden would be decreased and grafting enhanced [32]. However, ASCT is not curative, as it only improves median overall survival [32]. Patients ineligible for ASCT would undergo 8-12 cycles extending the original induction therapy [33].

Despite efforts, all MM will eventually relapse. Patients are usually put on different combinations of drugs to prolong their lifespan. Relapses occur after a remission period of a few months to years,

|          | %  |
|----------|----|
| Aspartate AT increase | 5% |
| Alanine AT increase   | 3% |
| Neurotoxicities      | 9% |
where the patients eventually develop resistance to commonly used treatments. Therefore, the development of CAR-T therapies granted great hope for MM patients, for the CAR-T responses are robust and prolonged. Studies with Abecma and Carvykti also showed promising response rates and negative MRD after initial treatment, which could greatly lengthen the patients’ lifespan with repeated applications.

5. Limitations and future development

While CAR-T therapies are effective against multiple myeloma and other blood cancers, the side effects associated with the treatment pose risks to the patient. Cell toxicities from CAR-T treatments range from constitutional to organ-specific effects, of which the severity varies from fatigue and fever to respiratory failures [11]. While most effects are reversible, the adverse events mark great limitations to the wide application of CAR-T based therapies. Most often, CAR-T is used as the third or fourth line of therapy provided if previous treatments fail.

The toxicity can be attributed to various factors. CAR-T cells actively produce multiple cytokines which can elicit a prolonged immune response, such as the pro-inflammatory cytokines IFN-γ, IL-1, IL-2, tumour necrosis factor (TNF)-α [34]. The activation can result in uncontrolled inflammation that may potentially damage the tissues. Several complications tied to neurotoxicity were also found, for instance, delirium, seizures, and motor dysfunctions [11], [34]. While this pathogenesis is largely not understood, these adverse events are almost exclusively associated with the occurrences of CRS, indicating the complicated nature of CAR-T induced toxicities [34].

Various optimization strategies are underway for CAR-T therapies. Studies have proposed potential amelioration for CAR-T associated toxicities by altering CAR structure [35]. These options include decreasing the antigen-binding affinity of the CAR, tailoring the co-stimulatory domain based on tumour type, and using human-derived antibody fragments instead of animal-derived [36]. Co-administration of CAR-T with drugs can also ease the inflammatory responses. For example, lenzilumab controls the macrophage and monocyte activating cytokine GM-SCF. This decreases CRS and the associated neurotoxicity [36]. Other options involve the construction of an “off-switch” in the CAR, usually through the production of a CD20 surface antigen. Upon extreme adverse events, rituximab can be administered to trigger cell death through association with CD20, facilitating the depletion of transfused CAR-T cells [11].

Additional CAR-T optimizations involve enhancing their functionality, such as reducing the instances of antigen escape, on-target off-tumour effects, and more efficient delivery. Antigenic specificity is usually enhanced by the usage of tandem CARs, as seen with the design of Carvykti [35]. On-target off-tumour effects are characterized by the targeting of healthy cells possessing the same targeted epitope as the tumour cells. Prevention tactics involve investigation of tumour-specific post-translational modifications and designing CARs targeting these characteristic domains [36]. The delivery of CAR-T is a crucial aspect in regard to advancement in treating solid tumours. Currently, most CAR-T options are designed for treating blood cancers, as CAR-T exhibit poor effects on tumour infiltration. However, studies have shown engineering of CAR-T with heparinase or fibroblast expression enhances their abilities to penetrate physical barriers, such as those created by the tumour stroma [36]. Recently, a study reported the usage of hydrogel in CAR-T injection showed an enhanced effect on solid tumour targeting and elimination. The injection provides a hydrogel niche, allowing maximized activation and effectiveness of CAR-T cells [15]. These advances provided new prospects for CAR-T based therapy, and additional ongoing investigations for optimizing CAR-T will better the treatment into a mature therapy.

6. Conclusion

The advancements in immunotherapy reformulated clinical treatments for cancer. Of these therapy options, CAR-T offered a novel approach for various malignant blood tumours, such as acute
lymphoblastic leukemia and non-Hodgkin lymphomas (NHL). Recently, the research and development of CAR-T against multiple myeloma have undergone massive progress, with two drugs receiving FDA approval. The administration of Abecma or Carvykti showed effective control of myeloma progression by drastically reducing the tumour cell counts, and the side effects were within the prediction. With a response rate close to 100%, CAR-T therapy provided a viable option that can counter this relapsing and refractory disease. Furthermore, various additional therapies being subjected to ongoing research or early clinical trials showed promising results against multiple myeloma. While effective, CAR-T is associated with a wide range of toxicity to the hosts despite being autologous. The adverse events can range from mild fever to severe respiratory failures, which hinders the wide application of CAR-T. Currently, many optimizations are underway to minimize damage to the host. These include increasing specificity, decreasing affinity, and co-administration of drugs to temper the overreacted immune responses. Furthermore, research is underway to improve CAR-T effectiveness against solid tumours for a wider range of therapeutic targets. The efforts combined will promote discoverers of the full potential of CAR-T, which can revolutionize cancer treatments.

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[37] Figures were created with biorender.com.