CAR T cell immunotherapy for human cancer

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Adoptive T cell transfer (ACT) is a new area of transfusion medicine involving the infusion of lymphocytes to mediate antitumor, antiviral, or anti-inflammatory effects. The field has rapidly advanced from a promising form of immuno-oncology in preclinical models to the recent approval of chimeric antigen receptor (CAR) T cells to treat leukemia and lymphoma. This Review describes opportunities and challenges for entering mainstream oncology that presently face the CAR T field, with a focus on the challenges that have emerged over the past several years.

By applying sophisticated ex vivo culture and cellular engineering approaches to adoptive T cell transfer (ACT), durable clinical responses of otherwise treatment-refractory cancers have recently been achieved, revealing the power and potential of ACT. On the basis of dramatic results, autologous T cells engineered to express a chimeric antigen receptor (CAR) specific for the CD19 B lymphocyte molecule have recently been approved by the U.S. Food and Drug Administration (FDA) for treatment of refractory pre-B cell acute lymphoblastic leukemia and diffuse large B cell lymphoma. In this Review, we focus on (i) the prospects for universal CAR T cells, (ii) the use of CAR T cell therapy for solid tumors, and (iii) emerging disparities in the use and commercialization of CAR T cell therapy.

Three forms of ACT are being developed for cancer therapy; these include tumor-infiltrating lymphocytes (TILs), T cell receptor (TCR) T cells, and CAR T cells. TILs have been shown to induce durable complete responses in patients with metastatic melanoma in a variety of clinical trials. The rationale for TIL therapy has been strengthened by recent data demonstrating that TILs can target neoantigens in melanoma (7); the status of TIL therapy is further discussed in (2). Similarly, gene transfer technology has been applied to peripheral blood T lymphocytes to generate cells with transgenic TCRs or CARs. A number of pharmaceutical and biotechnology companies are now commercializing these various forms of ACT (9).

Genetically engineered T cells: TCR versus CAR T cell immunotherapy

TCRs consist of an α- and a β-chain noncovalently associated with the CD3 complex on the T cell surface (Fig. 1). Activation of T cells occurs when the TCR recognizes peptides noncovalently bound to major histocompatibility complex (MHC) on the surface of antigen-presenting cells or tumor cells. The first TCR T cell cancer immunotherapy used in the clinic was tested against metastatic melanoma and utilized a TCR that bound a human lymphocyte antigen A2 (HLA-A2)–restricted peptide from a melanocytic differentiation antigen (4). Subsequently, a higher-avidity TCR targeting the MART-1 (melanoma antigen recognized by T cells 1) epitope was developed, with the aim of achieving enhanced recognition of malignant cells with lower MART-1 expression. Although an improved response rate was demonstrated, it came with a cost of also targeting normal melanocytes in the skin, eye, and cochlea (5). Such on-target, off-tumor toxicity occurred in more than half of the treated patients, providing the first clues that the line between efficacy and toxicity when targeting shared antigens may be thin. The onset of fatal neurotoxicity and cardiotoxicity associated with two separate TCR-based therapies directed to the cancer-testis antigen MAGE-A3 further highlighted the challenge (6, 7). However, targeting the cancer-testis antigen NY-ESO-1 with T cells expressing an affinity-enhanced TCR specific for an HLA-A2–restricted peptide produced evidence of clinical efficacy without appreciable toxicity. These observations raised hope that the therapeutic window may not be so narrow for all shared antigenic targets (8); engineered NY-ESO-1 T cells are now under evaluation in a late-stage clinical trial (NCT01343043, clinicaltrials.gov). Efforts to develop TCR T cell therapies with TCRs specific to particular tumor neoantigens would likely be safer than targeting shared antigens (9); however, this has not been tested clinically.

A CAR combines antigen-binding domains—most commonly, a single-chain variable fragment (scFv) derived from the variable domains of antibodies with the signaling domains of the TCRζ chain and additional costimulatory domains from receptors such as CD28, OX40, and CD137 (Fig. 1). CARs overcome some limitations of engineered TCRs, such as the need for MHC expression, MHC identity, and costimulation. Groups led by Kuwana and Eshhar first showed that these types of synthetic receptor molecules enabled MHC-independent target recognition by T cells (9, 10). The independence of CAR recognition from MHC restriction endows the CAR T cell with a fundamental antitumor advantage, because a major mechanism of immunoevasion by cancer is loss of MHC-associated antigen presentation by tumor cells (11). One limitation of current CAR T cell strategies is that they require extracellular surface targets on the tumor cells. The characteristics of CAR and TCR T cells are compared in Table 1.

B cell malignancies: Unexpected success with CAR T cells

The results from the initial clinical trials using first-generation CAR designs in patients with various cancers were disappointing. However, in 2011, second-generation CAR T cells targeting CD19 and encoding costimulatory domains emerged as the lead paradigm for engineered T cell therapies in cancer (12–15). Several features make CD19 a nearly ideal target. It displays frequent and high-level expression in B cell malignancies, it is required for normal B cell development in humans (16), and it is not expressed outside of the B cell lineage. Patients successfully treated with CD19 CARs often have profound B cell aplasia (13) with some preservation of plasma cells and prior humoral immunity (17). The loss of B cells after CAR T cell therapy is largely managed by replacement therapy with intravenous immunoglobulin, not unlike the treatment for individuals

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**Table 1. Characteristics of CAR- and TCR-engineered T cells.**

<table>
<thead>
<tr>
<th>CAR T cells</th>
<th>TCR T cells</th>
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<tr>
<td>Signal amplification from synthetic biology: 200 targets can trigger CAR T cells (57)</td>
<td>Sensitive signal amplification derived by evolution of the TCR</td>
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<td>Avidity-controllable</td>
<td>Low-avidity, unless engineered (58)</td>
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<td>CAR targets surface structures: proteins, glycans</td>
<td>TCR targets intracellular proteome</td>
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<td>MHC-independent recognition of tumor targets</td>
<td>Requires MHC class I expression and HLA matching on tumor</td>
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<td>At least decade-long persistence (59)</td>
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<td>Serial killers of tumor cells (60)</td>
<td>Serial killers of tumor cells (60)</td>
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<td>Cytokine release syndrome more severe than with TCR-based therapy</td>
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with genetic deficiencies in B cells owing to CD19 mutations (16).

Early results from CAR T cell trials evaluating other targets indicated that the CD19 off-tumor cross-reactions are not a singular example, but may be generally observed with other lineage-dependent targets. Multiple myeloma, which expresses low levels of CD19, has responded to CD19 CAR T cell therapy (18). In ongoing trials (NCT02546167, clinicaltrials.gov) with CARs targeting B cell maturation antigen (BCMA or CD22) in advanced myeloma, the nonmalignant plasma cells that also express BCMA are eradicated in addition to the malignant myeloma cells (19). The tolerability of off-tumor reactions will depend greatly on the types of noncancerous cells that are targeted.

Most patients with relapsed leukemia achieve complete remission after CD19-specific CAR T cell treatment. However, two forms of resistance to this therapy have emerged. In patients with acute leukemia, the loss of the antigenic epitope on CD19 that is targeted by CAR T cells appears to be a dominant mechanism of tumor escape. This is analogous to mechanisms of antigen escape due to acquired defects in antigen presentation or antigen loss observed with TCR T cell-based therapies (15, 20). The frequency of relapse with CD19-negative variant was 28% in the international trial for young adult and pediatric patients with acute leukemia (21). CD19 loss has not been reported as a form of resistance in patients with chronic lymphocytic leukemia (CLL); resistance in CLL is likely due to a failure of the CAR T cells to proliferate after infusion (22).

Table 2 lists several important translational challenges that need to be overcome in advancing CAR T cell therapy to clinical fruition.

**CAR T moving beyond B cells**

CAR T technology has now been shown to have broader applications beyond CD19, and early-phase clinical trials of CAR T cell targeting BCMA and CD22 have reported similarly potent antitumor activity in multiple myeloma and acute lymphoblastic leukemia, respectively (19, 23). However, BCMA and CD22, like CD19, are highly restricted to the B cell lineage, which resides in tissue that can be targeted with manageable toxicity. Attempts to target tumor-associated antigens in solid tumors have achieved limited success so far.

The ERBB2/HER2 protein is a receptor tyrosine kinase that is frequently overexpressed in cancer and is a validated target for antibody or antibody-drug conjugates. CAR T cell therapy targeting ERBB2/HER2 led to a fatal toxicity in the first patient treated. By using a third-generation CAR with a high-affinity scFv based on Herceptin and CD28 or 4-1BB intracellular signaling domains, it was revealed that the toxicity was apparently caused by recognition and killing of ERBB2-positive cells expressed at low density on the lung epithelium, triggering pulmonary failure and massive cytokine release (24). Lower doses of CAR T cells that have a scFv with lower affinity than the Herceptin-based CAR have proven safe in sarcoma patients but only have modest clinical activity (25).

A phase 1 trial of T cells expressing a first-generation CAR targeting the carbonic anhydrase IX (CAIX) antigen on renal cell carcinoma also encountered unexpected hepatotoxicity, owing to low-density expression of the CAIX antigen on normal biliary epithelium that was not discovered in preclinical studies (26). Delayed respiratory toxicity coinciding with peak T cell expansion in a trial of CAR T cell therapy targeting CEACAM5 also suggested the potential for on-target, off-tumor toxicity with this cancer-associated antigen (27).

Clinical trials of CARs targeting other shared antigens associated with solid tumors including mesothelin, carcinoembryonic antigen, and the GD2 ganglioside have not reported notable toxicity; however, the antitumor activity observed in these trials has also been minimal. GD2-specific CAR T cells with enhanced antitumor activity are capable of inducing fatal neurotoxicity in preclinical models, highlighting the challenge (28). Local-regional injection of CAR T cell therapy targeting the interleukin (IL)-13 receptor c2 on glioblastoma multiforme demonstrated on-target activity without the appreciable toxicity that would be expected if intravenous administration were performed, suggesting that the therapeutic index may be enhanced by direct intratumoral injection for some antigens (29). CAR T cell therapy targeting a tumor-specific antigen, the alternatively spliced variant of epidermal growth factor receptor (EGFRvIII), has demonstrated that this antigen can be safely targeted, but EGFRvIII antigen loss
within the tumor was also observed in some treated subjects, further illustrating the need to target multiple antigens to prevent antigen escape (30).

The tumor microenvironment presents additional barriers to the successful application of ACT, especially in solid tumors. Well-described pathways that inhibit T cell immunity within tumors include immune checkpoints (e.g., expression of PD-L1, a ligand for the programmed death 1 receptor), alterations in the tumor metabolic environment (e.g., hypoxia or expression of indolamine-1-oxidase and arginase), regulatory T cells, and suppressive myeloid cells (37). Many of these immunologic and metabolic checkpoints increase in tumors after ACT, suggesting adaptive resistance (30). Clinical trials that combine PD-1/PD-L1-blocking antibodies with CD19-specific CAR T cell therapies are under way (e.g., NCT02926833, NCT02650999, and NCT02706405; clinicaltrials.gov). In addition to combinations with other checkpoint inhibitors, alternative approaches to disrupting these suppressive pathways, such as switch receptors or gene editing, are also under study (32).

Toxicities with CAR T cell therapy

Although some degree of immune stimulation and inflammation was expected with T cell activation after ACT, severe cytokine release syndrome (CRS) has been observed with CD19-specific, BCMA-specific, and CD22-specific CAR T cells (Fig. 2). This syndrome can be more severe than the influenza-like syndrome commonly observed with TIL- and TCR-based therapies (33). The severity of the CAR T cell–associated CRS correlates with tumor burden (14, 34). In the most severe form, CRS shares many features with hemophagocytic lymphohistiocytosis and macrophage activation syndrome (35).

Although CRS was an expected toxicity with T cell immunotherapy, unexpected neurologic complications ranging in severity from mild to life-threatening have also been reported across different clinical studies with CD19- and BCMA-specific CAR T cells. The neurologic toxicities described with CD19-specific CAR T cells have been largely reversible (Box 1). It is not known whether the cerebral edema resulting from CAR T cell therapy is an extreme manifestation of CRS or whether there is a separate mechanism of action. In support of the latter, there is evidence for endothelial injury, perhaps related to inflammatory cytokines, contributing to the onset of neurotoxicity (36). The mechanisms underlying T cell immunotherapy–mediated CRS and cerebral edema are poorly understood, in part because the field lacks informative animal models to study these important toxicities.

Improving engineered T cells through cellular engineering

The strength of binding between a ligand and its receptor (affinity) is a fundamental biophysical parameter affecting the outcome of most receptor signaling. Characterizing the affinity of a single TCR for its cognate peptide presented within MHC (pMHC) is complex. In the most simplistic form, the binding reaction between TCR and pMHC can be represented by the equation

\[ \text{TCR} + \text{pMHC} \xrightleftharpoons[k_{	ext{off}}]{k_{	ext{on}}} \text{TCR:pMHC} \]

However, there is considerable debate regarding whether the equilibrium binding constant (\( K_D = k_{\text{on}}/k_{\text{off}} \), where \( k_{\text{on}} \) and \( k_{\text{off}} \) are the association and dissociation rates, respectively) or the dissociation half-life (\( t_{1/2} = 0.693/k_{\text{off}} \)) is the most important parameter affecting the outcome of TCR signaling. Using surface plasmon resonance measurements of TCR affinity, the apparent \( K_D \) values of most functional TCRs for pMHC range from 1 to 100 \( \mu \text{M} \) (37). The role of TCR affinity may be especially relevant to tumor-associated cancer-testis antigens, which are nonmutated self-antigens to which some degree of tolerance likely exists, unlike for foreign antigens (38).

Similar to TCRs, affinity engineering may also be applicable in CAR design to increase the antitumor potency of CAR T cells and modulate on-target, off-tumor toxicity. \( \text{ERBB2/HER2} \) encodes a cell surface receptor implicated in the pathogenesis of numerous epithelial malignancies (39). Varying the affinity of a CAR against \( \text{ERBB2/HER2} \) increases the discrimination between low antigen density, such as that found on healthy epithelial cells, and higher antigen load on tumor cells (40). However, identifying the optimal affinity is not straightforward, as evidenced by improved antitumor activity (but with the emergence of severe neurotoxicity) associated with enhanced binding of a GD2-specific CAR in a preclinical model (28). Beyond affinity, substantial effort has been expended to evaluate the impacts of CAR ectodomain structure, transmembrane domain, and signaling (41), which all can affect CAR function. Unfortunately, few standards have been defined, and CAR design remains largely empirical. In many cases, the functional consequences are also not fully apparent in preclinical experiments, further complicating the CAR design process.

Universal CAR T cells

Although ACT evolved from allogeneic bone marrow transplantation, ACT strategies have focused on autologous T cells owing to the inherent barriers imposed by the MHC. A return to allogeneic donor or “universal” T cells could provide considerable advantages over autologous T cells if the MHC barriers could be eliminated. Universal CAR T cells derived from healthy donors have the potential to overcome the many immune defects associated with cancer treatment. In addition, the use of universal CAR T cell therapies might provide...
opportunities to simplify the manufacturing of engineered cells, perhaps even allowing for the creation of “off-the-shelf” ACT products (42), facilitating more rapid and less expensive treatment compared with autologous patient-specific T cells.

The first study to report the use of gene editing to generate universal CAR T cells without a functional endogenous TCR was by Torikai et al. (43). A pilot trial using TALEN (transcription activator–like effector nuclease)–based engineering in two patients recently demonstrated the feasibility of applying off-the-shelf universal CD19-specific CAR T cell therapy (44). Engraftment of the genetically universal CAR T cells was limited in both subjects, constraining the therapeutic efficacy of the approach in the pilot study. Importantly, subject 1, who was mismatched at all MHC class I alleles, experienced graft-versus-host disease that was associated with the expansion of contaminating, nonedited T cells that retained the endogenous TCR, indicating that more complete editing will be required for the success of this approach. Recognition of MHC class I–deficient cells by natural killer (NK) cells also might have limited engraftment, despite profound immunosuppression induced by alemtuzumab. One appealing strategy to prevent NK lysis of universal CAR T cells is to insert HLA-E and delete HLA-A, -B, and -C, which prevents host T cells from killing the universal CAR T cells (45). Given the rapid progress in the field, it is likely that universal CAR T cells will become widely used. However, the major question remaining is whether the approach will be sufficiently potent to serve as a stand-alone therapy, or whether it will rather act as a bridge for a definitive therapy, such as a stem cell transplant or autologous CAR T cell therapy.

**Genome editing and multipurpose CARs**

Many technologies can introduce targeted double-stranded breaks in DNA, permitting efficient creation of insertion or deletion mutations, which generally inactivates the targeted gene. Homology-directed repair can be used to insert genes of interest at the targeted site. There are many genome-editing tools, including zinc-finger nucleases, mega-nucleases, TALENs, homing endonucleases, and CRISPR-Cas9 nucleases. These technologies have all been successfully applied to engineer T cells. The major issue in the field now is whether bacterially derived Cas9 will be sufficiently immunogenic to interfere with the delivery of CRISPR-Cas9–edited T cells (46).

Human genome editing offers the opportunity to eliminate immunosuppressive signals such as CTLA-4 and PD-1, enhancing the function of T cells, possibly without the toxicity associated with global blockade of immune checkpoint molecules (47). Gene editing has also been used to eliminate genes for CAR targets that are also expressed by the T cell, which may allow targeting of tumor-associated antigens that would otherwise not be amenable to T cell immunotherapy (48). Recently, Eyquem et al. introduced a CAR into the TCR locus so that receptor expression could be controlled under physiologic conditions of the endogenous TCR promoter, thereby markedly enhancing CAR T cell function (49).

Single antigen–based approaches are limited in their ability to discriminate tumor cells from healthy tissue. To provide enhanced specificity toward tumors, combined sensing approaches are increasingly being developed that target two or more antigens. One of the earliest strategies involved splitting of the primary CD3ζ and costimulatory signals from second-generation CARs into two separate chimeric receptors that are coexpressed within the same T cell (50). A synthetic Notch receptor system has also been described that integrates the dual antigenic signals through transcription rather than signaling (51) (Fig. 3). Understanding the pharmacokinetic features of CAR expression in combinatorial antigen-sensing systems will be important because trafficking between physiologic compartments can occur within hours after CAR infusion, when cells retaining CAR expression might still be capable of mediating toxicity.

To mitigate the potential risk of self-reactive immunity associated with ACT, synthetic molecular systems for achieving inducible death of the genetically engineered T cells, often called “suicide switches,” have been developed. The most notable approach uses the pro-apoptotic protein caspase-9 fused to a domain of FKBP12 (inducible caspase-9, or iCasp9). Upon introduction of a dimeric small molecule such as rimiducid, the FKBP12 domains of iCasp9 dimerize, and the T cells undergo rapid apoptotic cell death. The iCasp9 approach has been evaluated in allogeneic donor lymphocyte infusions after hematopoietic stem cell transplantation and has demonstrated

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**Fig. 3. Conditionally expressed CAR using Notch as a signal induction and response pathway system.** The extracellular ligand-binding domain of CAR 1, upon engagement with its cognate ligand (i), induces proteolysis of the intracellular domain of a synthetic Notch (synNotch) receptor, which contains a transcriptional regulator. Upon release, the notch intracellular domain is translocated to the nucleus (ii) to regulate transcription (iii) of the gene encoding CAR 2 downstream of the transcription factor binding site. Translation of the protein (iv) is followed by the surface expression of the CAR (v). In this manner, a conditional CAR expression specific to a second antigen in the presence of the first antigen–specific ligand safely arms the T cell for highly specific recognition.

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**Box 1. Cerebral edema associated with CAR T cell therapy.**

An unanticipated toxicity from CAR T cell therapy has been cerebral edema. Five deaths attributed to cerebral edema were reported in patients treated with JCAR015, the CD19 CAR originally developed by Brentjens and colleagues (14). The company Juno announced that it terminated clinical development of JCAR015 in March 2017. The cause of the cerebral edema occurring in patients treated with JCAR015 was a capillary leak owing to endothelial damage that was restricted to the central nervous system (36). Edema has classically been accepted as a consequence of some forms of physiologic immune activation. Swelling of tumor masses followed by tumor regression occurs after checkpoint therapy (65). However, swelling of tumors in patients treated with CAR T cells has not been reported. The underlying cause of cerebral edema after CAR T cell treatment remains unknown, and the lack of a suitable animal model to study the toxicity hinders research in this area.
robust T cell elimination with the ability to abrogate graft-versus-host disease (52).

**Commercialization of CAR T Cells**

The field of immuno-oncology has emerged as one of the great success stories of the past decade. However, the advent of numerous but often non-curative targeted therapies will increase life span and the prevalence of patients living with cancer (53). There are now more than 250 clinical trials worldwide testing CAR T cells. It is notable that there are disparities in the geographic locations of the trials, with hotspots for translational research occurring in China and the United States and far fewer trials taking place in Europe, Japan, and the Southern Hemisphere (Fig. 4). The reasons for the geographic disparity are likely complex and related to the willingness to adopt and invest in new therapies, divergent regulatory policies by health authorities, and societal differences.

The financial burdens imposed by effective but noncurative therapies that are encountered by patients with hematologic malignancies, particularly CLL and multiple myeloma, are also present by patients with hematologic malignancies. CAR T cells are the most common form of leukemia in the United States; about 100,000 patients were living with the disease in 2000, and, because of improved but noncurative targeted therapies such as ibritumombib and idelalisib, an increase to ~200,000 cases in the United States is projected (54). However, targeted therapies for CLL present a substantial economic burden for both patients and the economy, now estimated at a lifetime cost of $604,000 per patient, and the total cost of CLL management in the United States alone is estimated to exceed $2 billion per year by 2025 (54). It is likely that CAR T cell therapies are more cost-effective than current standard-of-care therapies for leukemia and lymphoma. The bespoke manufacturing processes now used for highly personalized engineered T cell therapies incur high costs. The cost of manufacturing CAR T cells is expected to decrease (55).

A detailed analysis of the public health considerations of the pricing of gene-modified cells is beyond the scope of this Review, but some aspects have recently been summarized (56).

**Future opportunities and applications**

The advent of CAR T cells for leukemia and lymphoma is noteworthy from several perspectives. Perhaps most important is that CAR T cells are the first form of gene transfer therapy to gain commercial approval by the U.S. FDA. Because of the risk of CRS and neurologic toxicities, CAR T cells were approved contingently with a risk evaluation and mitigation strategy, whereby the FDA requires that physicians complete training for management of adverse effects. One of the greatest challenges in developing cell-based therapeutic approaches is the paucity of preclinical models to evaluate the safety and efficacy of these complex therapies before human studies or in response to safety issues that are uncovered in early-phase clinical studies. Although CAR T cells are transforming the management of hematologic malignancies, there are still many hurdles to successfully applying these therapeutic approaches more broadly to solid tumors. Ongoing advances in T cell engineering, gene editing, and cell manufacturing have the potential to broaden the cell-based therapies to other cell types such as induced pluripotent stem cells, hematopoietic stem cells, and NK cells and to foster new applications beyond oncology in infectious diseases, organ transplantation, and autoimmunity.
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