Chimeric Antigen Receptor T-cell Therapy to Target Hematologic Malignancies

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Abstract

Several decades of humoral immunotherapy using monoclonal antibodies and cellular immunotherapy using hematopoietic cell transplantation have recently culminated in a successful merger: the development and clinical application of genetically engineered antibody–T cell chimeras. Also known as chimeric antigen receptor T cells (CAR T cells), these entities combine the exquisite antigen specificity of antibodies with the polyfunctionality and potency of cellular immunity and are a prime example of the potential for synthetic biology to treat disease. CAR T cells overcome several of the biologic obstacles that have historically hampered immunotherapy while providing fundamental mechanistic insights into cellular immunology and revealing new challenges in genetic engineering and target selection. Results from early-phase CAR T-cell–based clinical trials demonstrate the significant potential for this approach to affect dramatic and complete clinical responses while revealing novel toxicities associated with activation of potent and specific antitumor immunity. Cancer Res; 74(22); 6383–9. ©2014 AACR.

Introduction

Synthetic biology combines diverse biologic components in a novel engineered construct and endows each of its basic components with new functionalities (1). In this review, we summarize the state of the field for adoptive cellular therapy with chimeric antigen receptor–modified T cells (CAR T cells), focusing primarily on published results obtained from the most advanced CAR T-cell–based clinical studies for the treatment of hematologic malignancies.

Until recently, there have been three broadly efficacious clinical approaches based on adoptive cellular therapy. The first approach has involved hematopoietic stem cell transplantation, as pioneered at the Fred Hutchinson Cancer Research Center (Seattle, WA) in the 1970s and applied with curative potential in centers around the world (2). The second approach is the treatment of malignancies expressing viral antigens, particularly those of Epstein-Barr virus (EBV), using T cells enriched for anti-EBV specificity (3). This strategy depends on the presence of high-affinity T-cell receptors (TCR) specific for viral antigens, as T cells bearing high-affinity TCR for these non-self-antigens are not deleted during central tolerance and thus remain available for anti-viral responses throughout life. The third example is the transfer of autologous T-cell populations expanded from surgically resected tumor deposits, so-called tumor-infiltrating lymphocyte (TIL) infusions. TIL infusions have been most successful in melanoma (4) and in conjunction with the effect of monoclonal antibodies blocking the immune checkpoints CTLA4 or PD-1 in melanoma suggest that melanoma escapes immunosurveillance by “peripheral tolerance” (5).

The ideal adoptive immunotherapy modality would therefore obviate issues both of central tolerance and of peripheral tolerance. The advent of clinically feasible gene transfer and reliable ex vivo T-cell expansion techniques has led to the current state of affairs, where T cells can now be tailor-made for a particular histologic type of malignancy without needing to be unique to a given patient’s cancer, in clinically relevant quantities using good manufacturing practice (GMP)-compliant techniques.

Endowing Gene-Modified T Cells with Designer Specificity

There are two general approaches to introduce designer antigen specificity: (i) TCR specific to known antigens can be cloned and affinity-enhanced to overcome central tolerance and (ii) single-chain variable fragments (scFv) from antibodies specific to known surface antigens can be cloned into an expression cassette incorporating T-cell signaling domains that render T cells able to respond to antibody-mediated recognition (termed CAR T cells). As defined by the University of Pennsylvania (Philadelphia, PA) group and throughout this review, CAR refers to the transgene construct, whereas “CAR T” refers to the T cells engineered to express the transgene construct.
Affinity-enhanced TCR-engineered T cells can recognize tumor antigens that are presented on the human leukocyte antigen (HLA) molecules of antigen-presenting cells (APC) and of target cells. This approach has led to dynamic activity in a number of cases, its potency highlighted by occasional instances of significant off-tumor toxicity (6, 7). The fact that each construct is specific for a tumor antigen within the context of a particular HLA molecule means that each construct is HLA-restricted. Because in vitro affinity enhancement of TCR does not involve biologic selection, this approach has the potential to generate TCR with novel recognition of alternative protein motifs, as highlighted by the cardiogenic death of two patients treated with T cells engineered to target the tumor antigen MAGE-A3 but which were subsequently found to be cross-reactive to the cardiomyocyte antigen titin (7, 8).

CARs are synthetic polypeptides with a modular design. An extracellular ligand recognition domain is typically derived from an scFv isolated from a monoclonal antibody, although receptor ligands can also be used, such as when using IL13-transduced T cells to target IL13Rα1-expressing tumor cells (9). The scFv binds to a membrane ligand on the target cell with the same affinity as the parent monoclonal antibody, although the presence of multiple copies of the chimeric immunoreceptor on the genetically engineered T cells likely conveys greatly enhanced functional avidity. The scFv is attached to a hinge region that provides flexibility and transduces signals through an anchoring transmembrane moiety to an intracellular signaling domain. The signaling domain is classically the zeta chain of the TCR complex but can be constructed from any activating immunoreceptor signaling molecule.

There are several different approaches to the design and manufacture of CAR T cells. Figure 1 presents a general methodological schema for the ex vivo generation of CAR-engineered cells based on the approach used at the University of Pennsylvania, and a general overview of the in vivo events that lead to tumor destruction and establishment of long-term immunological memory. It is likely that the diverse approaches used by different groups impact on subsequent T-cell functionality and antitumor efficacy.

**Design and Manufacture of CAR T Cells**

Early CAR constructs signaled via the CD3ζα signaling domain without any co-stimulation (10). While these "first-generation" CAR T cells were able to specifically target antigen, they had very modest clinical activity and poor in vivo persistence (11). To overcome these limitations, co-stimulatory molecules such as CD27, CD28, CD134 (OX40), or CD137 (4-1BB) have been incorporated ("second-generation CAR T"; ref. 12). These co-stimulatory molecules may provide different effector functions such as proliferation and cytokine production (13). Third-generation CAR T contain an additional co-stimulatory domain, such as CD28 plus 4-1BB or CD28 plus OX40 although it is unclear whether additional co-stimulation is beneficial or deleterious (14–16).

The optimal design of the hinge and transmembrane domains has spurred less research than other aspects of the CAR T structure but likely impacts CAR T function. The transmembrane domain has been shown to be critical for CAR expression on the surface and the hinge affects scFv flexibility. The CD28 transmembrane domain led to higher expression of CAR as compared with the OX40 transmembrane domain (17), whereas the CD8 hinge appeared superior to IgG4 in preclinical evaluation of CD19-directed CAR T cells (13). Second-generation CAR T cells using CD28 (18, 19) or 4-1BB (20) have been the most frequently used constructs in clinical trials. Most constructs contained CD8 (20) or IgG hinge (21, 22) and CD8 (20, 23) or CD28 (18, 19) transmembrane domains. Preclinical data suggest that the length of the CAR construct may be inversely correlated with its ability to stimulate T cells (16). The modular design of new CAR constructs facilitates evaluation of a wide range of different design configurations. The optimal design of any given CAR remains an area of active investigation and it is conceivable that different tumor targets and different clinical settings may be best served by different combinations of scFv, hinge, transmembrane, stimulator, and co-stimulatory molecules.

Molecular engineering of the desired CAR must be complemented by efficient gene transfer into T cells. Gammaretroviral and lentiviral transduction techniques rely on the propensity of these viruses to integrate permanently into the host genome. GMP-grade viral vector production is a costly, time-consuming, and specialized technique. This approach does lead to acceptable transduction efficiencies and is the basis of most clinical trials to date. Despite early concerns about insertional mutagenesis, decades of experience with retroviral or lentiviral transduction of primary T cells have failed to show a single case of this theoretical complication (24). Unlike retroviral transduction, lentiviral vectors can integrate into nondividing cells, are less susceptible to silencing by host restriction factors, and can deliver large DNA sequences (25, 26).

Nonviral gene transfer techniques represent an alternative and relatively cheaper approach. The most commonly used approaches are transposon/transposase systems such as Sleeping Beauty. This system results in sustained expression of CAR on T cells and anti-CD19 transposon CAR T cells are in early-phase clinical trials (27).

Another novel approach is the electroporation of T lymphocytes with in vitro transcribed RNA (28). This leads to high but transient expression of the CAR, resulting in "biodegradable" CAR T cells due to natural decay of the mRNA species. This approach has been used in a few patients at our institution with preliminary evidence of efficacy (29) and maybe particularly useful in a setting where transient CAR expression is desired, for example, where the safety of the construct has not been previously demonstrated in humans.

With the exception of the abovementioned mRNA electroperoration technique, gene transfer into T cells is typically carried out at the beginning of a period of in vitro culture and stimulation. This system leads to more efficient transduction and to a greatly expanded number of T cells for subsequent adoptive transfer. This strategy also potentially allows the a priori selection of an optimal T-cell subset for manipulation before expansion. T-cell phenotype may be an important
variable in the final CAR T cell product and plays a role in antitumor efficacy and in vivo persistence. For example, the adoptive transfer of CD8\(^+\)CD62L\(^+\) central memory cells (Tcm) resulted in enhanced persistence in vivo compared with more differentiated effector memory cells in non-human primate models, and a clinical trial of anti-CD19 CAR T cells using this concept has been initiated (30, 31). Use of cytokines such as IL7 or IL15 during in vitro expansion and transduction can confer superior antitumor activity (32). A subpopulation of stem cell memory T cells has been described as capable of extensive proliferation and self-renewal and may prove a useful population to study for adoptive immunotherapy (33). These preclinical data are intriguing, and further research is warranted into the optimal T-cell subpopulation for adoptive transfer.

However, it is notable that the most potent and durable clinical responses to date have been attained with bulk unselected populations of CD8 and CD4 T cells (20, 23).

Clinical Experience with CAR T-cell Therapy in Hematologic Malignancies

Clinical trials of CD19-specific CAR T-cell therapy in B-cell hematologic malignancies

Most of the clinical successes of CAR T-cell immunotherapy have been achieved in the setting of B-cell malignancies, targeting CD19 (18–20, 23). CD19 represents an ideal target for immunotherapy as it is expressed on all B cells from early pro-B progenitors to mature B cells and thus can be used for...
any B-cell malignancy. Extensive experience with the monoclonal antibody rituximab has shown that patients tolerate prolonged depletion of normal B cells. Several groups have used different protocols that vary in regard to the CAR design, culture techniques, lymphodepleting strategies, and disease targeted.

The NCI group used CD19-directed second-generation CAR that included a CD28 co-stimulatory domain and used retroviral transduction of T cells expanded with OKT3 and IL2. Objective responses were observed in 6 of 8 patients with non-Hodgkin lymphoma (NHL), accompanied by transient B-cell ablation (19). The Memorial Sloan Kettering Cancer Center (New York, NY) group has treated 16 patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia (B-ALL) with CD28 and CD3zeta anti-CD19 T cells that were retrovirally transduced and expanded using anti CD3/CD28 beads. Complete responses were recorded in 88% of patients, with most of these patients proceeding to allogeneic stem cell transplantation shortly after CAR T-cell treatment (34). Over the past few years, our group at the University of Pennsylvania has reported results in the setting of adult chronic lymphocytic leukemia (CLL) and pediatric ALL (20, 23, 35). In contrast to the NCI or MSKCC groups, the UPenn group used a second-generation CAR construct that incorporated the 4-1BB co-stimulatory molecule, based on preclinical data that showed superior T-cell persistence for the 4-1BB co-stimulated CAR T cells (36). T cells were expanded using anti-CD3/CD28 beads and transduced with a lentiviral vector. Results from the UPenn group were recently updated at the American Society of Hematology annual meeting in December 2013, with dramatic clinical responses in CLL as well as in pediatric and adult ALL, including ongoing complete clinical and molecular remissions in a substantial subset of patients. Because to-date reported complete response rates were higher in patients with ALL than in patients with CLL, it is tempting to conclude that the diverse subtypes of B-cell malignancy treated to date show differential susceptibility to CAR T-cell therapy. However, it is important to note that patients in the reported trials were treated at different stages of disease, after different prior treatment protocols, and in different institutions using somewhat different regimens. Although the number of patients treated at this point is insufficient for a formal comparison, there does not appear to be a dose-dependent response to CAR T cells, as significant clinical responses have been observed independent of the total dose of infused cells or of the dose of engineered T cells. Anecdotally, some of the most profound and durable responses were observed in patients with high tumor burden who received a relatively low number of T cells with low transduction efficiency. A randomized 2-dose level study is currently underway at the University of Pennsylvania to formally address the issue of dose–response effect for CART19 therapy (NCT01747486).

The early success of CAR T-cell–based trials that target CD19 has led to the recruitment of more patients to current clinical trial protocols as well as the establishment of trials at additional institutions, both in the United States and internationally. Collective results from these trials will be crucial for informing future progress in the CAR T-cell field as a whole.

Other antigen targets in B-cell hematologic malignancies

Antigens other than CD19 are under evaluation as CAR T-cell targets in B-cell malignancies. One of the first reports used a first-generation anti-CD20 CAR and was subsequently updated using a third-generation anti-CD20 CAR. A total of 10 patients were treated on 2 different clinical trials. The patients experienced transient stabilization of disease and poor long-term persistence of CAR T cells (14). Secondary targets such as CD22, CD23, or CD38 may also be used in B-cell malignancies (16, 37, 38). It may be desirable in future to avoid or minimize the expected normal B-cell aplasia induced by CAR T cells. To this end, more selective anti-B-cell targets are being developed. ROR1 is thought to be specific for malignant B cells and therefore anti-ROR1 CAR T cells could potentially spare normal B cells (39). Kappa light chain–directed CAR capitalize on the monoclonality of malignant B cells and the polyclonality of normal B cells and are being evaluated in a phase I clinical trial. Preliminary results indicate that this clever approach has led to two complete responses and one partial response among five patients treated (40).

CAR T-cell–Related Clinical Toxicities

B-cell aplasia is an expected on-target toxicity of CD19–CAR T-cell therapy and has been used as a surrogate to assess the persistence and effectiveness of T cells. To mitigate this toxicity, patients receive monthly immunoglobulin replacement, and to our knowledge, there have been no reports of unusual infections.

Anti-CD19 CAR T-cell therapy can lead to a profound antitumor effect, including the elimination of large and disseminated tumor burdens. One collateral effect has been the development of clinical tumor lysis syndrome that is coincident with the maximal in vivo T-cell expansion and antitumor activity (20). A unique complication that results from antigen-specific immune activation is the development of cytokine release syndrome (CRS), which manifests in high fever, hypotension, and hypoxia and is related to production of several proinflammatory cytokines including IL6, TNFα, and IFNγ secondary to CAR T-cell activation. At present, it is unclear whether the primary source of these cytokines is the CAR T cells or other components of the immune system, although in vitro studies clearly show that all CAR T cells can produce these cytokines after antigen exposure (36). Other groups have found the serial monitoring of C-reactive protein levels to be useful in identifying patients who require intensive medical monitoring and pharmacologic management (34). The clinical features of CRS overlap with those of the macrophage activation syndrome (MAS) that is additionally characterized by marked elevation of serum ferritin. Careful, real-time analysis of serum cytokines in CART19-treated patients revealed the potent salutary effect of an anti-IL6 receptor antibody, tocilizumab, on these syndromes, and this drug is now used off-label to control severe CRS/MAS (23). The correlation of CRS/MAS with complete responses in many cases raises a
concern that premature interruption of this cytokine cascade could impair antitumor activity. The exact nature of the relationships between observed elevation in systemic cytokine levels, CAR-mediated T-cell activation, tumor cell targeting, CRS/MAS, and tumor burden are at this point very much undefined. In this regard, it will be of considerable interest to evaluate the extent and breadth of cytokine activation in patients with lower disease burden.

Neurologic sequelae including altered mental status, aphasia, or seizures have been noted in a handful of patients treated with anti-CD19 CAR T cells in some centers (34). These sequelae occurred in the setting of MAS, which is known to be associated with neurologic complications. The mechanism of this important complication is unclear and warrants careful study.

The consequences of "on-target off-tumor" toxicity, that is normal B-cell aplasia, have been very mild. In a broader sense, off-tumor toxicity remains the major obstacle to the development of CART cell therapy, as few truly tumor-specific targets have been identified, and thus healthy tissue expressing the targeted antigen may be susceptible to T-cell-mediated injury. The most informative study in this regard targeted carbonic anhydrase IX (CAIX) in the setting of renal cell carcinoma, with off-tumor expression of CAIX on bile ducts leading to significant dose-limiting hepatotoxicity despite limited persistence of engineered cells (41). In another report, a patient receiving anti-ERBB2 CAR T cells for colorectal cancer suffered fatal pulmonary toxicity due to low-level lung epithelial expression of ERBB2 (42).

Lessons Learned and Challenges Facing CAR T-cell Therapy in Hematologic Malignancies

Published results underscore several important characteristics and challenges related to CART cells. First, as highlighted by studies at the University of Pennsylvania, in vivo persistence appears to be required to achieve a sustained and complete antitumor response. Long-term functional persistence of CD19-directed CAR T cells was documented and correlated with durable complete remissions and ongoing B-cell aplasia (23, 35). At this point, the field is limited by the absence of direct clinical comparisons between different CAR constructs and different infusion strategies.

Treatment of CRS/MAS and of potential neurologic complications, while almost uniformly successful, remains empiric and the optimal time to intervene remains unclear. It may be that cytokine release and macrophage activation are required for the optimal antitumor effect, but this is difficult, if not impossible, to evaluate in mouse models and is best investigated in well-designed clinical trials.

A rare but significant observation has been the development of CD19-negative, antigen loss relapses in the setting of B-ALL after initial complete response to CART19 therapy and in the face of persisting CART19 cells (23), representing immune selection in action and also the exquisite on-target specificity of this treatment approach: In at least one instance, the relapsed CD19 disease was molecularly related to the original CD19-positive disease (23). It will be important to evaluate whether malignancies that are more mature and less proliferative than B-ALL will be prone to the outgrowth of antigen-loss variants.

Future Directions

The greatest challenge in extending the application of CAR T-cell therapy beyond B-cell malignancies is target discovery. To be useful and clinically feasible, a potential CAR T-cell target must be expressed on a majority of cells in the majority of patients within a particular histology and be absent from normal tissues. In multiple myeloma, several CAR targeting BCMA (43), CD38 (44), CD138, or CS-1 (45) have shown preclinical efficacy but only the first of these targets truly fits these criteria. In Hodgkin disease, there are currently two clinical trials of CD30-directed CAR T-cell therapy (46); notable in this approach is that CD30 is also expressed on activated T cells. In acute myeloid leukemia (AML), the lack of leukemia stem cell–specific markers has been a major obstacle. Four patients were treated with anti-Lewis-Y CAR T cells (47); of these, one had reduction in blast count, one had a transient cytogenetic response, and two had stable disease. CAR T-cell therapy is currently under preclinical evaluation in AML with promising results using anti-CD123 CAR and anti-CD4v6 CAR, although neither of these antigens are truly tumorspecific and significant hematopoietic or epithelial toxicity may be expected (48–50).

In the absence of truly tumor-specific antigens, off-target toxicity could potentially be mitigated using combinatorial CAR. If 2 different antigens are co-expressed only on tumor cells, CAR constructs could be designed to trigger only when the T cell is exposed to both antigens simultaneously (51). Another approach to divert off-target toxicity is the use of inhibitory CAR, which combine an antigen recognition domain with the signaling domain of inhibitory receptors CTLA-4 or PD-1, thus limiting T-cell function at off-target sites and diverting the immune system away from unwanted toxicity (52).

Where unwanted CAR T-cell activity must be promptly extinguished, there are several approaches. One is the use of "biodegradable" CAR T cells that are electroporated with in vitro transcribed RNA rather than lentiviral DNA, wherein CAR expression lasts only for the half-life of mRNA (28). Other options include suicide mechanisms incorporating an inducible caspase-9 switch that is activated upon the administration of a dimerizing agent (48) or by designing CAR constructs that contain the extracellular antibody-binding domain of a molecule such as EGFR, so that the transgenic T cells are susceptible to depletion using a clinically available anti-EGFR antibody (50).

CAR T-cell therapy, as exemplified by anti-CD19 targeting for B-cell malignancies, represents a new paradigm in cancer therapy. The early-phase clinical trials using this modality have led to very exciting results in patients with poor-prognosis B-cell malignancies. Careful analysis of these results has uncovered novel toxicities and continues to inform the next generation of CAR T-cell trials. These observations will prove useful in the quest to extend this modality to other hematologic malignancies and indeed solid tumors within the next few years.
Disclosure of Potential Conflicts of Interest

M. Kalos has ownership interest (including patents) in potential royalties from CART19-related IP licensed to Novartis Corporation. No potential conflicts of interest were disclosed by the other authors.

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