

Review article

Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor

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Infusions of antigen-specific T cells have yielded therapeutic responses in patients with pathogens and tumors. To broaden the clinical application of adoptive immunotherapy against malignancies, investigators have developed robust systems for the genetic modification and characterization of T cells expressing introduced chimeric antigen receptors (CARs) to redirect specificity. Human trials are under way in patients with aggressive malignan-

cies to test the hypothesis that manipulating the recipient and reprogramming T cells before adoptive transfer may improve their therapeutic effect. These examples of personalized medicine infuse T cells designed to meet patients' needs by redirecting their specificity to target molecular determinants on the underlying malignancy. The generation of clinical grade CAR⁺ T cells is an example of bench-to-bedside translational science

that has been accomplished using investigator-initiated trials operating largely without industry support. The next-generation trials will deliver designer T cells with improved homing, CAR-mediated signaling, and replicative potential, as investigators move from the bedside to the bench and back again. (*Blood*. 2010; 116(7):1035-1044)

Introduction

The systematic development and clinical application of genetically modified T cells is an example of how academic scientists working primarily at nonprofit medical centers are generating a new class of therapeutics. In this context, gene therapy has been used to overcome one of the major barriers to T-cell therapy of cancer, namely tolerance to desired target tumor-associated antigens (TAAs). This was achieved by the introduction of a chimeric antigen receptor (CAR) to redirect T-cell specificity to a TAA expressed on the cell surface. The prototypical CAR uses a mouse monoclonal antibody (mAb) that docks with a designated TAA and this binding event is reproduced by the CAR to trigger desired T-cell activation and effector functions. Multiple early-phase clinical trials are now under way or have been completed to evaluate the safety and feasibility of adoptive transfer of CAR⁺ T cells (Table 1). These pilot studies have revealed challenges in achieving reproducible therapeutic successes which may be solved by (1) reprogramming the T cells themselves for improved replicative potential, effector function, and in vivo persistence, (2) manipulating the recipient to improve TAA expression and survival of infused T cells, and (3) adapting the gene therapy platform to deliver (1) CARs capable of initiating an antigen-dependent fully-competent activation signal, and (2) transgenes to improve safety, persistence, homing, and effector functions within the tumor microenvironment. Academic investigators who work to both develop and deliver investigational biologic agents such as CAR⁺ T cells are poised to further tighten the pace of discovery between the bench and the bedside to improve the therapeutic potential of genetically modified T cells with redirected specificity. This review builds upon recent articles that describe the immunobiology of CAR⁺ T cells (Table 2) and we highlight how the CAR technology has been adapted to meet the challenges of infusing genetically modified T cells in medically fragile patients with aggressive malignancies and what new

directions the field will need to embrace to undertake multicenter trials to prove their therapeutic efficacy.

Redirecting T-cell specificity through the introduction of a CAR

The generation of T bodies, or CARs, by Eshhar et al has been adapted by investigators as a tool to enable T cells, as well as other immune cells, to overcome mechanisms (eg, loss of human leukocyte antigen [HLA]¹) by which tumors escape from immune surveillance of the patient's endogenous (unmanipulated) T-cell repertoire.^{2,3} The specificity of a CAR is achieved by its exodomain which is typically derived from the antigen binding of a mAb linking the V_H and V_L domains to construct a single-chain fragment variable (scFv) region. The exodomains of CARs have also been fashioned from ligands or peptides (eg, cytokines) to redirect specificity to receptors (eg, cytokine receptors), such as the IL-13R α 2-specific "zetakine."⁴ The exodomain is completed by the inclusion of a flexible (hinge) sequence, such as from CD8 α or immunoglobulin sequence^{5,6} and via a transmembrane sequence, the exodomain is fused to 1 or more endodomain(s) which may include cytoplasmic domains from CD3- ϵ , CD3- γ , or CD3- ζ from the T-cell receptor (TCR) complex or high-affinity receptor Fc ϵ RI.⁷⁻⁹ When CARs are expressed on the cell surface of genetically modified T cells, they redirect specificity to TAA (including TAA on tumor progenitor cells¹⁰) independent of major histocompatibility complex (MHC). This direct binding of CAR to antigen ideally provides the genetically modified T cell with a fully-competent activation signal, minimally defined as CAR-dependent killing, proliferation, and cytokine production. Given that T cells targeting tumor through an endogenous $\alpha\beta$ TCR (and even introduced TCR

Table 1. Clinical trials in the United States infusing CAR⁺ T cells under IND

	Antigen	Tumor target	Viral-specific T cell	Lympho-depletion	CAR generation	ClinicalTrial.gov identifier	Enrolling	SAE	Gene transfer
1	Kappa light chain	B-NHL and B-CLL	No	Yes	First and second	NCT00881920	Yes	TBM	Virus
2	CD19	Lymphoma/leukemia (B-NHL) and CLL	No	No	First and second	NCT00586391	Yes	TBM	Virus
3	CD19	Advanced B-NHL/CLL	Yes	No	First and second	NCT00709033	Yes	TBM	Virus
4	CD19	Lymphoma and leukemia	No	Yes	Second	NCT00924326*	Yes	TBM	Virus
5	CD19	ALL (post-HSCT)	Yes	No	Second	NCT00840853	Yes	TBM	Virus
6	CD19	Follicular NHL	No	Yes	First	NCT00182650	No	No	Electroporation
7	CD19	CLL	No	Yes/no	Second	NCT00466531*	Yes	Yes (1 Death)	Virus
8	CD19	B-NHL/leukemia	No	Yes	First and second	NCT00891215	Yes	TBM	Virus
9	CD19	B-cell leukemia, CLL and B-NHL	No	No	Second	NCT01087294	Yes	TBM	Virus
10	CD19	B-ALL	No	Yes	Second	NCT01044069	Yes	TBM	Virus
11	CD19	B-lymphoid malignancies	No	Yes	Second	NCT00968760	No	TBM	Electroporation (SB system)
12	CD20	Relapsed/refractory B-NHL	No	Yes	First	NCT00012207*	No	No	Electroporation
13	CD20	Mantle cell lymphoma or indolent B-NHL	No	Yes	Third	NCT00621452	Yes	TBM	Electroporation
14	GD ₂	Neuroblastoma	Yes	Yes	First	NCT00085930*	Yes	No	Virus
15	CEA	Adenocarcinoma	No	No	First	NCT00004178	No	No	Virus
16	PSMA	Prostate cancer	No	Yes	First	NCT00664196	Yes	TBM	Virus
17	CD171/L1-CAM	Neuroblastoma	No	No	First	NCT00006480	No	No	Electroporation
18	FR	Ovarian epithelial cancer	No	No	First	NCT00019136	No	No	Virus
19	CEA	Stomach carcinoma	No	No	Second	NCT00429078	Yes	TBM	Virus
20	CEA	Breast cancer	No	No	Second	NCT00673829	Yes	TBM	Virus
21	CEA	Colorectal carcinoma	No	No	Second	NCT00673322	Yes	TBM	Virus
22	IL-13R α 2	Glioblastoma	No	NA	Second	NCT00730613	No	No	Electroporation
23	ERBB2 (HER2/neu)	Metastatic cancer	No	Yes	Third	NCT00924287	No	Yes (1 Death)	Virus
24	HER2/neu	Lung malignancy	Yes	No	Second	NCT00889954	Yes	TBM	Virus
25	HER2/neu	Advanced osteosarcoma	No	No	Second	NCT00902044	Yes	TBM	Virus

ALL indicates acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; GD₂, disialoganglioside; FR, alpha folate receptor; CEA, carcinoembryonic antigen; L1-CAM, L1 cell adhesion molecule; PSMA, prostate-specific membrane antigen; ERBB2, receptor tyrosine-protein kinase erbB-2; Her2, human epidermal growth factor receptor; HSCT, hematopoietic stem cell transplantation; NA, not available; SB, Sleeping Beauty; TBM, to be monitored; SAE, serious adverse event; and IND, investigational new drug.

*Studies have been described to demonstrate a CAR-mediated antitumor effect based on reduction in tumor size. Other trials demonstrated a biologic effect of CAR⁺ T cells based on reduction in biologic markers of tumor activity.

Table 2. Published reviews since 2003 on immunobiology and clinical applications of CAR⁺ T cells

	References	Authors	Year published
1	11	Rossig and Brenner	2003
2	12	Willemsen et al	2003
3	13	Baxevas and Papamichail	2004
4	14	Rossig and Brenner	2004
5	15	Rivière et al	2004
6	16	Thistlethwaite et al	2005
7	17	Kershaw et al	2005
8	18	Dotti and Heslop	2005
9	19	Cooper et al	2005
10	20	Foster and Rooney	2006
11	21	Biagi et al	2007
12	22	Rossi et al	2007
13	23	Varela-Rohena et al	2008
14	24	Eshhar	2008
15	25	Marcu-Malina et al	2009
16	26	Berry et al	2009
17	27	Sadelain et al	2009
18	28	June et al	2009
19	29	Dotti et al	2009
20	30	Till and Press	2009
21	31	Vera et al	2009

chains³⁴) exhibit such a fully-competent activation signal, investigators have iteratively designed and tested CARs, for example, with 1 or more activation motifs, to try and recapitulate the signaling event mediated by $\alpha\beta$ TCR chains. These changes to the CAR are enabled by the modular structure of a prototypical CAR and this has resulted in first-, second-, and third-generation CARs designed with 1, 2, or 3 signaling endodomains (Figure 1) including a variety of signaling motifs, such as chimeric CD28, CD134, CD137, Lck, ICOS, and DAP10.^{6,35-37} A listing of such CARs is provided by Berry et al²⁶ and Sadelain et al.²⁷ While the optimal CAR design remains to be determined, at present it is believed that the first-generation technology, in which a CAR signals solely through immunoreceptor tyrosine-based activation motif (ITAM) domains on CD3- ζ , is insufficient to sustain in vivo persistence of T cells.³⁸ This is supported by early clinical data which demonstrate that CD19-specific,³⁹ CD20-specific,⁴⁰ GD₂-specific (not Epstein-Barr virus [EBV] bispecific),⁴¹ and L1-CAM (cell adhesion molecule)-specific⁴² T cells had apparently short-lived persistence in peripheral blood. The decision regarding which second- or even third-generation CAR design to use in clinical trials is predicated on the ability of a CAR to activate T cells for desired T-cell effector function, which at a minimum includes CAR-dependent killing. However, it is possible that next-generation CARs can be engineered to provide a supraphysi-

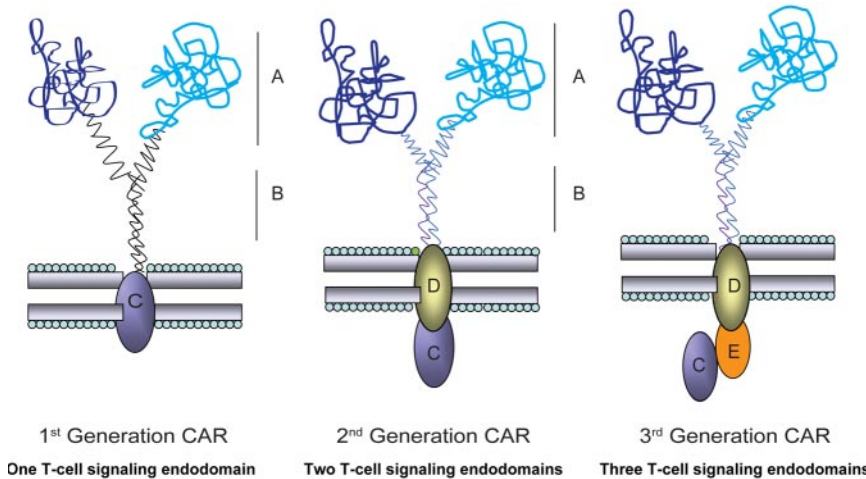


Figure 1. Modular structure of prototypical CAR. CAR shown dimerized on the cell surface demonstrating the key extracellular (A-B) and intracellular (C-E) domains. CARs may express 1, 2, or 3 signaling motifs within an endodomain to achieve a CAR-dependent fully-competent T-cell activation signal. The modular structure of the CAR's domains, for example, the scFv (V_L linked to V_H) region (A) and the flexible hinge and spacer, for example, from IgG4 hinge, C_H2, and C_H3 regions (B), allow investigators to change specificity through swapping of exodomains and achieve altered function by varying transmembrane and intracellular signaling moieties (C-E).

Modifying the CAR to achieve a fully competent activation signal and reduce immunogenicity

The inclusion of 1 or more T-cell costimulatory molecules within the CAR endodomain is in response to the appreciation that genetically modified and ex vivo-propagated T cells may have down-regulated expression of desired endogenous costimulatory molecules (eg, CD28) or that the ligands for these receptors may be missing on tumor targets (eg, absence of CD80/CD86 on blasts from B-lineage acute lymphoblastic leukemia). By way of example, chimeric CD28 and CD3- ζ ,^{38,44} CD137 and CD3- ζ ,^{23,36} or CD134 and CD3- ζ ,^{45,46} have been incorporated into the design of second-generation CARs with the result that these CARs with multiple chimeric signaling motifs exhibited effector function stemming from a both a primary signal (eg, killing) and costimulatory signal (eg, IL-2 production) after an extracellular recognition event.^{47,48} Animal studies have demonstrated that T cells expressing a second-generation CD19-specific^{36,38} and carcinoembryonic antigen-specific CAR⁴⁹ exhibited an improved antitumor effect compared with T cells bearing a first-generation CAR. In addition to altering the endodomains, investigators have also made changes to the scFv to improve affinity based on selecting high-affinity binding variants from phage arrays.^{50,51} The approach to developing CAR⁺ T cells with a calibrated increase in functional affinity may be necessary to enable genetically modified T cells to target tumors with low levels of antigen expression or perhaps to target a cell-surface molecule in the presence of soluble antigen.^{52,53} Because a CAR typically contains a murine scFv sequence which may be subject to immune recognition leading to deletion of infused T cells, investigators have developed humanized scFv regions to target carcinoembryonic antigen (CEA)^{54,55} and ERBB2

(a member of epidermal growth factor receptor family).⁶ However, a benefit for using human or humanized scFv regions is yet to be established in the clinical setting.

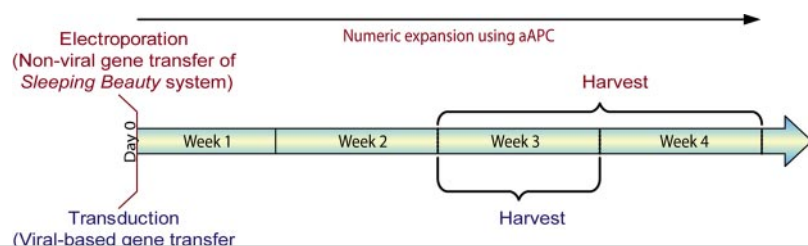
Imaging CAR⁺ T cells by positron emission tomography

The ability to genetically modify T cells to redirect specificity provides investigators with a platform to express other transgenes such as for noninvasive imaging. Such temporal-spatial imaging is desired as a surrogate marker for a CAR-mediated antitumor effect and to serially determine number and localization of infused T cells. One imaging transgene coexpressed with CAR is thymidine kinase (TK) and associated mutants from herpes simplex virus-1 (HSV-1)⁵⁶ which can be used to enzymatically trap radioactive substrates within the cytoplasm to image the locoregional biodistribution of T cells using positron emission tomography.⁵⁷⁻⁵⁹ The expression of TK also renders CAR⁺ T cells sensitive to conditional ablation using ganciclovir in a cell-cycle-dependent manner.⁶⁰⁻⁶²

Gene transfer of CARs

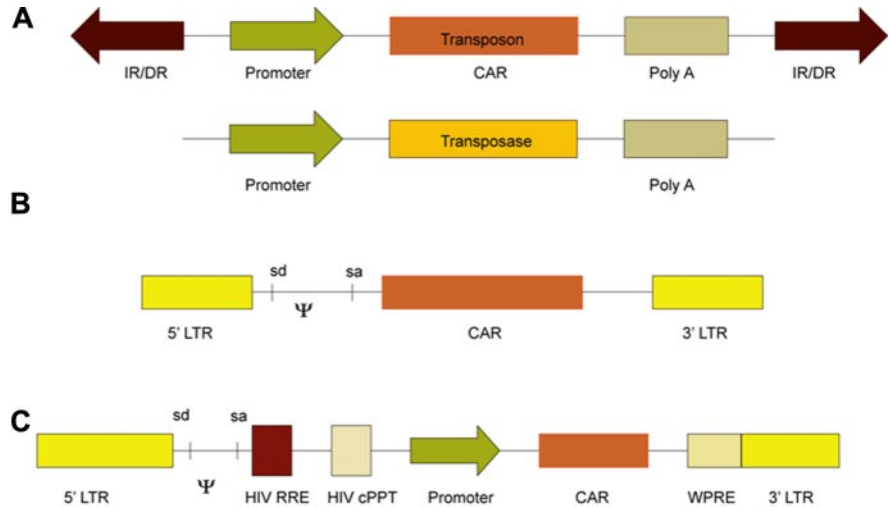
Approaches to the genetic manipulation of T cells for the introduction of CAR transgene use either viral-mediated transduction, or nonviral gene transfer of DNA plasmids or in vitro-transcribed mRNA species. The advantage of retrovirus⁶³⁻⁶⁵ or lentivirus^{23,36,66,67} to modify populations of T cells lies in the efficiency of gene transfer, which shortens the time for culturing T cells to reach clinically-significant numbers (Figure 2). Gamma retroviruses, the

Figure 2. Timeline for in vitro gene transfer and propagation of CAR⁺ T cells. The electrotransfer of transposon/transposase systems has narrowed the gap between nonviral and viral-based gene transfer for the amount of time in tissue culture needed to generate a clinically sufficient number of genetically modified CAR⁺ T cells. Cells transduced with virus (blue text) are typically



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Figure 3. Schematic of vector systems to express CAR transgenes used in clinical trials. (A) Two SB DNA plasmids expressing a (CAR) transposon and a hyperactive transposase (eg, SB11). Transposition occurs at a TA dinucleotide sequence when the transposase enzymatically acts on the internal repeat flanking the transposon. (B) A recombinant retroviral vector showing the long terminal repeats (LTR) containing the promoter flanking the CAR. SD and SA are the splice donor and splice acceptor sites, respectively, and ψ is the viral packaging signal. (C) A self-inactivating recombinant lentiviral vector construct containing the LTR, ψ , SD and SA sites, HIV Rev response element (RRE), HIV central polyurine tract (cPPT), CAR under control of an internal promoter, and the wood-chuck hepatitis virus posttranscriptional regulatory element (WPRE).



most common vector system used to genetically modify clinical grade T cells (Figure 3), have introduced CAR into T cells with a proven ability to exert a therapeutic effect.⁴¹ Self-inactivating lentiviral vectors (Figure 3) hold particular appeal as they apparently integrate into quiescent T cells.²⁸ These recombinant viruses often have a high cost to manufacture at clinical grade in specialized facilities skilled in current good manufacturing practice (cGMP) which may preclude investigators from undertaking clinical trials. As an alternative to transduction, we and others have adapted electroporation as an approach to the nonviral gene transfer of DNA^{42,70,71} resulting in CAR⁺ T cells that have been attributed to have an antitumor effect in a clinical trial.⁴⁰ Previously, the electrotransfer and integration of naked plasmid DNA into T cells was considered inefficient because it depended on illegitimate recombination for stable genomic insertion of nonviral sequences. As a result, lengthy in vitro culturing times were required to select for T cells bearing stable integrants^{72,73} during which period the T cell became differentiated, and perhaps terminally differentiated, into effector cells which may have entered into replicative senescence. This time in tissue culture can now be considerably shortened by using transposon/transposase systems such as *Sleeping Beauty* (SB)^{70,74,75} and *piggyback*^{76,77} to stably introduce CAR from DNA plasmids (Figure 3).^{71,76,78-84} We have shown that the SB system can be used to introduce CAR and other transgenes into human T cells with an approximately 60-fold improved integration efficiency compared with electrotransfer of DNA transposon plasmid without transposase⁷⁴ and this provided the impetus to adapt the SB system for use in clinical trials.⁸⁵ After electroporation, T-cell numbers from peripheral and umbilical cord blood can be rapidly increased in a CAR-dependent manner by recursive culture on γ -irradiated artificial antigen-presenting cells (aAPC) achieving clinically sufficient numbers of cells for infusion within 3 to 4 weeks after electroporation (Figure 2). Given that T cells transduced with γ -retrovirus are typically cultured with OKT3 and IL-2 for approximately 3 weeks before infusion, the use of the SB system with aAPC does not appear to greatly lengthen the ex vivo culturing process.

cell-surface TAAs as described in recent reviews (Table 2). To enable these CAR⁺ T cells to achieve their full therapeutic potential in clinical trials, there are 3 major challenges to be overcome.

Persistence

The adoptively transferred CAR⁺ T cells must survive and perhaps also numerically expand to achieve a robust antitumor effect. One approach to improving persistence is to alter the host environment into which the T cells are infused. For example, rendering the recipient lymphopenic, or even aplastic by chemotherapy and/or radiation therapy, improves the persistence of adoptively transferred T cells (and natural killer [NK] cells).⁸⁶⁻⁹⁰ Presumably, the infused T cells proliferate in the lymphopenic recipient through homeostatic mechanisms mediated by the removal of regulatory/suppressor cells and the ready availability of previously scarce homeostatic cytokines. This approach likely improves in vivo persistence and thus efficacy of CAR⁺ T cells as a published trial and animal studies have hinted.⁴⁰ However, combining chemotherapy and T-cell therapy might increase toxicity and compromise the interpretation of an antitumor effect for it may be difficult to definitively attribute efficacy to the infused CAR⁺ T cells in trials enrolling small numbers of patients who received concomitant chemotherapy. Trials infusing T cells expressing a first-generation CAR design (signaling solely through chimeric CD3- ζ endodomain) revealed that these T cells appear to have limited long-term persistence and one way of circumventing this limitation is to engineer CAR endodomains to deliver an activation signal for sustained proliferation. This has been achieved by the design of second-generation CARs that signal through 2 signaling domains and these are now in multiple clinical trials. Third-generation CARs are now being evaluated in humans in a few trials (Table 1) which are composed of 3 chimeric signaling moieties.^{6,35} Rather than modifying the CAR design to sustain proliferation, investigators have used signaling molecules alongside CARs to achieve improved persistence. For example, constitutively expressed recombinant CD80 and CD137L alongside the CAR demonstrated coordinated signaling between these 2 introduced receptors with endogenous ligands CD28 and CD137 within the immunologic synapse on or between T cells, which could improve antitumor effects.⁹¹ CAR has been introduced into T cells expressing endoge-

Improving the therapeutic potential of CAR⁺ T cells

achieve an improved antitumor effect delivered by the CAR as was recently demonstrated by infusing bispecific T cells which recognized EBV-specific antigens via the endogenous $\alpha\beta$ TCR and GD₂ on neuroblastoma cells by the introduced CAR.⁴¹ Because T cells may require exogenous cytokines to sustain *in vivo* persistence,⁹⁶ investigators have enforced expression of cytokines that signal through the common γ cytokine receptor chain. Animal experiments⁹⁷ as well as clinical experience^{98,99} have shown that long-lived T cells are associated with expression of the IL-7R α chain (CD127), but genetically modified and cultured T cells tend to down-regulate this receptor. Therefore, to enhance the ability of T cells to respond to IL-7, made available after inducing lymphopenia,¹⁰⁰⁻¹⁰² investigators have enforced the expression of IL-7R α to demonstrate improved survival of EBV-specific T cells in an animal model¹⁰³ or introduced a novel membrane-bound variant of IL-7 that when expressed on the cell surface improved persistence of CAR⁺ T cells.¹⁰⁴ Furthermore, overexpression of receptors for IL-2¹⁰⁵ and IL-15¹⁰⁶ as well as enforced expression of the cytokines themselves¹⁰⁷ have improved persistence of T cells.¹⁰⁸⁻¹¹⁰ However, when this approach was tested in a clinical trial infusing tumor-infiltrating lymphocytes (TIL) genetically modified to constitutively secrete IL-2, the persistence of the adoptively transferred T cells was not improved compared with genetically unmodified TIL.¹¹¹ As constitutive expression of cytokines and/or cytokine receptors is tested in early clinical trials, investigators will need to consider coexpressing transgenes for conditional ablation of these genetically modified T cells to guard against aberrant proliferation as unopposed cytokine signaling may lead to aberrant T-cell growth.^{109,112} The type of T cell into which the CAR is introduced may also impact persistence after adoptive immunotherapy. This has been demonstrated in the monkey by infusing autologous preselected central memory T cells which despite *ex vivo* numeric expansion retained superior *in vivo* persistence compared with adoptive transfer of differentiated effector T cells.¹¹³ These observations have been expanded upon by Hinrichs et al who showed that an infusion of naive murine T cells was associated with improved T-cell persistence.¹¹⁴ These animal observations are likely to influence the design of trials infusing genetically modified T cells as investigators seek to introduce CARs into T cells that preserve the functional capacity of central memory or naive T cells.

Homing

To act on and within the tumor, genetically modified T cells must home to the site(s) of malignancy. Migration may be compromised by the loss of desired chemokine receptors during genetic modification and passage *ex vivo*, or may result from the selection of T cells that are inherently unable to localize to certain tissues. Panels of tissue-specific homing receptors which are typically composed of integrins, chemokines, and chemokine receptors are associated with T-cell migration to anatomic sites of malignancy, and flow cytometry can therefore be used to describe the potential migration patterns of T cells before infusion.^{115,116} It is unclear whether T cells capable of expressing a desired matrix of endogenous homing receptors can be genetically modified to express CAR. Therefore, investigators are manipulating the homing potential of T cells through the enforced expression of chemokine receptors such as CCR4.^{117,118}

Overcoming mechanisms of resistance

an ability to overcome the adverse regulatory effects within the tumor microenvironment. For example, investigators have introduced a dominant-negative receptor for TGF β to enable genetically modified T cells can resist the suppressive effects of this pleiotropic cytokine.¹¹⁹ Recognizing that the tumor microenvironment contains regulatory T cells (T_{regs}), the CAR signaling motif has been adapted to resist the suppressive effects of these cells by the expression of chimeric CD28.¹²⁰ The ability to engineer CAR⁺ T cells to successfully function within tumor deposits remains relatively underexplored as most clinical trials to date have used CAR⁺ T cells with specificity for hematopoietic malignancies. However, trials have been published and are under way which seek to treat solid tumors^{41,42,121} (Table 1) and systematic approaches to enabling infused T cells to effectively operate within a tumor microenvironment will be needed to reliably eliminate large tumor masses.

Reprogramming T cells

The *ex vivo* gene transfer and propagation (Figure 2) of T cells provides an opportunity to further manipulate T cells before infusion. The *in vitro* propagation of T cells provides investigators with an opportunity to numerically expand T cells from scant starting numbers, such as when T cells are genetically modified from umbilical cord blood with the intent to augment the graft-versus-tumor-effect after allogeneic hematopoietic stem cell transplantation (HSCT).⁶¹ However, the *in vitro* culturing process can also be adapted to modify T cells for desired effector function by the selective addition of a subset of soluble cytokines, for example, that bind via common γ chain receptor to the culture media during *ex vivo* culture.^{122,123} In addition, to help maintain a desired T-cell phenotype after gene transfer, investigators have provided costimulatory signals by the addition of CD28-specific mAb in addition to OKT3, often using beads conjugated to these mAbs. As an alternative, we and others have used immortalized cells in tissue culture, such as 3T3^{124,125} and K562^{74,126-128} which can be genetically modified to express desired T-cell costimulatory molecules and function when irradiated as aAPC.

Expressing CARs in cells other than $\alpha\beta$ TCR⁺ T cells

Populations of hematopoietic cells other than T cells expressing $\alpha\beta$ TCR, such as NK cells, cytokine-induced killer (CIK) cells, monocytes, and neutrophils have been genetically modified to express CARs.^{27,28} Given the inherent lytic potential of NK cells, redirection of their specificity via a CAR is appealing.¹²⁹⁻¹³³ CAR signaling endodomain(s) can be specifically adapted or chosen to enhance NK-cell signaling versus those that activate T cells.¹³⁴ One drawback to adoptive transfer of NK cells has been their limited survival *in vivo* after transfer. However, *ex vivo* NK-cell culturing using aAPC adapted from K562 may change this perception.^{135,136} Furthermore, the use of NK cells as a cellular platform for introducing CARs may be attractive after allogeneic HSCT as donor-derived NK cells do not appear to significantly contribute to graft-versus-host-disease (GVHD).^{137,138} T cells expressing $\gamma\delta$ TCR

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