Book Chapter

CAR-T Therapy, Where Do We Stand Now?

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Abstract

Chimeric antigen receptor-T (CAR-T) therapy targeting CD19, has revolutionized the treatment of advanced acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma (DLBCL). The ability to specifically target the cancer cells has shown high positive results as reported in the registration studies. The success of CAR-T therapy in the first two indications led to the initiation of a large number of studies testing CAR-T therapy in different hematologic tumors such as acute myelogenous leukemia (AML), Hodgkin’s disease (HD), chronic lymphocytic leukemia (CLL), multiple myeloma (MM), as well as different solid tumors. Unfortunately, relapses occurred in the patients treated with CAR-T therapy calling for the development of effective subsequent therapies. Likewise, this novel mechanism of action was also accompanied by a different toxicity profile such as cytokine release syndrome (CRS). Patients’ access to the treatment is still limited by its cost. Another big hurdle is the lack of positive results in different solid tumors. Notwithstanding, this did not prohibit further development of this new therapy and continuing the research activity in solid tumors. This review of research activity of CAR-T will provide an overview on the clinical development of CAR-T in different tumors.

Keywords

Chimeric Antigen Receptor-t; CAR-T Therapy; Hematologic Malignancies, Solid Tumors, Cytokine Release Syndrome

Introduction

In 2017, the FDA granted the first approvals of–two CAR-T therapies for the treatment of adult patients with relapsed or refractory DLBCL after two or more lines of systemic therapies, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma, and for patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse [1,2].
The registration studies enrolled patients who were previously treated and considered of poor prognosis with some having had prior stem cell transplantation. Yet the results that were obtained from the studies (albeit single arm studies) were quite encouraging and resulted in high responses in those difficult to treat patient populations [3]. The genetically engineered T-cells, which are injected into the patient’s body, result in engraftment and rapid proliferation. Subsequently, each CAR-T cell attacks many cancer cells. This unique mechanism of action, with the involvement of the patient’s own immune system, resulted in a very high response rate despite the late disease stages and poor prognoses [4,5]. Furthermore, CAR-T therapy was used to bridge other therapeutic strategies with further improvement of responses. With the high response rates, and durable responses with long term follow up [6], it was hoped that the responses would be for long durations with the possibility of cure for these patients. Unfortunately, the relapse rate was around 30-60% for patients treated with CAR-T therapy [7]. With limited treatment options for patients who relapse after CAR-T therapy, it is of paramount importance to understand the mechanism of resistance in those patients in order to develop a subsequent effective therapy.

**Mechanism of Relapse to CAR-T Therapy**

Patients who relapsed following CAR-T therapy fall into two main groups of relapse: CD19-positive cells or CD19-negative cells [6]. The CD19-positive cells relapse occurs when the response to CAR-T therapy is not sufficient, with minimal effect for CAR-T cells and only transient B-cell aplasia. With flow cytometry, CD19 can be detected on the surface of the cells. Different animal studies attempted to identify the cause of this weak response to CAR-T therapy. Some of the factors that were proposed are the quality of CAR-T cells synthesized from children and young adults compared to those from older adults. For younger patients, the quality of the CAR-T cells was better, which was reflected in the tendency to better responses (longer event-free survival times) in the younger age groups versus older age groups. Studies have shown that starting T-cell phenotype of CAR-T-cell manufacturing is crucial to patients’ prognosis. A
study conducted by Gardner et al. enrolled 43 children and young adults [7]. They looked for factors that contributed to a maintained response among those who responded, achieved, and maintained a minimal residual disease (MRD)-negative state beyond 63 days, versus the non-responders who did not achieve that [7,8]. The analysis of apheresis obtained from both groups showed that in non-responders, the percentage CD8+ T-cells, which expressed lymphocyte-activated gene-3 (LAG-3) and PD-1, had significantly increased while in the responders group the number of CD4+ CAR cells and CD8+ CAR cells was significantly higher at the time of peak implantation. They also found that the number of CD8+ T-cells expressing tumor necrosis factor-α (TNF-α) was much less in the non-responder group compared to the responder group. This led to the assumption that the increase in LAG-3 expressing cells (LAG-3+ T-cells) and decrease in the cytokines after stimulation leads to the production of ineffective CAR-T cells, or ones with low potency resulting in a CD19-positive relapse. This is in comparison to the responders who have less LAG-3 expressing cells and increase in cytokines after stimulation thus having a better response [9]. The presence of LAG-3+ T-cells is a biomarker for T cell exhaustion. T-cell exhaustion refers to a state where the T-cells produced are dysfunctional with the decrease in the effectors and increase in the expression of the inhibitory receptors. This phenomenon is induced by chronic stimulation as in patients with cancer. Chronic stimulation leads to the production of T-cells that are usually deficient in their intrinsic activity. Accordingly, CAR-T cells derived from patients with cancer will be defective in function which can lead to poor prognosis and relapse. The mechanism of CAR-T-cell exhaustion is still not clearly understood. It was suggested that the CARs on the CAR-T cells can form a cluster that is formed independently of the antigen stimulation which leads to the development of tonic CAR-CD3ζ signalling, that can cause CAR-T-cell exhaustion. Additionally, the endogenous T-cell receptor signal of CAR-T cells caused by a specific antigen stimulation can result in T-cell exhaustion [6,9].

The CD19-negative relapses represent up to 20% of the relapsed CAR-T cell treated patients. With CD19-negative relapse, CD19
is not detected on the surface of the cells. This occurs due to mutation in the exons 1-13 of CD19 gene [10]. The exons 1-4 carry the codes for the extracellular domains and the exons 5-13 carry those of the transmembrane domain. Studies have shown that patients who show CD19-negative relapse have developed mutations in exons 2-5 in the form of one insertion or deletion [11]. The percentage of CD19-negative cells can be measured by flow cytometry, which is also able to assess the frequency of allelic mutations and the percentage of cells where there is a homozygous loss and biallelic mutations —which are major reasons for the loss of the targeted epitope in the membrane of CD19—with the subsequent escape from the effect of anti CD19 CAR-T. Called antigen escape, this leads to the development of resistance to CD19-directed CAR-T therapies.

There are other mechanisms which were described as possible causes for the occurrence of CD19 gene mutation and antigen escape, such as alternative splicing with the subsequent development of CD19-negative resistance [12]. These resistance mechanisms are not unique to CAR-T therapy, they have been observed in other tumor types e.g., breast cancer tissues show splicing of exon 16 of HER2 leading to resistance to trastuzumab. Likewise, melanoma tissues show splicing of BRAF (V600E), which results in dimerization and consequently resistance to vemurafenib. Splicing of mRNA (e.g., in SF3B1, SRSF2) also happened in hematological tumors such as MDS and CLL resulting in resistance to treatment [13].

**CRS-a Limit to the Full Therapeutic Potential of CAR-T Therapy?**

Both approved CAR-T cells cause the condition called CRS, which includes a set of life-threatening or even fatal reactions to the infusion of CAR-T therapy. Generally, CAR-T therapy should not be administered to patients who have active infections or inflammatory disorders.

The treatment with CAR-T cells causes rapid activation of T-cells and the release of high levels of cytokines. The main cytokines involved in CRS are IL-6, IL-10, and interferon (IFN)-
Y. IFN-Υ, when secreted, causes the release of other cytokines such as IL-6, IL-10, and TNF-α. Its release is also accompanied by fever, chills, headache, malaise, and other symptoms occurring with CRS. IL-6 also is considered a key cytokine in the pathophysiology of CRS since it is highly elevated in cases suffering from CRS. It is also responsible for the occurrence of many of the severe symptoms such as vascular leakage and disseminated intravascular coagulation (DIC). The occurrence of life-threatening CRS mandates immediate treatment with tocilizumab or tocilizumab and corticosteroids. [1, 2, 14]. Tocilizumab, an anti-IL-6 receptor antagonist, is the standard for CRS management, although, the optimal timing of administration is still unclear [14, 15] (Figure 1). Management also is depending on the severity of the symptoms. The commonly used scheme for the assessment of the severity of CRS is the one developed by the National Cancer Institute (NCI).

CRS can affect different body systems and organs, other medical specialties such as neurology, nephrology, cardiology in addition to the treating hematologist are required for the effective management of patients with severe CRS. Intensive care facilities should also be available when needed. The early and appropriate management of severe cases of CRS generally can lead to a good outcome [15].
Figure 1: Pathophysiology of CRS with the activation of different cytokines. The clinical picture can differ from mild only representing with fever (Grade 1) to life threatening conditions (Grade 4). Reprinted from Alexander Shimabukuro-Vornhagen et al. J Immunother Cancer 2018;6:56 with permission from Journal for ImmunoTherapy of Cancer [16].

CRS can affect different organs, and the symptoms can range from mild to severe and debilitating [16]. Respiratory symptoms that are common with CRS can range from cough and hypoxia up to acute respiratory distress syndrome, which can be seen on lung images as opacities affecting large areas of the lung and can be bilateral [16-18].

Cardiac and circulatory manifestations can be in the form of hypotension and can progress to capillary leak syndrome with subsequent peripheral and pulmonary edemas and signs of cardiac failure [19].

The American Society of Clinical Oncology (ASCO) published guidelines for the management of immune-related adverse events (irAEs) in patients treated with CAR-T therapy. The guidelines discuss the management of the most common irAEs occurring during the treatment with CAR-T which includes CRS, immune effector cell–associated neurotoxicity syndrome (ICANS), infections, B-cell aplasia, and cytopenias. In addition to the CRS
discussed above, the guidelines discuss the neurological toxicities/ICANS which usually occur after 4 days of therapy [16]. ICANS is the second serious irAEs that is not infrequent in patients treated with CAR-T. The patient suffers from encephalopathy with other different symptoms e.g., behavioral changes, aphasia, fine motor impairment, headache [17]. In severe cases the patient suffers from seizures that warrant admission to the ICU to control it and possibly intubating the patient if necessary. ICANS can occur with CRS or alone, it can also occur up to 1 month after the treatment with CAR-T. Likewise, it can be self-limited with the symptoms resolving by 17 days or may become severe and cause permanent neurological damage [18]

Cases of ICANS are graded using the ASTCT Consensus grading. The ASTCT includes a 10-point Immune Effector Cell–Associated Encephalopathy (ICE) score. This scale is also able to assess other critical neurological problems such as motor weakness. For children younger than 12 years or for patients who suffer from delayed development milestones, another scoring system is used for the assessment of the neurological side effects- the Cornell Assessment of Pediatric Delirium score I not the ICE assessment [19].

Treatment of ICANS is by the administration of corticosteroids and supportive care. Tocilizumab is contraindicated for the treatment of neurotoxicity as it can worsen the symptoms. As CRS can occur with ICANS and CRS necessitates the treatment of Tocilizumab, accordingly if the ICANS is low-grade and is expected to spontaneously resolve, the treatment of CRS can be prioritized and tocilizumab treatment could be started. If the ICANS is severe and requires active measures, then managing ICANS is the first priority before the management of the CRS [20,21]. (Figure 2).
Figure 2: Clinical presentation of CRS. Symptoms can range from fever to the affection of multiple organs. Abbreviations: DIC: disseminated intravascular coagulation; INR: international normalized ratio; PTT: partial thromboplastin time. Reprinted from Alexander Shimabukuro-Vornhagen et al. J Immunother Cancer 2018;6:56 with permission from Journal for ImmunoTherapy of Cancer [16].

In their pursuit to decrease the CRS signs/symptoms of CAR-T therapy while maintaining its good therapeutic effect, investigators tried to change the design of the studies and administration of CAR-T therapy. Frey et al. conducted a study on 35 adults with relapsed/refractory (R/R) ALL who received CAR-T therapy [26]. Patients were enrolled in 1 of 3 dosing cohorts. The first cohort was a low-dose cohort where nine patients received a low dose of therapy either as a single dose or fractionated dose. In the second cohort, six patients received CAR-T therapy as a high single dose, and the third cohort had 20 patients enrolled and received high dose therapy either as a single dose or fractionated dose. Any fractionated dose was given on 3 consecutive days with 10%, 30%, and 60% given on days 1, 2, and 3, respectively. This schedule allowed for the
assessment of CRS occurrence, taking necessary measures if they occur and trying to avoid fatal complications.

The nine patients in the low single or fractionated dose had a complete remission (CR) rate of 33% with manageable toxicity. In the six patients who received the high single dose, three had fatal CRS complications and the other three achieved CR. The results in the third cohort, where patients received fractionated high CAR-T dose, were impressive with a CR rate of 90%, event free survival rate (EFS) of 49.5% (95% CI: 21% - 73%) and a 2-year overall survival (OS) rate of 73% (95% CI: 46% - 88%). The superior results were achieved for the patients who were dosed with a high fractionated dose of CAR-T cells, while the toxicity was manageable with only one patient out of the 20 patients having grade CRS grade 4 and no grade 5 CRS. The fractionation of CAR-T over 3 days allowed for early detection of clinical symptoms of CRS and withholding of consequent doses. Accordingly, in the third cohort seven out of the 20 patients received all 3 doses, nine patients received only 1 dose and four patients received 2 doses. Only two patients in this cohort did not achieve CR and both only received 2 doses. The authors concluded that CAR-T cell fractionation allowed for early dose modification and consequently for optimizing the safety of patients with R/R ALL who received CAR-T therapy without compromising the efficacy of treatment [26].

Gardner et al. tested the early intervention of tocilizumab plus or minus corticosteroids with the first occurrence of mild CRS symptoms in a study of 43 patients. The first 23 patients received tocilizumab with or without corticosteroids if there was an occurrence of any grade 4 toxicity, not meeting the study’s defined dose limiting toxicity (DLT) and lasting > 48 hours, or there was a DLT lasting > 48 hours, and in either situation the CRS was not effectively managed by medication. Corticosteroids were also given to any non-hematologic toxicity > grade 3 that was considered a result of CAR-T infusion, lasting for > 48 hours, and not controlled despite active medical intervention. The subsequent 20 patients received concomitant tocilizumab and corticosteroids every 6-12 hours for persistent mild CRS symptoms according to the requirement of a protocol
modification. The investigators reported that despite the patients being treated with tocilizumab and steroids, there was no increase in incidence of infection. Likewise, the efficacy was not affected by the early treatment with tocilizumab and steroids or the expansion and engraftment of T-cells. They also reported a decrease in severe CRS in the 20 patients who had early intervention and concomitant tocilizumab and corticosteroids, versus the first 23 patients who had their CRS managed as above (15% vs 20%) [27].

In conclusion, the therapeutic effect of CAR-T therapy is well established and has been proven in a wealth of studies, however, the fact that there is a high percentage of patients suffering from relapse and CRS presents serious complications for the treatment of CAR-T therapy. It represents huge challenges for the treating physicians and the patients. Ongoing research activity is trying to address the mechanism of relapse and the best way to administer CAR-T therapy in order to reduce the incidence of CRS. Similarly, the occurrence of ICANS represents a major challenge for the treatment of patients with CAR-T therapy, especially when both CRS and ICANS occur in the same patient. The treating physician needs to assess the patient’s condition carefully in order to decide which of these 2 events needs to be treated first [20,21,28,29].

**CAR-T Therapy in Other Hematologic Malignancies, is there a Role?**

The unique mechanism of CAR-T therapy in addition to the high positive results mentioned above also triggered wide development programs assessing this novel therapy in different hematologic malignancies as well as solid tumors.

**CAR-T Therapy in AML**

In 2020, the American Cancer Society estimated AML to represent 1% of all cancers. It is mainly a disease of the older population, being uncommon before the age of 45, with the average age at first presentation of 58 years. Its incidence is slightly more common among men that women. It is the most
common leukemia in adults and the second most common leukemia in children [30,31].

Currently with the standard 7+3 regimen (consisting of cytarabine 7 days and anthracycline 3 days), a CR rate of up to 80% has been achieved in young adults and up to 60% in older adults who are 60 years of age and above. This is followed by post-remission induction which differs according to different factors e.g., patient’s age, general condition, molecular prognostic stratification, etc. [32,33]. However, patients who do not achieve remission from the first line regimens and those who relapse pose a serious problem for the hematologists with the continuous need for the development of effective therapies [34].

In ALL, the presence of a specific target such as CD19 supported the scientific concept behind the development of a specific targeted CAR-T therapy However, the lack of a specific cell target for AML makes it difficult to treat this disease with targeted immunotherapy [35,36]. Currently different researchers are attempting to identify an antigen or a group of antigens that are predominantly expressed on the myeloblast and not the normal tissues. This concept of specifically targeting surface antigens on myeloblasts is important to be able to target AML disease while avoiding the exposure of normal tissues to unnecessary immunotherapy which could result in toxicity [37].

The concept of using targeted immunotherapy for patients with AML was successfully applied by treating CD33-positive AML patients (adults and children over 2 years of age) with chemotherapy and the CD33-targeted antibody–drug conjugate, gemtuzumab ozogamicin [38,39]. The positive results of this combination led to the approval of this combination in adults and children over 2 years of age who suffer from CD33 positive AML [40,41].

In an attempt to identify the ideal candidates for CAR-T therapy in AML, Perna et al. did an extensive analysis of large datasets of transcriptomics and proteomics from malignant and normal tissues [42] (Figure 3). The researchers performed surface-specific proteomic studies in a diverse panel of AML cell lines
(THP1, Mono-mac, Kasumi, Molm13, OCI/AML3, and TF-1). The team performed a mass spectrometric analysis, which led to the identification of 4,942 proteins. They enriched their protein sets by adding the findings from other similar studies conducted on other AML cell lines e.g., (NB4, HL60, THP1, PLB985, CD32, CD33, CE 96, CD99, and K562). The researchers used data from the Human Protein Atlas, the Human Proteome Map and the Proteomics Database to identify the protein expression information in different normal tissues/organs such as liver, gallbladder, pancreas, stomach, gut, rectum, testis, epididymis, prostate, breast, etc. [43-45]. Consequently, they looked-for CAR-T therapy targets that are over expressed in AML versus normal tissue, and they were able to identify 24 molecules that fulfil this criterion. Using flow cytometry to analyze the expression of the 24 candidates in AML specimens, they managed to detect nine targets that are detected in >75% of the cells (expression range: 78%-99%, mean 82%): CD82, TNFRSF1B (also known as CD120b), ADGRE2 (also known as EMR2 or CD312), ITGB5, CCR1 (also known as CD191), CD96, PTPRJ (also known as CD148), CD70, and LILRB2 (also known as CD85d). Six of the nine targets were expressed in <5% in normal tissues while the other three were expressed in the normal tissues at a slightly higher level with the maximum expression of 20%. Still, all were much less expressed than their corresponding expression in the primary AML cells [42].

Figure 3: Flow Cytometric Analyses in Primary AML Samples and Normal Hematopoietic Cells. (A–D) Expression (% positive) of the 24 candidate antigens and the three CAR targets in current clinical investigation (most right
three) in bulk AML population (A), in leukemic CD34+CD38- cells (B), in normal BM CD34+CD38- CD45RA- CD90+ HSCs (blue), CD34+ CD38+ progenitor cells (light blue) (C), or in freshly purified (green) or activated (brown) normal CD3+ peripheral blood T cells (D). Data are represented as mean ± SD. (E) Summary of expression levels (mean ± SEM) of four top targets in indicated cell populations. ****p < 0.0001 (Student’s t test). Reprinted from Perna F, Berman SH, Soni RK, et al. Integrating proteomics and transcriptomics for systematic combinatorial chimeric antigen receptor therapy of AML. Cancer Cell 2017; 32: 506–519.e505 with permission from Elsevier [42].

Many preclinical studies were also conducted to assess the different possible targets for CAR-T therapy in AML populations which led to the start of the Phase I clinical studies. These studies tested a wide range of ages, from as young as 6 months and up to 90 years of age. The number of patients treated in these studies is small. Cummins et al. treated six patients with CAR-T therapy, two patients achieved complete remission (CR), three patients achieved partial remission (PR) and one patient had progressive diseases (PD) [46]. Richie et al. also treated four patients with CAR-T with all of them initially achieving PR or stable disease (SD), then they relapsed [47]. Of note, both Cummins et al. and Richie et al. used autologous T-cell source. Numerous studies are ongoing with data still to be presented [48, 49].

**CAR-T Therapy in CLL**

In order to assess if CAR-T therapy can target CLL cells, a group of researchers from the NCI in Bethesda managed to construct two CARs. They subsequently chose the one that has shown the best anti-tumor activity in vitro for further testing in CLL clinical trials [50].

One the earliest observations of clinical activity in CLL was reported by Porter et al in 2011 [51]. A patient who had R/R CLL was infused with a low dose (approximately 1.5×10^5 cells per kilogram) of autologous CAR-T cells. A real-time polymerase chain reaction detected DNA encoding anti-CD19 CAR-T after 1 day of the infusion of the cells. The infused CAR-T cells expanded to a level more than 1000 times the initial engraftment. The patient achieved CR. The toxicity profile was
as expected with the only grade 3/4 adverse event related to CAR-T therapy being lymphopenia as well as hypogammaglobulinemia, which was a chronic effect. The patient also suffered from a tumor lysis syndrome. Leukemia as well as normal B cells expression CD19 disappeared from the patient’s blood and bone marrow cells. A high level of CAR-T cells was maintained in the patient’s blood and bone marrow for 6 months and the patient’s remission was ongoing at 10 months post engraftment [52].

Following that, CAR-T therapy was investigated in different clinical trials with more than 130 patients being tested in the trials. Most of these patients were heavily pre-treated, some relapsed post hematopoietic cell transplant [53,54]. Others were cases treated as they progressed to Richter syndrome. Despite the heterogeneity of the patient population and the adverse prognostic factors, a CR rate in the range of 20-30% of the patients with estimated progression free survival (PFS) at 18 months of 25% was achieved. These studies have shown the potential activity of CAR-T therapy in the CLL patient population [55,56].

Ibrutinib, which is a breakthrough treatment for patients with CLL, was found to improve response in clinical trials. In one of the trials, treatment with CAR-T therapy following ibrutinib in three patients resulted in responses in all three patients, with one achieving CR [57]. Two other studies combining ibrutinib and CAR-T therapy have shown a very promising response rates of 80% in two series of 19 patients with a MRD eradication in the bone marrow of around 90% among responders. The safety profile was not different from that of other patients with other indications when treated with CAR-T therapy with the CRS being the main concern [58,59]. Other studies are ongoing to explore the full potential of CAR-T therapy in patients with CLL [60].

**CAR-T Therapy in Hodgkin Lymphoma (HL)**

Data from two studies testing CAR-T therapy, targeting CD30 antigen, in patients with HL were recently presented in
Transplantation & Cellular Therapy Meetings of ASBMT and CIBMTR and highlight the potential role of anti-CD30 CAR-T cells for this disease [61,62]. In one of the trials the investigators treated the patients with a lymphodepletion chemotherapy that included cyclophosphamide and fludarabine (FC) before CAR-T cell infusion. With 14 patients enrolled into this Phase I study, the median age was 30 years (range: 17-69 years). Patients were CD30 positive HL and heavily pretreated with a median of five prior regimens. Most patients previously received a checkpoint inhibitor and the monoclonal antibody brentuximab targeting CD30. Patients received a single infusion of 1 of 3 dose levels: $2 \times 10^7$ cells/m$^2$, $1 \times 10^8$ cells/m$^2$, or $2 \times 10^8$ cells/m$^2$. The investigators found the expansion and persistence of the CAR-T cells to be dose dependent. Of the 14 patients treated, important safety findings were seen in four patients who developed grade 1 CRS, and some patients developed maculopapular rashes that disappeared without any treatment. Other adverse events were in the form of alopecia, gastrointestinal toxicities and transient cytopenias. At 6 weeks, 12 patients were evaluable for response, seven patients achieved CR and one patient achieved PR. One of the CR patients showed the response by 6 weeks and maintained the remission for more than a year [61].

In another Phase I/II study, patients received one of two dose levels in a standard 3+3 design. The doses tested in Phase I were $1 \times 10^8$ cells/m$^2$ or $2 \times 10^8$ cells/m$^2$ and the Phase II part of the study tested the selected Phase I dose in more patients [62]. In both Phases of the study a total of 29 patients were enrolled with a median age of 35 years (range: 23-69 years). All patients had refractory disease and were heavily pre-treated with a median of 8 previous regimens. There were 28 evaluable patients of which 26 (eight in Phase I and 18 in Phase II) received CAR-T cells infusion (24 patients had classic HL, and the other two had T-cell lymphomas). In the eight patients enrolled in the Phase I part of the study three patients received the lower dose of CAR-T cells ($1 \times 10^8$ cells/m$^2$) and all progressed. Of the other five patients who received the high dose ($2 \times 10^8$ cells/m$^2$), three patients had CR, one patient had SD, and one patient had PD. As there were no dose limiting toxicities, the $2 \times 10^8$ cells/m$^2$ was selected for the Phase II part of the study. Bendamustine (B)
single agent was given for lymphodepletion in Phase I of the study. However, as response and CAR-T cell expansion were suboptimal the investigators decided to add fludarabine (F) i.e., bendamustine and fludarabine combination (BF).

Out of the 18 patients treated in Phase II, four developed CRS (three grade 1 that resolved spontaneously, and one was grade 2 that was managed by tocilizumab). There were also nine patients who had mild rashes after the infusion. Patients also had cytopenias in the form of grade 3 or high neutropenia in 3 patients (12%), thrombocytopenia and lymphopenia each occurring in four patients (15%). Of the 18 patients who received CAR-T cell infusion 14 patients achieved CR (78%) of which two had a response longer than 1 year. Another two patients (11%) achieved PR; one patient had SD (5%). Only one patient had PD (6%). Of note, the CR rate was higher in the patients who had the combination of BF (78%) versus the patients who received B monotherapy (37%), with survival also being longer in those who had BF than those who had F alone (median of 389 days vs 55 days). After a median follow-up of 108 days (range not reported), progression-free survival was 164 days for 19 evaluable patients who had active disease at the time of lymphodepletion. Patients who received the higher dose of CAR-T cells with the combination lymphodepletion regimen appeared to have a longer survival than those treated with a lower dose and single agent bendamustine (median = 389 days vs. 55 days; p=0.0004) [62].

These data suggest the potential role of CAR-T therapy targeting CD30 HL. However, further studies with larger numbers of patients and longer follow up periods are needed to identify the role of anti-CD30 CAR-T cell infusion in HL.

**CAR-T Therapy in MM**

MM is a plasma cell cancer and despite the recent advances in treatment and the use of immunomodulatory, proteosome inhibitors and other novel therapies there is still no cure for it [63,64]. Current available therapies improve disease outcomes,
but patients will ultimately relapse. Each relapse negatively affects survival chances for the patients [65,66].

The superior results seen with CAR-T therapy in non-Hodgkin lymphoma and ALL encouraged the testing of CAR-T cell therapy in the disease of MM. B-cell maturation antigen (BCMA) is a protein and a tumor necrosis factor that is expressed by normal and malignant plasma cells including MM cells [67,68]. A specific CAR-T cell therapy (bb2121) was produced by the transduction of autologous T cells with a lentiviral vector that encodes a second-generation CAR-T that incorporates an anti-BCMA single-chain fragment, a CD137 (4-1BB) costimulatory motif and a CD3-zeta signaling domain [69,70].

CAR-T therapy has shown the potential to benefit patients with MM. In a pre-clinical Phase I study conducted by Raje et al., bb2121 (a CAR-T agent that targets BCMA) showed promising activity in patients with R/R MM [71]. The study consisted of 2 Phases - dose escalation and dose expansion Phases. In the dose escalation phase, a single infusion of CAR-T cells was tested in groups of patients in a dose escalating manner. The doses that were tested were 50x10^6, 150x10^6, 450x10^6, and 800x10^6. In the dose expansion Phase, only two doses were tested: 150x10^6 and 450x10^6. The study enrolled heavily pre-treated patients who received at least 3 previous lines of therapy (the median was 7 and the range was 3-14). With the exception of one patient, all patients previously failed an autologous stem cell transplant. All patients previously received and relapsed or were refractory to an immunomodulatory agent and a proteasome inhibitor. Some patients also had extramedullary disease (27%) and 45% of the patients had high risk cytogenetic profile with the presence of t(4;14), t(14;16) or del(17p). Safety was the primary endpoint of the study, and the safety results of 33 evaluable patients (out of 36 enrolled into the study) were reported. The main toxicity was hematologic toxicity with events of grade 3 or higher reported for 85% of the patients in the form of neutropenia. Other grade 3 or higher toxicities were reported in the form of leukopenia, anemia, and thrombocytopenia in 58%, 45% and 45% of the patients, respectively. The non-hematologic toxicities were in the
form of CRS which occurred in 25 patients (76%). Most of the patients (23 patients-70%) had a maximum of grade 2 CRS, and only two patients (6%) had grade 3 CRS. Neurologic toxicities occurred in 14 patients (42%) with only one patient suffering from a reversible grade 4 event and all the remaining patients having only grade 1 or 2. A CR was achieved in 15 patients (45%), one patient had PR (3%), and a median of PFS of 11.8 months was achieved. Of note, six patients out of the 15 patients ultimately relapsed. It is important to note that some responders had MRD negativity. The investigators concluded that BCMA-directed cellular immunotherapy for patients with R/R MM had toxicities similar to what was reported in other CAR-T cell therapies used for other indications. The therapy also resulted in promising responses in the form of CR/PR with doses of 150x10^6 in this heavily pretreated patient population of R/R MM [71,72].

In another first in human study (FIH) the anti-B-cell maturation antigen BiTE molecule was assessed in patients with R/R MM [72]. Patients who relapsed after at least 2 prior lines of therapies and no extramedullary disease were treated in the study. Patients received up to 10 cycles of treatment, given as an infusion every 4 weeks. The length of the cycle was 6 weeks. MRD was assessed using flow cytometry and MRD negativity was defined as the presence of <1 cell/10^4 bone marrow cells. Forty-two patients were enrolled into the study. The median age of the patients was 65 years. Patients had a median duration of MM of 5.2 years. The median number of cycles received by the patients was 1 (range: 1-10 cycles), while the responders received a median of 7 cycles. The doses received by the patients were in the range of 0.2-800 µg/d. Reasons for treatment discontinuation were disease progression in 25 patients (60%), adverse events in seven patients (17%), and death in four patients (10%). Likewise, three patients (7%) withdrew from treatment as they received the maximum allowable cycles in the study (10 cycles), one patient (2%) withdrew consent for continuation of treatment and two patients (5%) were still on treatment at the time of the presentation of these data. Safety results have shown that the 800 µg/d was not tolerated with two out of the three patients enrolled having a DLT, with one having a grade 3 CRS and the other one
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suffering from neurotoxicity (polyneuropathy) which started as grade 2 and progressed to grade 3. Both events later resolved. For the patients who received the 400 µg/d dose, one patient had grade 1 polyneuropathy which progressed to Grade 3 but ultimately resolved by week 12. CRS was seen in 16 out of the 42 patients (38%), however it was treated with glucocorticoids, antihistamines, antipyretics, analgesics with one patient receiving tocilizumab for grade 2 CRS. Additionally, there were five patients who had increases in liver enzymes (ALT and/or AST > 3x times upper limit of normal), among them four patients received the 400 µg/d. However, one of the four patients who had increased liver enzymes had increased enzymes at baseline. At the 400 µg/d dose which was received in 10 patients, seven achieved a response rate of 70%. Among the seven responders there were five patients who achieved MRD negative CRs. For the remaining two patients, one had a PR, and one had a very good PR. All the responses occurred in the first cycle and some responses lasted > 1 year. The investigators concluded that these results are promising and warrant further investigation [73,74].

The CARTITUDE-1 was a Phase Ib/II trial that enrolled adult patients who were heavily pre-treated with 3 or more prior lines of therapy or were double-refractory to an immunomodulatory drug and proteasome inhibitor. Patients received a single low dose of ciltacabtagene autoleucel (cilta-cel), an autologous CAR-T therapy. Cilta-cel is a bioengineered T-cell receptor construct with a CD3ζ signaling domain, a 4-1BB costimulatory domain, and 2 BCMA binding domains. The Phase I study objectives were safety and defining a dose to carry forward to the Phase II part of the study. Patients underwent apheresis and cell collection to produce the CAR-T cells. The patients had lymphodepletion with 3 days of fludarabine 30mg/m² plus cyclophosphamide 300mg/m². The dose received of cilta-cel was a single dose of 0.75×10⁶ cells/kg (range: 0.5-1.0×10⁶ cells/kg). At the time of the cut-off level of 1st of September 2020, 113 patients were enrolled with 97 receiving CAR-T (Phase Ib 29 patients and Phase II 68 patients). The median age of the patients was 61 years (range: 43-78 years) and the median number of prior lines of therapies was 6 (range: 3-18). Hematologic adverse
events were the most common with neutropenia, anemia, and thrombocytopenia occurring in 96%, 81%, and 79% of the patients, respectively. Most hematologic adverse events resolved quickly without complications. Non-hematologic adverse events were CRS (95% of patients) and neurotoxicity (21% of patients). Most CRS events were grade 1/2 (95%). All CRS events resolved within 14 days of their onset in all but one patient. Only 10% of the patients who suffered from neurotoxicity had their events as grade ≥3. The number of deaths was 14, five of which were due to PD, three due to adverse events unrelated to the CAR-T therapy and six due to adverse events related to the therapy. The efficacy results have shown that 67% of the patients achieved stringent CR, 26% achieved a very good PR and 4% had PR. There were 57 patients who were evaluable for the assessment of MRD, 93% of which became MRD negative. PFS at 12 months was 77%. The investigators recommended the testing of this CAR-T therapy in a larger clinical trial [75,76].

The KarMMA Phase II study also reported the results of Ide-cel, another CAR-T therapy targeting BCMA. Based on the Phase I promising activity, Phase II study enrolled 140 heavily pretreated patients with MM. The median age of the patients was 61 years. For lymphodepletion, the patients received cyclophosphamide 300 mg/m² and fludarabine 30 mg/m² for 3. This was followed by the infusion of CAR-T cells in the range of 150─450 × 10⁶. Of the 128 heavily pretreated patients (median of prior 6 regimens) who received Ide-cel, and with a median follow up period of 11.3 months, the ORR (the primary endpoint of the study) was 73% and the PFS was 8.6 months. The most common adverse events of any grade were cytopenias and CRS occurring in 97% and 84% of the patients respectively. CRS was mainly grade 1/2 with only 5% having grade 3, 1 patient had grade 4 and 1 patient died of CRS at the 300× 10⁶ dose of the CAR-T. The response was durable with 36% having CAR-T cells detected at 12 months. The investigators concluded that Ide-cel has demonstrated deep, and durable responses in this heavily pretreated patient population with an acceptable safety profile [77,78].
CAR-T Therapy Solid Tumors, is there a Role?

CAR-T therapy was assessed for the treatment of different solid tumor types e.g., colorectal cancer (CRC), breast cancer (BC), thoracic tumors, hepatocellular carcinoma (HCC), ovarian cancer, etc. This review will focus on 4 of the common tumor types and the trial of CAR-T therapy in its management.

In CEA positive CRC: A CAR-T was specifically developed for the carcinoembryonic antigen (CEA) which is a tumor marker expressed in the majority of CRC. Currently, there are several ongoing Phase I studies evaluating the safety and efficacy of CEA directed CAR-T therapy in advanced CRC. The results of these studies are still awaited. Minimal data comes from a case report of a patient with CRC who had a complete metabolic response within the liver lasting for 13 months and the preliminary results of a phase I study showing that out of 15 patients with unresectable metastatic CRC who received CAR-T therapy, there were 2 partial responses and 9 had stable disease. [79].

In prostatic cancer, a Phase 1 trial; all patients were treated with lymphodepletive chemotherapy. That was followed by CAR-T therapy received as a continuous infusion in a dose escalating manner. The treatment was tolerable and 2 out of 5 patients (40%) achieved PR and a PSA decline of 50-70%. Another patient achieved a minor response to treatment [80].

In hepatocellular carcinoma (HCC) a CAR-T was developed against a potential antigen target which is glypican-3 (GPC3). In two Phase I studies assessing the effect of CAR-T therapy on GPC3+ HCC, patients were given infusion of cyclophosphamide and fludarabine-based lymphodepletion. There were 13 patients enrolled in the study, with 9 of the 13 patients (69%) suffering from CRS. There were no grade 3/4 neurotoxicities. The overall survival at 6 months was 50.3%, for 1 year it was 42% and 10.5% survived for 3 years. Of note, one patient from the study maintained stable disease and was alive 44 months.
In another Phase I study, patients with advanced metastatic solid tumors were treated with CD133 targeting CAR-T. The study enrolled patients with different solid tumors among them were 23 patients who had HCC. Three patients achieved partial remission-PR (13%), and 14 patients achieved stable disease-SD (61%). The median PFS was 5 months, and the 3-month disease control was 65% [81,82].

For the treatment of thoracic cancer, a wide variety of targets are currently being evaluated for CAR-T cell therapy in lung cancer that include EGFR, HER2, MSLN, MUC1, CEA, ROR1, and PD-L1. Among these, EGFR and MSLN specific CAR-T cells seem to be more promising compared to the others due to the antigen’s higher specificity and lower on-target, off-tumor toxicity concern.

An open-label phase I investigated the use of regionally delivered autologous mesothelin-targeted CAR-T with pembrolizumab for malignant pleural mesothelioma (MPM). Pembrolizumab was given based on pre-clinical work that has shown PD-1 blockade has enhanced mesothelin CAR-T activity and rescues the function of exhausted CAR-T cells [83]. In this study, 27 patients with malignant pleural disease (either as primary or pleural metastases) received intrapleural mesothelin targeting CAR-T; 25 of these patients had the diagnosis of malignant pleural mesothelioma (MPM), among them 18 patients received pembrolizumab after CAR-T cell therapy. The median overall results were based on 23 out of the 27 patients in the intrapleural mesothelin targeting CAR-T and then the 18 patients who received the combination of Pembrolizumab and CAR-T therapy. At a median follow-up of 20 months, the CAR-T treatment median OS was 17.7 months, and the 1-year survival rate was 74%. Pembrolizumab was given every 3 weeks to promote CAR-T function. With the addition of pembrolizumab, the patients’ results were further enhanced with a median OS achieved of 23.9 months, and a 1 year-survival rate of 83% [84,85].

Table 1 shows some of the ongoing CAR-T studies in solid tumors based on the clinicaltrials.gov [79].
Table 1: Non-comprehensive list of ongoing CART cell therapy studies in solid organ malignancies.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>NCT number</th>
<th>Recruiting status</th>
<th>Brief title</th>
<th>Cell target</th>
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<tbody>
<tr>
<td>Brain Cancers (Glioblastoma)</td>
<td>NCT02203862</td>
<td>Ongoing Phase I</td>
<td>Genetically Modified T-cells in Treating Patients with Recurrent or Refractory Malignant Glioma</td>
<td>IL13Rα2</td>
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<td>NCT03730615</td>
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<td>CART-EGFRvIII + Pembrolizumab in GBM</td>
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<td>NCT01454394</td>
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<td>CAR T-Cell Receptor Immunotherapy Targeting EGFRvIII for Patients with Malignant Gliomas Expressing EGFRvIII</td>
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<tr>
<td>NCT01109059</td>
<td>Completed recruiting/phase I</td>
<td>CMV-specific Cytotoxic T Lymphocytes Expressing CAR Targeting HER2 in Patients With GBM</td>
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<td>Gastrointestinal Cancers</td>
<td>NCT01373047</td>
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<td>CEA-Expressing Liver Metastases Safety Study of Intraperitoneal Infusions of Anti-CEA Designer T Cells</td>
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<tr>
<td>NCT03682744</td>
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<td>CAR-T Intraperitoneal Infusions for CEA-Expressing Adenocarcinoma Peritoneal Metastases or Malignant Ascies (IPC)</td>
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<td>NCT03323944</td>
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<tr>
<td>NCT03159819</td>
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<tr>
<td>NCT02744287</td>
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<td>Renal Cancer</td>
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<td>Carbohydrate-xylose IX (CAIX)</td>
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<tr>
<td>Prostate Cancer</td>
<td>NCT03939336</td>
<td>Ongoing phase I</td>
<td>Safety and Efficacy of CTC301 CAR-T in Adult Subjects with Recurrent or Refractory Stage IV Renal Cell Carcinoma</td>
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<td>NCT02499112</td>
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<td>HER2-CAR T Cells in Treating Patients with Recurrent Brain or Leptomeningeval Metastases</td>
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<td>Autologous huM2-CAR44 T Cells for Breast Cancer Targeting Cleared Form of MUC1</td>
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<td>NCT03054239</td>
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<td>NCT02706039</td>
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<td>Genetically Modified T-Cell Therapy in Treating Patients with Advanced ROR1+ Malignancies</td>
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</table>

*List updated on 09/29/2021 from clinicaltrials.gov.


What is next?

The previously discussed studies in different hematologic malignancies represent a huge opportunity for the patients who are suffering from those diseases. The fact that most of these studies are early Phase studies with promising results, and that
there are planned Phase III studies, represents great research opportunities for the scientific community and a chance for patients to be treated with a highly effective CAR-T therapy. These opportunities come with some challenges; the CRS is one of them. Some ongoing studies try to minimize this side effect while maintaining the superior outcome seen with CAR-T therapy [26]. Cost still represents a challenge to treatment with CAR-T therapy. Treatment with CAR-T cells is still not affordable for many patients in the approved indications. With the potential of more indications to come the cost of treatment, either for the patients or any health care system, should be thoroughly evaluated, which also includes the cost of hospitalization during treatment with CAR-T therapy and possible costs for the management of side effects [85]. For the treatment of solid tumors with CAR-T, the road is still not clear as none of the studies have shown good results similar to those seen in hematologic malignancies. However, the clinical trial activity is ongoing, and it is hoped that with the results of these studies it will clarify the way forward and the possible future designs/doses of CAR-T in solid tumors.

Conclusions

Treatment with CAR-T therapy has started a new concept for the treatment of patients with advanced ALL and DLBCL. Although CAR-T therapy is currently approved for advanced/heavily pre-treated patient populations, the research activities will potentially be conducted in earlier disease settings. For solid tumors, a success similar to that seen in hematologic malignancies is still to be seen, but there is a large numbers of ongoing studies in different solid tumors e.g., CRC, prostatic cancer, HCC, Thoracic cancers, etc. These studies in addition to the ongoing and planned activities in hematologic malignancies will open the door to a new era of research and treatment with gene/targeted therapy.
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