Chimeric Antigen Receptor T Cell Therapy: Challenges to Bench-to-Bedside Efficacy

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Chimeric Antigen Receptor T Cell Therapy: Challenges to Bench-to-Bedside Efficacy

Shivani Srivastava and Stanley R. Riddell

Immunotherapy with T cells genetically modified to express chimeric Ag receptors (CARs) that target tumor-associated molecules have impressive efficacy in hematological malignancies. The field has now embraced the challenge of applying this approach to treat common epithelial malignancies, which make up the majority of cancer cases but evade immunologic attack by a variety of subversive mechanisms. In this study, we review the principles that have guided CAR T cell design and the extraordinary clinical results being achieved in B cell malignancies targeting CD19 with a single infusion of engineered T cells. This success has raised expectations that CAR T cells can be applied to solid tumors, but numerous obstacles must be overcome to achieve the success observed in hematologic cancers. Potential solutions driven by advances in genetic engineering, synthetic biology, T cell biology, and improved tumor models that recapitulate the obstacles in human tumors are discussed. The Journal of Immunology, 2018, 200: 459–468.

Innovations in gene transfer and adoptive T cell transfer (ACT) have converged in a novel approach to cancer therapy in which a patient’s T cells are genetically modified to express synthetic chimeric Ag receptors (CARs) that redirect T cell specificity toward tumor-associated Ags. CAR T cells have shown remarkable success in some hematologic malignancies and serve as an example of how advances in immunology can inform a new class of cancer therapeutics (1). In this study, we review the principles underlying CAR T cell therapy, discuss obstacles to further improve results in hematologic cancers, and extend this approach to common cancers that are the major cause of cancer mortality.

Principles of CAR design and T cell engineering

A CAR is a synthetic construct that, when expressed in T cells, mimics TCR activation and redirects specificity and effector function toward a specified Ag. For cancer therapy, this is accomplished by linking an extracellular ligand-binding domain specific for a tumor cell-surface Ag to an intracellular signaling module that activates T cells upon Ag binding. The earliest first-generation CARs contained only a CD3ζ or Fc receptor γ signaling domain (2), and the addition of one (second generation) or more (third generation) costimulatory domains such as CD28, 4-1BB, or OX40 induced more cytokine production and T cell proliferation (3–5). The constellation of signaling modules in a CAR is usually selected based on analysis of tumor recognition in vitro and in preclinical in vivo models (6–8), and advances in synthetic biology are likely to improve upon constructs currently in clinical trials. For example, strategies for small molecule–mediated regulatory control of CAR expression (9), combinatorial Ag sensing (10), targeted integration of the CAR transgene into defined loci (11), logic gating of CAR recognition to improve tumor selectivity (12, 13), and suicide mechanisms for targeted elimination of transferred T cells (14, 15) have been described and could provide more potent and safe CARs.

The immune cell chassis used to express a CAR is most commonly a T cell derived from the peripheral blood. Peripheral T cells can be broadly divided by surface phenotype into naïve T cell (T N), memory T cell, and effector T cell (T E) subsets. Memory T cells are further subdivided into stem cell memory cells, central memory cells (T CM), effector memory T cells (T EM), and tissue-resident memory cells, each of which has a distinct role in protective immunity (16–18). Current data support a progressive differentiation model such that activation of T N by Ag gives rise to long-lived stem cell memory cells and T CM that can self-renew and provide proliferating populations of shorter-lived T EM and T E (19–21). This understanding has led several groups to focus on defining the starting population of T cells that are genetically modified with CARs and used for ACT, initially in preclinical models and subsequently in clinical trials (22–27). Accumulating data suggest that engineering less differentiated T N and/or T CM, or culturing T cells in conditions that preserve these phenotypes, provides CAR T cell products with superior persistence in vivo (22–28). Thus, as with CAR design, cell product composition can be manipulated to improve potency and potentially reduce
Clinical efficacy: B cell malignancies and beyond

Clinical trials of CAR T cells have proceeded rapidly in B cell malignancies. B cell malignancies are an attractive target for CAR T cells because they express B cell lineage-specific molecules such as CD19, CD20, and CD22, which are not expressed on other tissues, and preclinical data demonstrated that human B cell tumors could be eradicated in immune-compromised mice treated with CAR T cells (29–32). To prepare CAR T cell products for the treatment of patients, T cells are obtained from the blood, activated in vitro to facilitate gene insertion, and modified to express the CAR by viral or nonviral gene delivery. CAR T cells are then reinfused into the patient, often after the administration of lymphodepleting chemotherapy to promote engraftment and proliferation of transferred cells (Fig. 1). Initial reports in patients with relapsed and/or refractory chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and non-Hodgkin lymphoma (NHL) showed remarkable antitumor effects of CD19-specific CAR T cells (33–36). Subsequent larger phase 1/2 trials at a number of centers confirmed the high level of efficacy of CAR T cells, particularly in ALL where complete remission (CR) rates of 70–93% are achieved (26–28, 37–40). CAR T cells administered in these studies varied in T cell subset composition, method of gene delivery, cell manufacturing platform, and used either CD28/CD3ζ or 4-1BB/CD3ζ costimulatory domains. Further studies are necessary to determine optimal product characteristics and CAR design.

A majority of patients with CLL and NHL demonstrate tumor regression after treatment with CD19 CAR T cells; however, the CR rates for these lymph node–based malignancies are lower than for ALL (27, 28, 34, 41–43). Defining the reasons for incomplete response in CLL and NHL is important to improve outcomes and is the subject of ongoing research. Combination therapy with checkpoint inhibitors, cytokines, modulators of the tumor microenvironment (TME), improved CAR design, and/or further genetic modifications of the T cells are being studied to improve efficacy. The initial response rates in patients with refractory leukemia and lymphoma are impressive, but the durability of responses will only be established with longer follow up. CRs have been reported for up to 56 mo after CD19 CAR T cell therapy; and CR continues even after the disappearance of functional CD19 CAR T cells and recovery of normal B cells (44). Understanding the factors that correlate with long-term CR or with relapse will be critical to enhancing the efficacy of CAR T cell therapy.

The eradication of large tumor burdens by CAR T cells is not accomplished without toxicity. Cytokine release syndrome (CRS) is a common complication initiated by release of IFN-γ, TNF-α, and IL-2 by activated CD19 CAR T cells and is associated with fever, hemodynamic compromise, and macrophage activation with production of IL-6 and additional cytokines (45). The severity of CRS correlates with tumor burden, and interventions to block IL-6 signaling, or to suppress cytokine production by immune cells with dexamethasone, are the mainstays of therapy. Algorithms for timing interventions based on clinical and laboratory parameters are rapidly evolving. Neurologic adverse events are observed concurrent with or following CRS in a subset of patients treated with CD19 CAR T cells, and rare fatal cases have occurred. The pathogenesis of neurotoxicity remains to be elucidated: current data suggest that cytokines released by activated T cells play a role by affecting endothelial integrity (46). Finally, an anticipated side effect of targeting CD19 is that normal CD19+ B cells are eliminated. Transient and even prolonged loss of normal B cells can be managed clinically. However, vector designs that permit elimination of CAR T cells and restoration of B cell numbers are effective in animal models and could be applied in patients that achieve durable remission of their malignancy and have persisting CAR T cells (14).

The success of CAR T cells in ALL, CLL, and NHL has encouraged translation of this approach to other malignancies. CARs have been designed to target molecules such as CD123 and Lewis Y on acute myeloid leukemia (47, 48). However, none of the targets are as attractive as CD19 due to their expression on other critical hematopoietic cells and/or lack of uniform expression on the tumor. Multiple myeloma expresses several candidate molecules to target with CAR T cells including BCMA and CS1 (49, 50), and early clinical data targeting BCMA are promising (51). The application of CAR T cells to common solid tumors has proceeded cautiously following a fatal toxicity in a patient treated with a high dose of ErbB2-specific CAR T cells due to recognition of normal epithelial cells (52). Subsequent studies in patients with glioblastoma and sarcoma suggested it may be possible to target ErbB2 safely, although antitumor efficacy was limited in these studies (53, 54). In glioblastoma, a dramatic response was observed after local intracranial administration of CAR T cells specific for IL-13Ra2, and activity of systemically administered CAR T cells specific for EGFRVIII has been reported (55, 56). CAR T cells targeting gD2 have shown activity in patients with Ewing’s sarcoma and neuroblastoma (57, 58). Trials using CAR T cells to target mesothelin, Muc16, Muc1, and ROR1 are in progress (59–63) but, as discussed below, may need to overcome unique obstacles compared with hematologic malignancies.

Barriers to CAR T cell efficacy and potential solutions

Tumor Ag loss. A challenge for CAR T cell therapy in solid tumors is identifying target Ags expressed homogeneously

FIGURE 1. Adoptive cell therapy with CAR-modified T cells.
throughout the tumor and not on normal vital tissues. The success of CAR T cells in B cell malignancies targeting CD19 is tempered by outgrowth of CD19− tumor cells in some ALL patients (26, 64, 65). Few targets with homogeneous expression on epithelial cancers have been identified, and outgrowth of Ag-null tumor cells after CAR T cell therapy is an anticipated resistance mechanism. A strategy to circumvent tumor escape is to target multiple Ags simultaneously, such that only tumor cells that lack expression of all target molecules would escape an antitumor immune response (66). One way to target multiple Ags is to use promiscuous receptors as the Ag-binding portion of the CAR. NKG2D CARs, for example, target multiple ligands expressed on both tumor cells and immunosuppressive cells, whereas CARs using the promiscuous ErbB ligand TIE as the extracellular domain can bind multiple ErbB1-based homo- and heterodimers that are often overexpressed in tumors (67, 68). Another strategy is to link multiple single chain variable fragments (scFvs) in tandem. Several groups have demonstrated that cotargeting CD20 or CD123 in addition to CD19 with bispecific CAR T cells eliminates CD19 loss variants and is superior to targeting CD19 alone in xenograft models (69, 70). Bispecific CAR T cells were also superior to monospecific CAR T cells when targeting Ags with nonuniform expression on solid tumors, such as Muc1 and PSCA for pancreatic tumors, or Her2 and IL-13Ra2 for glioblastoma (71, 72). Of interest, bispecific T cells showed superior activity in vivo compared with 1:1 mixtures of monospecific CAR T cells targeting the same Ags, although the mechanism behind functional superiority remains unclear. Bispecific CARs showed enhanced ZAP70 phosphorylation and downstream signaling when both target Ags were engaged, suggesting that dual-positive tumor cells activate bispecific CAR T cells more efficiently (72). CD19, CD20, and CD22 are attractive for multivalent targeting because they are often coexpressed on B cell malignancies, but identifying other pairs of tumor-associated Ags that are coexpressed on common epithelial tumors but not normal tissues remains a challenge.

Minimizing the escape of Ag-null tumors may also depend on the ability of CAR T cells to induce epitope spreading and engage an endogenous immune response against other tumor-associated Ags. It is possible that CAR T cell–mediated lysis of tumor cells will result in release and cross-presentation of other tumor Ags to endogenous T cells, resulting in a more effective polyclonal antitumor response. Some preclinical studies using CAR T cells have demonstrated epitope spreading and even resistance to rechallenge with Ag-null tumors, suggesting the development of immunological memory to other tumor-associated Ags, although this has yet to be demonstrated in patients (73, 74). Mesothelin-targeting CAR T cells were reported to induce humoral epitope spreading in some patients, although not to Ags overexpressed by the tumor or involved in tumorigenesis (60). Cotreatment of CAR T cells with modulators that enhance cross-presentation or activation of the endogenous immune system may enhance the probability of epitope spreading. For example, CAR T cells secreting IL-12 were able to activate macrophages that mediated elimination of Ag-negative tumor cells in preclinical models (75). Likewise, T cells engineered to express CD40L may better activate cross-presenting CD80+/ dendritic cells, whereas those expressing 4-1BBL can provide direct costimulation to bystander tumor-specific T cells (76, 77). Future studies will be needed to determine whether CAR T cells can be engineered to better engage an endogenous antitumor response and whether this can help combat tumor heterogeneity more effectively.

**Toxicity to normal tissues.** Because few truly tumor-specific targets have been identified, applying principles in synthetic biology that might enable CAR T cells to discriminate between tumor and normal cells expressing the same Ag could improve both the efficacy and safety of therapy. Tuning the affinity of the CAR scFv can allow T cells to distinguish between Ags that are overexpressed on tumor cells but expressed at lower levels on normal cells (78). Tumor Ags thought to be unsafe to target due to wide normal tissue expression, thus, may be targetable if expression levels are sufficiently higher on tumor versus normal cells. CARs designed from scFvs targeting either CD38 or EGFR with >1000-fold reduced affinity conferred effective lysis of tumor cells but spared Ag-positive normal cells (79, 80). Whether this could truly achieve discrimination of tumor and normal cells based on level of Ag expression in clinical settings without the outgrowth of Ag low tumor cells remains to be determined.

Another strategy to increase tumor-specificity is to use AND logic gates that require recognition of two different Ags on the same target cell to elicit full CAR T cell activation (13). The success of this strategy requires identifying Ag pairs that are selectively coexpressed on tumor cells but not normal tissues. One implementation of this strategy is to split the CD3ξ signaling and CD28 costimulatory domains across separate receptors, with each signaling domain linked to an scFv specific for a different Ag (81). However, several studies employing such split-receptor systems have found that CD3ξ signaling alone is sufficient to induce some T cell effector functions, including lysis of single-positive cells (66, 81), suggesting toxicity to single-positive normal tissues may not be avoided. This problem could be solved by using a low-affinity scFv that is incapable of inducing T cell activation when linked only to the CD3ξ signaling domain (13). Development of such dual-signaling CAR T cells is likely to require further optimization of each individual scFv. Other aspects of Ag pairs, such as size and ability to colocalize in the synapse, might also affect their ability to properly activate T cells.

Several groups have built constructs in which CAR expression is regulated by a drug-inducible promoter or in which the recognition and signaling domains are only associated in the presence of a small molecule dimerizer (9, 82, 83). CAR T cells can be transiently activated in vivo by drug administration, and their activity can theoretically be halted if toxicity occurs by withdrawal of the drug. Alternately, timing and location of drug delivery can be adjusted to minimize toxicity. For example, Her2-specific CAR T cells induced rapid pulmonary toxicity as a consequence of recognition of Her2+ cells in the lung (52). If a CAR-inducing drug was delivered several days after infusion, when the majority of intravenously infused T cells have migrated out of the lung, toxicity to normal cells in the lung might be diminished or averted. Likewise, by delivering the drug locally rather than systemically, CAR T cell activity could be restricted to particular tissue compartments.

Engineering T cells in which CAR expression is regulated by input signals found primarily in the TME is another potential
Trafficking to solid tumors. Analysis of the TME has identified a variety of obstacles such as trafficking, immunosuppressive molecules and cells, and immune checkpoints that CAR T cells will need to overcome to be effective in solid tumors (Fig. 2). The efficacy of CAR T cells in hematological malignancies in part may reflect efficient access to tumor cells in the bone marrow and lymph nodes where T cells normally traffic. Recognition of solid tumors requires egress from the blood into the tumor site, and many malignancies evolve such that T cell infiltration is actively impeded (85–87). In situations where the tumor is localized, regional rather than systemic administration of CAR T cells might be effective. Intracranial delivery has been shown to be safe and to have antitumor activity in glioblastoma (56), and intrapleural delivery of CAR T cells was superior to systemic administration in preclinical studies of human pleural malignancy (88).

Improved understanding of mechanisms that promote or exclude T cell infiltration into tumors is likely to create opportunities to improve CAR T cell trafficking, either by additional genetic modification of T cells (89) or by combining CAR T cells with oncolytic viruses or other strategies that promote inflammation at the tumor site (90, 91). CAR T cells can be engineered to express receptors like CCR2 and CCR4 that are specific for chemokines naturally overexpressed by tumors, enabling them to traffic more efficiently to tumors (92–94). Rather than custom engineering T cells to the chemokine profile of individual tumors, a more generalizable strategy is to induce tumors to secrete chemokines to which CAR T cells are already responsive. An oncolytic virus has been used to deliver the chemokine CCL5 (RANTES) to the tumor. CAR T cells already express receptors (CCR1, CCR3, and CCR5) for CCL5, and combination therapy with CCL5-expressing oncolytic virus and CAR T cells synergistically improved survival and tumor clearance in preclinical models (90).

CAR T cell access to tumors might also be improved by combining adoptive therapy with drugs that induce immunogenic cell death (ICD) of tumor cells. Unlike physiological cell death, in which dying cells are cleared without an inflammatory response, ICD induces release of damage-associated molecular patterns, which directly activate dendritic cells to secrete T cell–attracting chemokines and cross-present tumor Ags (95). Local radiotherapy and certain chemotherapeutic agents induce ICD and activate endogenous T cell responses to tumor Ags (96, 97). These modalities also inhibit or eliminate immunosuppressive cell subsets in the TME, resulting in an overall shift to a proinflammatory state and improved immune responses (96–99). A similar regimen may improve CAR T cell infiltration by inducing production of chemokines and creating a favorable environment for CAR T cell function. Unlike genetic engineering–based approaches, such combination therapy has the advantage of modulating multiple immune pathways at once.

The differentiation state of the T cells selected for CAR modification can also influence CAR T cell function and migratory properties in vivo. TCM have superior antitumor function relative to TEM in xenograft models of hematological malignancies due to superior persistence and proliferation (22, 24, 25). However, TEM express higher levels of chemokine receptors and adhesion molecules required for homing to inflamed peripheral tissues and may be better poised to enter solid tumor sites. Despite these attributes, a recent study demonstrated that in vitro–generated TEM expressing a gp100-specific TCR were less effective on a per-cell basis than TCM of the same Ag-specificity against B16 tumors (23). Superior activity was dependent on the ability of TCM to traffic first to secondary lymphoid organs rather than peripheral tissues, which may be necessary to engage APCs in tumor-draining lymph nodes. CAR T cells, however, do not depend on interactions with APCs for activation, and one study demonstrated that CAR T cells engineered to CCR7− T cells accumulated better within solid tumors than those derived from CCR7+ T cells (100). These CAR T cells were more prone to activation-induced cell death, but when CD28 and OX40 costimulation were incorporated into the CAR construct, activation-induced cell death was reduced such that CCR7+ CAR T cells were more effective at clearing tumors than CCR7− CAR T cells. Thus, the best T cell subset for CAR T cell therapy for solid tumors may differ from the subset suited for hematological malignancies, or from the subset used for TCR-based T cell therapy. Further research is needed to define the genetic manipulations of specific T cell subsets that endow the cells with homing and functional properties needed to infiltrate and effectively target solid tumors.

Overcoming the immunosuppressive TME. Migration of CAR T cells into tumor sites is not sufficient to ensure tumor destruction because of the immunosuppressive TME (Fig. 2). Low pH, hypoxia, an absence of vital nutrients, and stromal and immune cells that release suppressive factors are characteristic of the TME and inhibit T cells. Additionally, tumor and infiltrating cells may express inhibitory receptor ligands like PD-L1 that can directly suppress tumor-specific T cells.

Several groups have attempted to enhance CAR T cell activity by combining ACT with modulators of the TME. A promising avenue is the use of checkpoint inhibitors that target the PD-1/PD-L1 or CTLA-4 pathways, which alone have shown efficacy in some cancers (101). Responsiveness to
checkpoint blockade was improved by enhancing priming of tumor-specific T cells and might logically be combined with adoptive transfer of CAR T cells, although the risk of toxicity to normal tissues may be increased (99, 102, 103). Other groups have engineered CAR T cells to secrete anti–PD-L1 Abs (104), knocked out PD-1 and LAG-3 using CRISPR (105–107), or coexpressed switch receptors linking the PD-1 ectodomain to the CD28 endodomain such that engagement of PD-L1 delivers an activating rather than inhibitory signal to the T cell (108, 109). Anti–CTLA-4 Abs can also boost endogenous T cell responses to tumors, but the context in which they might improve CAR T cell responses is unclear. CTLA-4 inhibits T cell responses in part by competing with CD28 for binding to CD80/CD86 on dendritic cells and by physically excluding CD28 from the synapse (110). Thus, CAR T cells with a CD28-signaling endodomain may not be intrinsically affected by CTLA-4 regulation. This is supported by a study demonstrating that short hairpin RNA–mediated knockdown of CTLA-4 improved the function of first-generation CAR T cells in vivo but not second-generation CAR T cells with CD28 signaling domains (111). However, anti–CTLA-4 Abs also promote immune responses in a cell-extrinsic fashion by depleting CTLA-4$^+$ regulatory T cells (Treg) (112, 113), which may benefit CAR T cells. In addition to inhibitory receptor expression, T cell dysfunction may be acquired in the TME by dysregulation of signaling pathways through upregulation of SHP-1 or diacylglycerol kinase, and pharmacologic inhibition of these enzymes can improve the antitumor function of CAR T cells (114, 115).

Overcoming immunosuppressive cells in the TME is likely to be necessary for CAR T cell efficacy. Depletion of Tregs and myeloid-derived suppressor cells with blocking Abs or genetic manipulation has improved the efficacy of T cell therapy in animal models (116–118). Cancer-associated fibroblasts (CAFs), which comprise a majority of tumor stromal cells and express high levels of fibroblast activation protein (FAP), play a central role in establishing the immunosuppressive microenvironment and depositing extracellular matrix proteins to limit T cell penetration. Targeting CAFs with FAP-specific CARs or engineering CAR T cells to secrete extracellular matrix–degrading enzymes improves their ability to infiltrate and lyse tumors (73, 119). Alternatively, engineering CAR T cells to express the proinflammatory cytokine IL-12 can modulate the TME and promote recruitment and activation of macrophages (73, 75, 120, 121).
A number of studies have focused on improving CAR T cell activity by altering their metabolic profiles to enhance cell function in hostile environments. Tumors are often characterized by high levels of adenosine and reactive oxygen species (ROS), both of which directly impair T cell responses (122, 123). Knocking down the adenosine 2A receptor with short hairpin RNA or cotransducing T cells with catalase to enable breakdown of ROS significantly improved CAR T cell persistence and function in vivo (124, 125). Likewise, tumors display elevated levels of extracellular potassium that directly impair TCR-driven Akt-mTOR phosphorylation and effector function. Engineering T cells to overexpress a potassium channel to enable greater potassium efflux effectively undoes this mode of suppression and improves T cell function within the tumor (126).

Overall, CAR T cells face a number of hurdles in combating solid tumors, but some obstacles may be easier to overcome than others. Advances in genetic engineering are proceeding rapidly, and our ability to engineer CARs that, for example, target multiple Ags to overcome tumor heterogeneity and Ag-loss or that coexpress modulators of the TME are now undergoing clinical evaluation. In contrast, one of the highest barriers to success may be the ability of CAR T cells to infiltrate solid tumors. A number of studies have shown that the success of checkpoint inhibitors depends on the presence of tumor-infiltrating T cells, and strategies aimed at enhancing T cell infiltration can overcome tumor resistance to checkpoint inhibition (99, 127). Increasing CAR T cell migration to tumors could potentially increase the response of tumors to other combination therapies as well, such as those aimed at targeting immunosuppressive cells, enhancing CAR survival, and activating the endogenous immune response. Thus, efficient infiltration of tumors is likely to be a rate-limiting step for CAR T cell therapy.

Moving beyond empirical testing

Given the myriad ways in which tumors can suppress T cells, the number of genetic manipulations and combination therapies that could be tested in the clinic are seemingly limitless. A challenge for the field is the need for faithful preclinical models to screen therapeutic combinations before clinical translation. Better tools to analyze posttreatment biopsies will also help maximize our understanding of what resistance mechanisms may evolve and inform the design of future combination therapies.

Models for CAR T cell therapy. A challenge for preclinical studies evaluating the efficacy of CAR T cells is having clinically relevant models that recapitulate the obstacles in human solid tumors (Fig. 3). Most studies have relied on transplanted human tumor xenografts in immune-compromised NOD/SCID/γc−/− (NSG) mice that lack T cells, NK cells, and B cells. The NSG model allows rapid analysis of human T cell recognition of tumor cells in vivo and is useful to evaluate T cell persistence and effector function. However, NSG models fail to develop a clinically relevant TME and do not inform the safety of targets that lack epitope homology and/or normal tissue expression. ACT is also studied in immune-competent syngeneic mouse models, but a majority of these models implant tumor cells into foreign anatomical sites where the tumors grow rapidly and do not coevolve with the host immune system in the same way as human tumors (128). Moreover, endogenous antitumor immune responses are higher in transplanted than autochthonous tumors for the

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<th>Model Type</th>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>Xenogeneic Transplantable</td>
<td>Predicts persistence and lytic ability of human CAR T cells</td>
<td>No host immune system</td>
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<td></td>
<td>Predicts drug response of human tumors</td>
<td>No tumor microenvironment</td>
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<td></td>
<td>Technically easy to use</td>
<td>Cannot answer questions about T cell trafficking, function within the tumor microenvironment, or interaction with host immune cells</td>
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<tr>
<td>Syngeneic Transplantable</td>
<td>Intact host immune system</td>
<td>Tumors are fully mature at implantation and do not co-evolve with host immune system</td>
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<td></td>
<td>Some TME develops</td>
<td>Transplantation process can be artificially immunogenic</td>
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<td></td>
<td>Cell lines can be engineered to express neoantigens or tumor targets</td>
<td>TME is present but artificial</td>
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<td>Technically easy to use</td>
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<tr>
<td>GEM Spontaneous</td>
<td>Tumors develop from clinically relevant mutations and evolve naturally with host immune system</td>
<td>Oncogenic mutations are present from birth, central tolerance may develop to mutations or neoantigens</td>
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<td>Realistic TME develops</td>
<td>Model is slow and variable</td>
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<td>Time to tumor development is variable</td>
<td>Introducing neoantigens or tumor targets requires complex breeding</td>
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<tr>
<td>GEM Inducible</td>
<td>Tumors develop from clinically relevant mutations and evolve naturally with host immune system</td>
<td>Model is slow and variable</td>
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<td>Realistic TME develops</td>
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<td>Tumor inflation can be synchronized by inducible event</td>
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same disease, suggesting the implantation process artificially increases immunogenicity (129, 130).

An alternative to transplantable models is to induce malignant transformation in normal cells in situ with defined oncogenic events. Such genetically engineered mouse (GEM) models recapitulate tumor initiation, progression, and the genetic and histopathological characteristics of human cancers (128). Oncogenic mutations in Kras and p53 can be introduced at birth through a Cre/lox system, such as in the KPC (KrasLSL—G12D/+; p53f/f, Pdx1-Cre) model of pancreatic adenocarcinoma, where a Pdx1-driven Cre restricts the mutations to the pancreas (131). A disadvantage of tissue-restricted Cre expression is that cancer is induced throughout the entire tissue, and the presence of mutations from birth may affect central tolerance and influence the evolution of immune responses differently than if mutations were acquired postnatally. To address this, Tyler Jacks’s group developed an inducible KP GEM model of lung adenocarcinoma in which intratracheal infection with Cre-expressing lentivirus initiates p53 deletion and KrasG12D activation in individual lung epithelial cells (132). Importantly, this model mimics both the development and therapeutic response of human lung adenocarcinomas (99). A drawback of GEM models, however, is their relative lack of CAR targets and neoantigens relative to carcinogen-induced models and human cancer (133). However, model Ags can easily be introduced in the KP model by engineering the lentivirus, and exposing Kras-mutant GEM mice to tobacco smoke can induce a more realistic mutational landscape.

GEM models that reflect the TME of human tumors may give more accurate estimates of treatment efficacy and offer insight into resistance mechanisms that evolve and pathways to target with combination therapy (134). For example, recent studies have used GEM models to study Tregs in tumor development and test strategies for Treg inhibition. One approach to Treg inhibition may be to block IL-35, an immunosuppressive cytokine secreted by tumor-resident Tregs that promotes T cell exhaustion in part by promoting expression of inhibitory receptors like PD-1, TIM-3, and LAG-3 (135). Interestingly, this model accurately predicted that IL-35 and PD-1 blockade would not synergize because IL-35 overexpression and PD-1 upregulation are part of the same suppressive pathway. Thus, it is anticipated that GEM models will be useful for studying impediments to CAR T cell therapy of solid tumors and for identifying rational combination therapies for clinical translation.

Advances in immune monitoring of clinical trials. Identifying methods to enhance CAR T cell therapy will be assisted by discovery-driven approaches to clinical trials. Collecting tumor biopsies and blood pre- and posttreatment, enables thorough analysis of tumors by flow cytometry, immunohistochemistry, and unbiased genome-wide RNA sequencing, and can identify correlates of clinical success or failure. Response to anti–CTLA-4 therapy of localized bladder cancer, for example, was associated with upregulation of ICOS on T cells (136, 137); subsequent studies in mice demonstrated that ICOS expression was required for the efficacy of anti–CTLA-4 in vivo and that activation of the ICOS/ICOSL pathway synergistically enhanced response to anti–CTLA-4 (138, 139). Similar analysis of pre- and posttreatment biopsies and transcriptomic and epigenetic analysis of CD19 CAR T cells is being performed to identify mechanisms of resistance in the ~50% of NHL patients that do not achieve a CR, and where CD19 loss is not the mechanism of escape (27, 28, 42, 140).

New technologies with improved sensitivity and systems analysis will facilitate the identification of pathways associated with therapy response or resistance. Single-cell RNA sequencing can provide unbiased insight into tumor responses, revealing differences in gene transcription that may be obscured by heterogeneity at the cell-population level. At the protein level, methods such as cytometry by time of flight allow analysis of 40 (and up to 100 theoretically) proteins simultaneously, providing high resolution of cell phenotype, and can be coupled to immunohistochemical methods to obtain spatial information of proteins and protein modifications at subcellular resolution (141). Additionally, the development of multiplex immunohistochemistry allows detection of multiple biomarkers simultaneously on tumor biopsies and visualization of cell subsets with tissue architecture preserved. Integrating longitudinal data from gene expression, epigenetics, flow and mass cytometry, and immunohistochemistry will provide a comprehensive understanding of patient responses to therapy and should guide the development of rational, rather than empirical, combinations.

Conclusions
Progress in immune-based therapies is improving outcomes for many patients with advanced malignancies. The development of CAR T cells represents a convergence of insights from multiple scientific fields, but success has thus far been limited to B cell malignancies. Extending this approach to other cancers will require the development of strategies based on understanding the obstacles posed by tumor heterogeneity and the TME that is emerging from sophisticated analytical tools and superior models. These strategies will take advantage of our unprecedented ability to genetically manipulate T cells to confer novel functions, enabling them to target tumor cells and persist and function in hostile circumstances.

Disclosures
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BRIEF REVIEWS: CHALLENGES TO CAR T CELL THERAPY


