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CD19-Targeted CAR T Cells as Novel Cancer Immunotherapy for Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia

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Abstract

Immunotherapy has demonstrated significant potential for the treatment of patients with chemotherapy-resistant hematologic malignancies and solid tumors. One type of immunotherapy involves the adoptive transfer of T cells that have been genetically modified with a chimeric antigen receptor (CAR) to target a tumor. These hybrid proteins are composed of the antigen-binding domains of an antibody fused to T-cell receptor signaling machinery. CAR T cells that target CD19 recently have made the jump from the laboratory to the clinic, and the results have been remarkable. CD19-targeted CAR T cells have induced complete remissions of disease in up to 90% of patients with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL), who have an expected complete response rate of 30% in response to chemotherapy. The high efficacy of CAR T cells in B-ALL suggests that regulatory approval of this therapy for this routinely fatal leukemia is on the horizon. We review the preclinical development of CAR T cells and their early clinical application for lymphoma. We also provide a comprehensive analysis of the use of CAR T cells in patients with B-ALL. In addition, we discuss the unique toxicities associated with this therapy and the management schemes that have been developed.

Keywords

Adoptive T-cell therapy; B-cell acute lymphoblastic leukemia; CD19; chimeric antigen receptor; immunotherapy

Introduction

Immunotherapy for cancer has generated significant excitement owing to unprecedented responses in patients with chemotherapy-refractory acute leukemia and solid tumors. The mechanism of action for most immunotherapy includes the activation of a T-cell response

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against a malignancy. Cancer spread can be mediated by blocking T-cell suppression signals or by redirecting a T cell to a tumor target with an antibody specific to both T cells and tumors. In the case of adoptive T-cell therapies, a patient's own T cells are isolated and manipulated in the laboratory and then reinfused. The 2 main types of adoptive T-cell therapies employ either tumor-infiltrating lymphocytes (TILs) or chimeric antigen receptor (CAR) modified T cells.

In TIL therapy, TILs are isolated from solid tumors and expanded over several weeks to months in a laboratory to generate a sufficient number of tumor-reactive T cells.[1] Some patients with metastatic tumors experience durable complete remission (CR), which is not possible with salvage chemotherapy.[2]

One of the major disadvantages of TIL therapy is the culture time required to generate a sufficient number of TILs to mediate treatment responses. Although TIL production has evolved from taking several months to taking several weeks, antigen stimulation and culture with interleukin 2 (IL-2) commonly results in terminal-differentiated T cells with limited in vivo persistence. This may explain why most patients have no antitumor effect from TIL therapy.[3] Also, requiring patients with refractory disease to wait for TIL infusion is problematic for those who are very ill.

Engineering T cells to express CARs overcomes this time disadvantage, and includes several other advantages over TIL therapy. The CAR is a hybrid protein that includes an antigen-binding domain, derived from an antibody, fused to a transmembrane domain followed by T-cell activation domains associated with the T-cell receptor (TCR).[4] A T cell modified with a CAR is endowed with a new antigen specificity, and binding its antigen supports T-cell activation and killing of the target cell. With robust gene-transfer technologies available for human T cells, a sufficient number of tumor-reactive T cells can be produced in as little as 1 week.[5] Also, CARs are universal antigen receptors that can be used in all patients owing to their antigen-binding domains being derived from antibodies. In contrast, TIL therapy and other T-cell therapies are human leukocyte antigen (HLA)-restricted, so they recognize tumor-specific antigen when it is presented by certain major histocompatibility complex (MHC) molecules. Furthermore, TCRs recognize short peptide sequences as tumor antigens, whereas CARs can recognize proteins, lipids, and/or carbohydrates as antigens. Finally, owing to the modular nature of the CAR, it can be continually modified and refined for optimization, or potentially to develop new functions.

Zelig Eshhar developed the first CAR using what he called a T-body approach. He combined a single-chain variable fragment (scFv), recreating an antigen-binding domain, with TCR-associated activation domains from CD3ζ or CD3γ.[6] Eshhar and colleagues validated the function of genetic-retargeted CAR T cells in vitro. Several groups later confirmed this by developing CARs against various tumor targets in vitro.[7] However, these first-generation CARs displayed limited persistence and poor tumor control in vivo.[8] Poor efficacy in mice prevented the clinical translation of this technology to patients. However, CAR T-cell investigators were able to overcome this in vivo inefficacy by engineering CAR T-cell activation to mimic more closely physiologic T-cell activation. The 2-signal rule of T-cell activation states that TCR activation via CD3ζ is insufficient for complete activation and

long-term T-cell persistence. Instead, a costimulatory signal, such as CD28, is required as signal 2. Modification of CARs to include both CD3ζ and CD28 resulted in similar in vitro function, but also supported robust tumor killing and long-term CAR T-cell persistence in vivo.[9–12] Although second-generation CARs paired CD28 with CD3ζ, researchers ultimately demonstrated that other costimulatory agents, such as OX40, 41BB, or CD27, could similarly enhance CAR T-cell function in vivo.[13,14] Third-generation CARs combining 2 costimulatory domains with CD3 also have been developed, but have not been clinically evaluated to the extent of second-generation CARs. This preclinical validation of CAR T cells supported clinical evaluation in early-phase clinical trials. Owing to the recent number of reports from multiple groups detailing outcomes of infusing CD19-targeted CAR T cells in nearly 100 patients with B-cell acute lymphoblastic leukemia (B-ALL), our review focuses on this disease. However, because early trials targeting non-Hodgkin lymphoma (NHL) with CD19-targeted CAR T cells provided important insights and set the foundation for the great success in treating B-ALL, the review begins by highlighting these efforts.

Clinical Evaluation of CD19-Targeted CAR T Cells for B-Cell Malignancies

Early Application of CAR T Cells in NHL and Chronic Lymphocytic Leukemia

The first trials evaluating CAR T cells for B-cell malignancies focused on NHL, and targeted CD19 or CD20 antigens. One of the most important observations came from a group at the Baylor College of Medicine, whose work confirmed the results of preclinical studies comparing first-generation and second-generation CAR T cells. Savoldo and colleagues[15] infused patients with NHL with a mixture of T cells that were modified with either a first-generation CD19-specific CAR or a second-generation CD19-specific CAR that contained a CD28 costimulatory domain. They demonstrated that the second-generation CAR T cells expanded better and persisted longer than the first-generation CAR T cells, although responses were modest. In addition, investigators at the Memorial Sloan Kettering Cancer Center (MSKCC) demonstrated that lymphodepletion with conditioning chemotherapy was required for optimal CAR T-cell function. Brentjens and colleagues[16] treated patients with T cells modified with a second-generation CAR that included a CD28 costimulatory domain. The first cohort was infused with CD19-targeted CAR T cells alone, whereas the second cohort was treated with cyclophosphamide conditioning chemotherapy (1.5 to 3.0 g/m^2) prior to CAR T-cell infusion. They demonstrated that expansion, persistence, and responses were improved in the conditioned cohort.[16]

Although these early trials and others established the safety of CD19-targeted CAR T cells, they lacked obvious evidence of T-cell mediated eradication of disease. However, a case report from the National Cancer Institute (NCI) that used a second-generation CAR with CD28, and a case series from the University of Pennsylvania (UPENN) that used a second-generation CAR with 41BB, established proof of principle that CAR T cells are a potent and targeted immunotherapy.[17–19] Both groups demonstrated rapid resolution of disease shortly after infusion with the CD19-targeted CAR T cells. However, what clearly established the role of the CAR T cells in mediating the treatment response was a concomitant B-cell aplasia observed in some patients that lasted longer than 1 year.[17–19] Such a long-term B-cell aplasia could not be attributed to chemotherapy, but was predicted as an on-target, off-

tumor toxicity of CD19-targeted CAR T cells. In addition, these case reports also detailed a unique set of toxicities, including fevers, hypotension, and hypoxia, that were the first hallmarks of an inflammatory response that we now recognize as cytokine release syndrome (CRS). These anecdotal results have been followed up with trials optimizing conditioning chemotherapy and focusing on certain NHL subtypes, which has resulted in objective response rates of up to 53% for patients with chemotherapy-refractory NHL.[20–22] More importantly, they provided the framework for trials evaluating CD19-targeted CAR T cells for relapsed or refractory B-ALL that likely will change the standard of care for this poor-outcome disease.

Application of CD19-Targeted CAR T Cells for Relapsed/Refractory B-ALL

In the last 3 years, 6 reports have detailed trials of CD19-targeted CAR T cells for patients with B-ALL.[23–28] The results have been uniformly remarkable, despite a myriad of trial differences that include a wide spectrum of ages treated, CAR T-cell production systems that were either lentiviral or gammaretroviral, and different costimulatory domains in the CAR (CD28 or 41BB). The first reports came from the MSKCC group and detailed outcomes from treating adults (n=16) with relapsed or refractory B-ALL.[23,24] This was followed by reports from investigators at UPENN,[25,26] who treated children and young adults (n=25) and adults (n=5), and the NCI, who reported their efforts with treating children and young adults (n=21).[27] Most recently, the group at the Fred Hutchinson Cancer Research Center (FHCRC) reported their outcomes after treating 30 adults.[28] Although all groups utilized second-generation CD19-targeted CAR designs, the differences included scFv and costimulatory domains. The UPENN, NCI, and FHCRC groups developed an scFv from the FMC63 hybridoma, and the MSKCC developed their scFv from the SJ25C1 hybridoma.[29,30] In addition, the CARs from the MSKCC and NCI groups utilized the same CD28 transmembrane and costimulatory domain paired to the CD3ζ intracellular activation domain, whereas those from the UPENN and FHCRC groups utilized the 41BB costimulatory domain paired to CD3ζ.[13,28,30,31]

The UPENN and FHCRC groups produced their CD19-targeted CAR T cells via lentiviral gene transfer, whereas the NCI and MSKCC groups used gammaretrovirus. Both viral techniques appeared equivalent, with efficient gene transfer and minimal production failures of required doses (MSKCC, 1; UPENN, unknown; NCI, 2; FHCRC, 0, but 1 unsuccessful CD8 enrichment).[23–28] Considering the results with NHL, all trials required lymphodepleting chemotherapy before CAR T-cell infusion, which included cyclophosphamide (MSKCC), physician's choice (UPENN), and fludarabine (FLU) plus cyclophosphamide (CY; the NCI). Although no obvious differences occurred that could be attributed to the different conditioning regimens used across these trials, the FHCRC group demonstrated that FLU/CY vs CY alone or with etoposide increased CAR T-cell persistence and expansion, and more importantly, disease-free survival. CAR T-cell doses ranged from 2×10^5/kg up to 2×10^7/kg, with MSKCC having a fixed-dose trial (3×10^6/kg) and the NCI and FHCRC groups having dose-escalation trials (1×10^6/kg followed by 3×10^6/kg, and 2×10^5/kg followed by 2×10^6/kg and 2×10^7/kg, respectively).[23–28] The maximum tolerated doses determined by the NCI and FHCRC groups were 1×10^6/kg and 2×10^6/kg, respectively. There was also a difference in cell formulation for infusion. Most study sites

infused bulk CAR T cells after production, however, the FHCRC group first isolated CD8 central memory and CD4 T cells separately before CAR T cell production.[23–28] Afterward, they formulated the product to be composed of an equivalent number of CD4 and CD8 CAR T cells before adoptive transfer into the patient. The rationale for derivation of the CAR T-cell product from CD8 central memory cells was from preclinical work that demonstrated this selected composition provided superior control of leukemia.[32]

Despite the differences between these trials for relapsed or refractory B-ALL, the outcomes and toxicities were similar. The CR rates were 88% (MSKCC), 90% (UPENN), 67% (NCI), and 90% (FHCRC).[23–28] In comparison, the expected CR rate for relapsed or refractory B-ALL treated with salvage chemotherapy is 30%. Even blinatumomab (Blincyto, Amgen), which recently was approved for this indication, had a CR rate of only 43%.[33,34] Furthermore, these CRs were high-quality molecular remissions, as suggested by lack of minimal residual disease when evaluated with high-sensitivity assays such as flow cytometry, quantitative polymerase chain reaction, and/or deep sequencing for immunoglobulin H rearrangements. The molecular CR rate ranged from 60% to 90%.[23–28] Consistent with the high quality of these remissions is their durability. The UPENN group reported that at 6 months, the probability of event-free survival was 67% and the probability of overall survival was 78%. The NCI group reported that overall survival was 52% at 10 months, and the FHCRC group reported that disease-free survival was greater than 60% at a median follow-up of 300 days.[25–28]

The most obvious difference detected between the correlative studies was the persistence of the CAR T cells. The CAR T cells from the NCI and MSKCC groups did not persist beyond 2 to 3 months from adoptive transfer, whereas the CAR T cells from the UPENN and FHCRC groups occasionally could be detected beyond 6 months. This difference may be related to the discrete biologic functions encoded by the costimulatory domains. Recent reports suggest that CAR T cells with CD28 drive more rapid expansion and effector-like functions, whereas 41BB drives more memory T-cell functions.[35,36] Regardless of the differences in CAR T-cell persistence, they do not appear to result in different efficacies because the initial remission rates are equivalent and, albeit with limited follow-up, the durability of remissions is similar as well. This suggests that robust CD28-mediated CAR T-cell expansion is sufficient to induce high-quality molecular remissions in a short period. Indeed, we (Drs Davila and Brentjens) were able to detect bone marrow molecular CR within as little as 8 days after adoptive transfer, whereas initial reports from UPENN suggested the 41BB-containing CAR required longer to induce a molecular CR.[23,26]

Despite these impressive response rates, follow-up has been long enough to detect some relapses. Early relapses were related to prolonged (eg, 1 month) corticosteroid administration, which resulted in decreased CAR T-cell expansion.[23] Similar relapses have not been ascribed yet to short pulses of corticosteroids. Other relapses appear to be related to immune escape. For example, out of the 7 relapses reported by the UPENN group, 3 had evidence of a CD19-negative immunophenotype.[25] Similarly, the FHCRC group reported 9 relapses, although all but 2 patients were given conditioning chemotherapy that lacked fludarabine. Two of these 9 relapses were categorized as CD19-negative ALL tumors, and 2 CD19-negative relapses were detected by the NCI group.[27,28] It has been reported that some

of the CD19-negative relapses were due to alternative splicing of CD19 exons that removed the epitope recognized by the CAR, although it is possible that the entire CD19 protein also may be downregulated.[37] However, CD19-negative acute leukemia relapses after CD19 CAR T-cell infusion also have been detected with a myeloid immunophenotype.[38] Based on genetic analysis of the leukemia before and after CAR T-cell treatment, it appears the immune escape was due to selective outgrowth of a pre-existing CD19-negative myeloid clone, or possibly a retro-differentiation of a B-ALL to a myeloid leukemia.

CAR T Cell–Associated Toxicities

Inflammatory Syndromes

Reinduction of CRs was associated with a unique set of clinical signs and symptoms of a massive inflammatory disorder. Shortly after infusion of CAR T cells, patients developed high-grade fevers that progressed, along with hypotension and respiratory distress. A large increase in cytokines coincided with these toxicities, so this disorder has been classified as CRS.[23–28] It is likely that the CRS is related to the widespread activation of a large number of tumor-specific T cells, considering that similar toxicities have been reported with blinatumomab and anti-CD28 antibodies.[39,40] Although some cytokines, such as interferon-γ, IL-6, and IL-10, are commonly increased after CAR T-cell infusion, there is no consistent pattern of cytokine upregulation from patient to patient, which is most likely due to the individualized nature of the CAR T-cell therapy. The CRS can become severe and require intensive medical management, which occurred at all trial sites. Grading schemes have been developed to identify CRS that requires aggressive monitoring and interventions. The MSKCC group identified severe CRS based on the presence of fevers, cytokine elevations, and clinical signs of severe cytotoxicity, such as hypotension requiring vasopressor agents or hypoxia requiring mechanical ventilation.[23] A collaborative group of CAR T-cell investigators developed a revised scheme based on the requirement of medical interventions to support patients; grade 1 is self-limiting, whereas grade 4 is life-threatening.[41] Comparison of the grading schemes suggests that grades 3 to 5 by the criteria of Lee and colleagues[41] would be classified as severe CRS by the criteria of Davila and colleagues.[23]

Severe CRS was reported by the MSKCC group in 7 out of 16 patients, by the UPENN group in 8 out of 30 patients, by the NCI group in 6 out of 21 patients, and by the FHCRC group in 7 out of 30 patients.[23–28] CRS has even resulted in fatal toxicities, although this has been rare. Only 2 deaths out of 97 B-ALL patients treated with CD19-targeted CAR T cells were attributed to CRS.[23–28] For most patients, mild to moderate CRS (ie, grades 1–2) is self-limiting and requires supportive care alone, but in severe cases medical intervention is required. Cytokine-directed therapy and corticosteroids are the mainstay of CRS medical management. Cytokine-directed therapy includes tocilizumab, which inhibits IL-6 receptor signaling, and etanercept (Enbrel, Amgen), which inhibits tumor necrosis factor signaling. Tocilizumab is more widely employed because IL-6 commonly increases during the intensification of CRS. Tocilizumab rapidly ameliorates the CRS without any reported deleterious effects on CAR T-cell expansion or persistence, or durability of remissions.

The MSKCC group treated 3 patients with tocilizumab (Actemra, Genentech), 2 with corticosteroids, and 1 with both tocilizumab and corticosteroids; the UPENN group treated 9

patients with tocilizumab and 6 with corticosteroids, the NCI group treated 2 patients with tocilizumab and 2 with both corticosteroids and tocilizumab, and the FHCRC group treated 7 patients with tocilizumab and 3 with corticosteroids.[23–28] There were reports that corticosteroids may inhibit CAR T-cell expansion and reduce the durability of remissions, but this was likely due to prolonged administration.[19,23,24] Considering that corticosteroids rapidly reduce cytokines and eliminate fevers, shorter pulses should attenuate the CRS without affecting CAR T-cell expansion and function.

Clinical investigators have evaluated whether laboratory markers could be used to predict patients who will have severe CRS. The MSKCC group determined that leukemia burden strongly predicted which patients have severe CRS.[23] All but 1 of the patients with morphologic residual leukemia (marrow blasts ≥5%) developed CRS, whereas none of the patients with MRD or in molecular CR had severe CRS. This observation has been confirmed by all the other CAR T-cell studies of B-ALL. The MSKCC group also determined that C-reactive protein could be monitored daily and used to identify patients who would shortly develop severe CRS toxicities.[23] All the CD19 CAR T-cell studies have confirmed that elevation of certain cytokines, such as IL-6 and interferon-γ, strongly correlate with severity of CRS.[23–28] The NCI group also demonstrated that CAR T-cell expansion correlated with CRS severity.[27] Based on clinical experience with CD19-targeted CAR T cells, management schemes for CRS have been developed. The guidelines from Lee and colleagues[41] reserve tocilizumab and/or corticosteroids for patients with severe CRS (grade ≥3) or patients with moderate CRS (grade 2) who have comorbidities. The guidelines from Davila and colleagues[23] similarly recommend tocilizumab and/or corticosteroids for severe CRS, but also include tumor burden as a branch point for management. Based on the extensive data correlating CRS severity with leukemia burden, patients with MRD can be infused and potentially discharged with minimal follow-up because their probability of CRS toxicity is very low.

There is also evidence that some patients experience another inflammatory disorder, hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), which is a toxicity mediated by abundant T-cell and macrophage activation and proliferation. Some of the laboratory abnormalities detected in patients treated with CAR T cells that suggest HLH/MAS include hyperferritinemia, coagulopathies, pancytopenia, and hemophagocytosis on bone marrow biopsies.[23–28] Whether this represents a separate inflammatory disorder from CRS or is potentially an overlap syndrome is difficult to distinguish based on the limited data on patient toxicities. Fortunately, because anti-inflammatory treatments such as corticosteroids are the mainstay of treatment for HLH/MAS, the distinction is not affecting management of clinical toxicity.

Neurotoxicity

All clinical trials evaluating CD19-targeted CAR T cells for B-ALL have reported neurologic toxicities after treatment. These toxicities include word-finding difficulty, aphasia, encephalopathy, obtundation, and generalized seizures.[23–28] The exact mechanism for neurotoxicity is currently unknown. Neurotoxicities and CRS are considered to be separate toxicities because they can occur at disparate times during the clinical course. The

UPENN group reported that 6 of 13 cases of neurologic complications occurred after CRS had completely resolved.[25] However, neurologic toxicities are still probably related to T-cell activation because similar complications develop in patients treated with blinatumomab.[42] Indeed, CAR T cells can be detected in the cerebrospinal fluid after treatment,[23–27] and Turtle and colleagues[28] reported that peak serum cytokine levels correlated with severity of neurotoxicity. This suggests that en masse activation of the CAR T cells either directly or indirectly endows the cells with the ability to traverse the blood-brain barrier. However, the presence of B-ALL in the central nervous system (CNS) also may contribute to neurologic toxicities because CAR T cells are increased in the CNS of patients with residual disease vs the CNS of patients without residual CNS disease. Severe (grade ≥3) neurotoxicity occurred in 15 out of 30 patients in the FHCRC group and 6 of 17 patients in the MSKCC group.[23,28] Neurotoxicity of any grade occurred in 6 of 21 patients in the NCI group and 13 of 30 patients in the UPENN group.[25,27]

Management of neurotoxicity in these patients includes prophylaxis and medical interventions. Many patients received seizure prophylaxis medications, but currently there is no evidence that prophylaxis has reduced the number of neurologic complications and/or severity. As with CRS, the medical interventions for neurologic toxicities are tocilizumab and corticosteroids.[23–28] Although nearly all patients ultimately respond to corticosteroids, it is unclear whether tocilizumab ameliorates neurologic toxicities, given that this antibody is unable to cross the blood-brain barrier. However, tocilizumab still may provide some benefit by reducing inflammation, which could impede the ability of CAR T cells to cross the blood-brain barrier. Fortunately, after the neurologic complications resolve, most patients have no long-term neurologic deficits except for partial amnesia regarding their hospital course.

B-Cell Aplasia

The majority of CAR T cells evaluated for B-cell malignancies target CD19, which in addition to being expressed on most B-cell malignancies, is also expressed on normal B cells. As such, it was expected that a robust CAR T-cell response would also deplete normal B cells. Indeed, that was confirmed in early studies of patients with NHL who had prolonged B-cell aplasia that lasted a year or longer after CAR T-cell infusion.[17,19] As more patients have been treated, there have been reports of B-cell aplasias lasting a year or longer.[23–28] These patients are managed with antibiotics and/or infusional gamma globulin until B cells recover. Presumably the lack of dangerous toxicities is related to the persistence of plasma cells that do not express CD19 and are able to secrete antibodies, thereby preserving humoral immunity.[43]

Conclusions

In a short time, CAR T cells have advanced from the bench to the bedside. The early-phase clinical trials revealed dramatic efficacy with durable remissions in patients with acute leukemias refractory to standard salvage chemotherapies. In the next couple of years, the first gene-modified cell therapy will likely be approved, with an indication for B-cell malignancies. As CAR T-cell evaluation expands to multicenter phase 2 trials, the clinical

expertise with this therapy will broaden. Other academic medical centers will mirror our institutions (Moffitt and Memorial Sloan Kettering), which have developed dedicated medical services to administer this therapy and manage the patients. With the ongoing development of multiple treatment sites, we expect further breakthroughs in understanding the nature of the toxicities and developing targeted supportive therapies that minimize the complications while preserving the therapeutic benefits. Furthermore, exciting preclinical work describing the next-generation of CAR T-cell therapeutics will hopefully allow this innovative, living drug to target not only B-cell malignancies, but other hematologic malignancies and solid tumors as well.

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