Adoptive Immunotherapy for Cancer or Viruses

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Abstract
Adoptive immunotherapy, or the infusion of lymphocytes, is a promising approach for the treatment of cancer and certain chronic viral infections. The application of the principles of synthetic biology to enhance T cell function has resulted in substantial increases in clinical efficacy. The primary challenge to the field is to identify tumor-specific targets to avoid off-tumor, on-target toxicity. Given recent advances in efficacy in numerous pilot trials, the next steps in clinical development will require multicenter trials to establish adoptive immunotherapy as a mainstream technology.
INTRODUCTION

Adoptive immunotherapy, or cell therapies, is undergoing a period of growth and enthusiasm following encouraging data regarding its clinical efficacy. Virus-directed cell therapies are under investigation for the treatment of chronic viral infections such as HIV and for viruses that cause morbidity and mortality in immunosuppressed settings such as bone marrow transplantation. In addition, cell therapies are poised to take a prominent role in both hematologic malignancies and solid tumors. Here we review the history and rationale of immunotherapy and advances in understanding the principles of T cell transfer that are thought to impact clinical results. We also discuss strategies and methods that are important in developing appropriate, effective, reliable, and scalable culture systems. Our current understanding of methodologies for engineering cells to redirect them to specific targets, endowing immune cells with additional functions and safety features, and combining cells with other immune and targeted therapies is discussed in this review (see Figure 1, below). Finally, we illustrate how immune monitoring and biomarkers can determine the effects and fate of cell therapies in the clinical setting. We conclude with a brief discussion of the elements required to establish a new pillar of medical treatments built around personalized cell therapies.

HISTORY AND RATIONALE FOR ADOPTIVE IMMUNOTHERAPY

Given the abilities of T cells to recognize and kill target cells, it is not surprising that most investigations of adoptive T cell therapy have targeted chronic viruses and cancer.

Viruses

Cell and gene therapy strategies have been proposed from the earliest days of the HIV epidemic (1, 2). The first clinical use of chimeric antigen receptor (CAR)-modified T cells was in HIV infection. In this setting, the CAR was composed of the receptor for the HIV envelope protein, namely the extracellular and transmembrane portions of the CD4 protein, fused to the T cell receptor (TCR)-ζ signaling molecule (CD4ζ CAR). The proposed mechanism of action was for transduced T cells to lyse HIV envelope–expressing T cells. Between 1998 and 2005, three clinical studies evaluated the CD4ζ CAR expressed in autologous CD4+ and CD8+ T cells via a retroviral vector in subjects with active viremia (3) or in T cell–reconstituted patients with chronic HIV-1 infection (4). These studies showed that infusion of redirected T cells was feasible and safe; in addition, T cells trafficked to reservoirs of infection (mucosa) and had modest effects on viremia. A decade later, analysis of the data collected from these protocols in a long-term follow-up study demonstrated the safety of retroviral modification of human T cells and the long-term persistence of redirected T cells.

Figure 1

Adoptive transfer of autologous, genetically engineered, ex vivo–expanded T cells: the “seed,” the “soil,” and the “fertilizer.” Autologous cells are harvested from the patient by apheresis. Following purification, cells undergo polyclonal in vitro activation and expansion as well as genetic modification to form the seed. Engineered cell populations (seeds), along with antibodies and/or cytokines (fertilizer), are refusined (“planted”) into the preconditioned patient (soil). Genetic modifications can take many forms, including introduction of transgenes or gene editing, and can be conferred to the T cells by a variety of genetic engineering platforms, such as RNA transfection or lentiviral transduction. (Abbreviations: APC, antigen-presenting cell; CAR, chimeric antigen receptor; CRISPR, clustered regularly interspaced short palindromic repeat; PBL, peripheral blood lymphocyte; TALEN, transcription activator–like effector nuclease; TCM, central memory T cell; TEM, effector memory T cell; TSCM, stem-cell memory T cell; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; ZFN, zinc-finger nuclease.)
Ex vivo culture and expansion of purified cells

- Bead stimulation
- Cytokine treatment
- Peptide treatment
- APC stimulation
- TIL culture

Transgenesis
- Chemokines
- Cytokines
- CAR
- TCR

Gene editing
- ZFN
- TALEN
- CRISPR

Lentivirus
- Lentiviral vectors
- Adenovirus vectors
- DNA transfection
- RNA transfection

Genetic engineering platforms

Transduction or transfection

SOIL
- Conditioning regimens

FERTILIZER
- Seed
  - PBL
  - Virus-specific
  - T
  - TEM

Engineered cell populations

Activation and expansion

Co-culture

Activation and expansion

Purification

Apheresis or solid tumor excision

Engineered cell populations

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of CAR-modified T cells, with an estimated half-life greater than 16 years (5). This study added to the literature indicating that T cells were less susceptible to retrovirus-mediated insertional mutagenesis than were hematopoietic stem cells (HSCs). In 2009, the remarkable story of the “Berlin patient” was published (6); this was the first report of a patient being functionally cured of HIV infection following an allogeneic HSC transplant for acute myelogenous leukemia. The donor was homozygous for the CCR5 Δ32 mutation, which confers genetic resistance to HIV infection. The findings from this report have challenged the field to develop cell therapy–based approaches that do not require myeloablative chemotherapy or allogeneic donors. One such approach has been to develop gene therapy strategies to reduce CCR5 expression, either through shRNA encoded by lentiviral vectors (7) or through gene-editing strategies using zinc-finger nucleases (ZFNs) to disrupt the CCR5 gene in T cells (8). In these cases, autologous gene-modified T cells are reinfused with the goal of reconstituting the T cell repertoire in HIV-infected patients. Interpretation of T cell effects on viremia and control of HIV may be affected by ongoing treatment with highly active antiretroviral therapy (HAART), and carefully designed trials with scheduled, thoroughly monitored treatment interruptions are under way.

Patients with hematologic malignancy undergoing allogeneic bone marrow transplantation are also at high risk for viral illness, particularly from reactivation of chronic viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus 6; primary adenovirus infection can also cause acute and severe illness in this immunocompromised population. Although pharmacologic treatments for these viruses are available, they often have limited efficacy, must be administered recursively, and have significant side effects. For these reasons, several transplant centers have focused on developing donor-derived virus-specific T cells that can be administered as a donor lymphocyte infusion (DLI), either prophylactically or as treatment (9, 10). Because of the limitations in approaching healthy donors and single-patient manufacturing lots of virus-specific T cells, some centers have developed “third-party” T cell banks derived from a panel of donors selected to span the most common HLA alleles (11–13). The Baylor group has pioneered the use of T cell lines that are specific for three to five viruses simultaneously and has administered these to patients either as donor-derived or as third-party-derived lymphocyte infusions (11, 14–16). Importantly, the incidence and severity of graft-versus-host disease (GvHD) have been limited or tolerable in all these studies. These forms of adoptive immunotherapy are the most clinically advanced, with publication of phase II, multicenter trials (11).

Cancer

Immunotherapy for cancer has a long and somewhat checkered history; the first observations that immune system engagement has antitumor effects are often attributed to William Coley, who observed regression of sarcoma following severe bacterial infections in the 1890s (17). However, the seminal finding that allogeneic immune reconstitution after bone marrow transplant had antileukemic effects (18) definitively identified the anticancer effects of immune cells. Allogeneic bone marrow transplant remains the most potent, widely available form of cellular immunotherapy and offers curative potential for hematologic malignancies. Researchers soon noted that the major mediators of the graft-versus-leukemia effect were T cells (19); though noted later (20), a contribution by natural killer (NK) cells is also quite potent (21). There is a strong rationale for combining T cell therapy with NK cell therapy because NK cells do not cause GvHD, and they may limit resistance to T cell therapy through the emergence of tumor cells that are major histocompatibility complex (MHC) class I deficient.

In the case of relapse after allogeneic transplantation, withdrawal of immunosuppressive therapy and/or DLIs are considered standard therapies, although they have the potential to cause
or worsen GvHD. Ex vivo activation and culture of donor lymphocytes have also been clinically evaluated and appear to have modest benefit over standard DLI (22), particularly in hematologic malignancies aside from chronic myeloid leukemia (CML). Although CML was formerly one of the most common indications for transplant, it tends to be the most responsive to immune manipulations such as DLI; in the modern era, CML is most often treated with tyrosine kinase inhibitors such as imatinib, dasatinib, and nilotinib. The limitation of these inhibitors is that they are expensive, and although they result in long-term remissions in most cases, they are not curative. The major opportunity for research in CML is to combine targeted agents such as kinase inhibitors with adoptive cell transfer therapy, with the goal of developing a curative regimen.

In solid tumors, investigators have hypothesized that tumor-infiltrating lymphocytes (TILs) are the result of a naturally occurring, yet ineffective, T cell response to the tumor. The observation of TILs has spawned three forms of immune-based clinical interventions designed to convert TILs into effective cells: (a) systemic administration of cytokines and immunologically active proteins such as IL-2 and interferon, which are currently approved for melanoma; (b) systemic administration of antibody therapies aimed at modifying T cell activation and relieving checkpoint blockade, such as ipilimumab (anti-CTLA-4), anti-PD-1 and anti-PD-L1, anti-4-1BB, and anti-CD40, to name a few; and (c) direct isolation and ex vivo activation of the TIL. Checkpoint blockade therapy has had remarkable results not only in melanoma (23), but also in tumors such as lung cancer that had previously been considered “immunologically silent” (24, 25). Even more encouraging, simultaneous blockade of two checkpoints (CTLA-4 and PD-1) in melanoma significantly improved the response rate and time to response over either therapy alone (26). Direct isolation and ex vivo activation of the TILs have also been tested in multiple early-phase studies and result in durable responses in melanoma (27). In most presenting cases, however, this approach cannot be undertaken, either because surgical material is not available or contains insufficient numbers of TILs, or because the patient cannot tolerate the conditioning regimen or the time required for manufacturing of their TIL product. As a result, researchers have been unable to conduct randomized controlled studies.

As discussed below, recent advances in the use of genetically engineered T cells and an understanding of the principles underlying effective T cell therapy have produced encouraging results in the use of T cell therapies for viruses, hematologic malignancies, and solid tumors. T cell therapy is now poised to advance from phase I trials to phase II and phase III trials. Multiple biotech and pharmaceutical companies have also begun active, clinical development of T cell therapy, with the goal of offering a standardized, quality-controlled, regulatory-body-approved treatment for the integration of cell therapies to benefit patients worldwide.


T cell transfer and engraftment into the host is a complex biologic process. By thoroughly understanding the role of the host immune system (the “soil”), growth factors and the balance against inhibitory cells (the “fertilizer”), and the transferred T cell product (the “seed”), investigators may be able to optimize this process (Figure 1).

**Preparation of the Soil: Host Conditioning**

Evidence from bone marrow transplantation and adoptive therapy trials of TILs demonstrated that “conditioning,” or lymphodepleting, the host enhanced engraftment of the transferred T cells. Multiple hypotheses indicate why conditioning, or preparing the soil for the incoming T cells, is...
attractive, particularly in the setting of malignancy. For example, host conditioning may reduce
tumor burden (thus improving the effector:target ratio in vivo), reduce the population of inhibiting
regulatory T cells (28), and induce production of homeostatic cytokines to facilitate proliferation
of the transferred T cells (29). Typical regimens for host conditioning include cyclophosphamide
with or without fludarabine; some centers also use total-body irradiation. All these techniques,
particularly the most intense ones that combine chemotherapies and irradiation (30), appear to
improve the persistence of the transferred T cells as well as clinical responses involving cancer.
Notably, however, host conditioning has not been required in cases of HIV infection to enable
long-term persistence of transferred T cells (5); similarly, low numbers of virus-specific T cells can
persist and expand in post-transplant settings (31). Furthermore, recent reports of CAR-modified
T cells administered in the absence of host conditioning have shown clinical effects for both
hematologic and solid tumors (32, 33).

The Fertilizer: Cytokines and T Cell–Modulating Antibodies

Cytokines provide important growth and homeostatic signals to T cells: IL-2, IL-7, and IL-15, in
particular, have been well studied. Recombinant human IL-2 as a single agent is FDA approved
for metastatic melanoma; because of its toxicity, however, it is administered in only select centers.
Furthermore, the biologic role of IL-2 is physiologically complicated; low-dose IL-2 may maintain
regulatory T cells and has been used to control GvHD (34). Thus, it is not clear that administration
of IL-2 will help the transferred cytotoxic T cells instead of the native regulatory T cells. Strong
preclinical data support the use of IL-7 and IL-15, both of which are also being explored in clinical
trials. IL-15, in particular, may relieve the inhibition of regulatory T cells while providing support
for adoptively transferred T cells (35).

The combination of adoptive cell therapies with newer agents, including checkpoint blockade
and/or small-molecule-targeted therapies, is still in its nascent stages but is bound to generate
excitement. Many combinations are possible: Coadministration of agonistic antibodies [CD40
(36) or 4-1BB (37)] to mediate costimulation or with a checkpoint blockade (anti-PD-1 or
anti-CTLA-4) is likely both to improve the effects of the transferred T cells and to stimulate the
native T cell responses to tumors. Typically, small-molecule drugs aimed at aberrant signaling
in the tumor effect rapid, but short-lived, tumor responses, whereas immunotherapy approaches
take longer to eliminate the tumor but are potentially long-lived. Combinations of treatment
with T cell transfer coupled with small-molecule drugs targeting tumor mutations [such as BRAF
inhibitors in melanoma (38) or Bruton’s tyrosine kinase or Bcl-2 inhibitors in lymphoma] have
the exciting potential to make cancer treatment chemotherapy-free (Tables 1–3 and Figure 2).

The Seed: The Cell Product

Because of the complexity of T cell activation, differentiation, and homeostasis, several groups
have tried to determine the optimal cell population to serve as the seed for adoptive cell therapy.
T cells that have been cultured extensively, whether stimulated with autologous dendritic cells
(DCs) or artificial antigen-presenting cells (APCs) or cloned and passaged on allogeneic feeder
cells, have a terminally differentiated phenotype with a loss of in vivo engraftment and proliferative
capacity; such cells also have limited in vivo function. Reprogramming of the T cell (39, 40) may
overcome these effects, but it is associated with its own complex culture system that will be difficult
to adopt widely. Currently, culture systems that rely on repetitive antigen stimulation to generate
a T cell product are not easily scalable, efficient, or reliable enough to generate functional T cells
for immunotherapy, except perhaps as third-party donor banks, which are expensive to generate
owing to the heterogeneity of HLA types in the population.
Table 1  Summary of current clinical trials of CD19 CAR T cells

<table>
<thead>
<tr>
<th>Target antigens</th>
<th>Cancers</th>
<th>CAR signaling domain</th>
<th>Combinatorial/engineering strategies (biologics, drugs)</th>
<th>Phase; ID</th>
<th>Sponsor</th>
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<td>CD19 B cell malignancies</td>
<td>Relapsed post-allo-HSCT, T cells from donor</td>
<td>CAR: CD28-CD3ζ, or CD137-CD3ζ</td>
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<td>Relapse/refractory CLL, NHL, or ALL</td>
<td>CAR: CD3ζ</td>
<td>None</td>
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<td>Fred Hutchinson Cancer Research Center</td>
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<td>CD19</td>
<td>ALL; DLBCL; MCL; NHL; CLL relapsed post-allo-HSCT</td>
<td>CAR: CD28-CD3ζ</td>
<td>CMV- or EBV-specific T cells derived from donor CD62L^+ TCM</td>
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<td>CLL; small lymphocytic lymphoma; MCL, Follicular lymphoma; large-cell lymphoma</td>
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<tr>
<td>CD19</td>
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<td>CD19</td>
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<td>CAR: CD28-CD3ζ</td>
<td>CD19 CAR-transduced PBLs and EBV-specific CTLs</td>
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<td>CD19</td>
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<td>CD19^+ malignancies</td>
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Table 1 (Continued)

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<thead>
<tr>
<th>Target antigens</th>
<th>Cancers</th>
<th>CAR signaling domain</th>
<th>Combinatorial/ engineering strategies (biologics, drugs)</th>
<th>Phase; ID</th>
<th>Sponsor</th>
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<tr>
<td>CD19</td>
<td>Auto-HSCT for NHL followed by T cell infusion</td>
<td>CAR: CD28-CD3ζ</td>
<td>T&lt;sub&gt;CM&lt;/sub&gt;-enriched CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>I/II; NCT01318317</td>
<td>City of Hope/National Cancer Institute</td>
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<td>CD19</td>
<td>ALL</td>
<td>CAR: CD3ζ</td>
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<td>CD19</td>
<td>Leukemia</td>
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<td>CD19/EGFRt</td>
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Abbreviations: ALL, acute lymphoblastic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplant; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; CTLs, cytotoxic T lymphocytes; DLBCL, diffuse large B cell lymphoma; EBV, Epstein-Barr virus; EGFRt, epidermal growth factor receptor, truncated; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphomas; NP, information not provided; PBLs, peripheral blood lymphocyte; T<sub>CM</sub>, central memory T cells.

To maintain the persistence and function of adoptively transferred T cells, some investigators have used T cells specific to a chronic virus such as EBV or CMV and redirected them to tumor-associated antigens (41, 42). Others have explored using phenotypically defined populations that may proliferate and survive for longer, such as central memory T cells (43) or naive T cells (44), to improve engraftment in preclinical studies. Recent data have identified and characterized early-differentiated, stem-cell memory T cells (T<sub>SCM</sub>) (45). These cells constitute the most undifferentiated human T cell compartment exhibiting bona fide memory functions and can survive for extended periods even after the loss of cognate antigens. These cells may also persist and support memory T cell functions, which would make them ideal candidates for long-term control of cancer, and may be engaged for viral vaccine purposes (46). However, it is not clear that the frequencies of the T<sub>SCM</sub> peripheral blood samples are consistent in large numbers of diverse cancer patients. Validated and clinically approved systems will be required to isolate these cells to form the basis of a cell therapy product (Figure 3).

Finally, although the efficacy of adoptive cell therapy is most often attributed to CD8<sup>+</sup> T cells, there are reports of pure CD4<sup>+</sup> T cell populations mediating tumor regression (47). Furthermore, immune effector cell types other than T cells have been used in cell-transfer protocols. For example, NK-based trials within autologous (48) and allogeneic settings (20) have been published, and redirection as well as engagement of NK cells are areas of active research.

STRATEGIES OF EX VIVO T CELL CULTURE

Inherent barriers to widespread clinical application include manufacturing difficulties and access to robust and efficient methods for the expansion of input T lymphocytes. Our laboratory has
<table>
<thead>
<tr>
<th>Target antigens</th>
<th>Cancers</th>
<th>CAR signaling domain</th>
<th>Combinatorial/engineering strategies (biologicals, drugs)</th>
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<td>HSV thymidine kinase (used for imaging and suicide gene)</td>
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</tbody>
</table>

Abbreviations: ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; CTLs, cytotoxic T lymphocytes; EBV, Epstein-Barr virus; HSV, herpes simplex virus; iCaspase9, inducible caspase 9; NHL, non-Hodgkin lymphomas; NP, information not provided; TGF-β, transforming growth factor β.
Table 3 Summary of recent clinical trials involving genetically redirected T cells

<table>
<thead>
<tr>
<th>Target antigens</th>
<th>Cancers</th>
<th>Receptor</th>
<th>Combinatorial/engineering strategies (biologics, drugs)</th>
<th>Phase; ID</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1</td>
<td>AML, CLL</td>
<td>TCR</td>
<td>None</td>
<td>I/II; NCT01621724</td>
<td>University College, London</td>
</tr>
<tr>
<td>WT1</td>
<td>AML, MDS, or CML</td>
<td>TCR</td>
<td>Aldesleukin; virus-specific CD8+ T cells</td>
<td>I/II; NCT01640301</td>
<td>Fred Hutchinson Cancer Research Center/ University of Washington Cancer Consortium</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>Melanoma</td>
<td>TCR</td>
<td>None</td>
<td>I/II; NCT01350401</td>
<td>Adaptimmune</td>
</tr>
<tr>
<td>NY-ESO-1/ LAGE-1</td>
<td>Multiple myeloma</td>
<td>TCR</td>
<td>None</td>
<td>I/II; NCT01892293</td>
<td>Adaptimmune</td>
</tr>
<tr>
<td>NY-ESO-1/ MAGE-A3/6</td>
<td>Multiple myeloma</td>
<td>TCR</td>
<td>None</td>
<td>I/II; NCT01352286</td>
<td>Adaptimmune</td>
</tr>
<tr>
<td>CEA</td>
<td>Metastatic cancers</td>
<td>IgCD28TCR</td>
<td>None</td>
<td>II; NCT01723306</td>
<td>Roger Williams Medical Center</td>
</tr>
<tr>
<td>MART-1</td>
<td>Metastatic melanoma</td>
<td>TCR</td>
<td>Administration of MART-126-35-pulsed dendritic cells and IL-2</td>
<td>II; NCT00910650</td>
<td>Jonsson Comprehensive Cancer Center</td>
</tr>
</tbody>
</table>

*Abbreviations: AML, acute myelogenous leukemia; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes; TCR, T cell receptor.

developed methods for efficient activation, expansion, and gene transduction of T lymphocytes (Figure 4).

Additionally, desired properties of adoptive immunotherapies include (a) demonstrated potency against a tumor or infectious organism, (b) efficient engraftment enabling a high effector to target ratio, and (c) long-term persistence and memory.

**T Cell Therapy and Ex Vivo Culture Methods**

The clinical application of T cell–based therapeutics has gained extensive momentum within the past 30 years as a result of a number of critical discoveries including the identification of T cell antigens that have also been tested as cancer vaccines (49). A large number of studies suggest that DCs, when appropriately activated and induced to present tumor-associated antigens, can elicit tumor-specific T cell immunity. This DC therapeutic approach is currently being pursued by several biotechnology companies (50–53), but it has limitations because the ability to generate DCs varies from patient to patient. As a result of this variability, short-term or insufficient T cell activation may not generate an effective immune response.

**Magnetic Bead–Based Artificial Antigen-Presenting Cells**

Recognizing that both a primary specificity signal via the TCR (Signal 1) and a costimulatory/regulatory signal via the CD28 receptor (Signal 2) are simultaneously required to generate full T cell effector function and a long-lasting immune response (54), we have developed efficient and reproducible methods of mimicking the signal that DCs provide to T cells, but without delivering a negative costimulatory signal. With artificial APCs (aAPCs), T cells can be grown rapidly ex vivo to clinical scale for therapeutic applications. Instead of indirect activation via...
Figure 2

Durable tumor regression may be achieved by combining immunotherapy with targeted therapeutic strategies. (a) Patients with metastatic melanoma exhibit improved survival with ipilimumab treatment (23) (Kaplan-Meier survival plot). Treatments are as follows: ipilimumab only; gp100 only; ipilimumab plus gp100. Arrow indicates how immunotherapy raises the tail of the curve, indicating prolonged effects. (b) Kaplan-Meier survival plot showing that improved survival can be achieved in melanoma with vemurafenib therapy (204). (Panels a and b are adapted with permission from the New England Journal of Medicine.) Patients were treated with either dacarbazine or vemurafenib. Arrow indicates direction of change effected by targeted therapy; overall survival is improved early, but effects are transient. (c) Hypothetically, the combination of immunotherapy with targeted treatment may increase survival in patients with metastatic cancers. The solid lines depict typical survival curves for standard cancer therapy, immunotherapy alone, and a targeted therapy alone. The dashed line depicts the potential enhanced survival that can be achieved using immunotherapy combined with targeted therapy. Arrows highlight the impact of the above therapeutic regimens on the tail of the curve or the spread between curves for different treatments. Thus, immunotherapy requires more time but increases survival; targeted therapy works rapidly but is not durable. Combining these strategies may ultimately improve the fraction of patients with long-term survival.

vaccines, this technology enables direct T cell activation, which can be modulated by controlling the cell dose as necessary to achieve a clinical response (55, 56).

The first generation of off-the-shelf aAPCs covalently linked clinical-grade antihuman CD3 and anti-CD28 monoclonal antibodies to magnetic Dynabeads® (Life Technologies), which serve to cross-link the endogenous CD3 and CD28 receptors on the T cell. Via this bead-based
Figure 3
Phenotypic and functional changes in T cells during progressive differentiation driven by chronic antigen stimulation. A state of persistent and frequent antigenic stimulation, such as that induced by tumor burden, facilitates a progressive differentiation pathway whereby naive T cells become terminally differentiated effectors. The following changes in the phenotypic markers that characterize progressive T cell differentiation are depicted: Superscripts indicate expressed (plus symbol), not expressed (subtraction symbol), expressed at high levels (high), expressed at low levels (low), long telomere length (L), long/intermediate telomere length (L/I), intermediate telomere length (I), short/intermediate telomere length (S/I), and short telomere length (S). Together with the gradual shortening of telomere length, T cells lose their proliferative and self-renewal capacities as well as their responsiveness to homeostatic mediators, and they ultimately become exhausted. Although cytotoxic potential/effectector functions increase with persistent antigen stimulation and T cells must be fully differentiated to possess antitumor activity, experimental evidence suggests that, in the context of adoptive cell therapy, increasing differentiation state is inversely correlated with antitumor efficacy (205).

aAPC, the most efficient growth of human polyclonal naive and memory CD4+ T cells has been reported (56). In terms of cell function, the expanded cells retain a highly diverse TCR repertoire and, by varying the culture conditions, can be induced to secrete cytokines characteristic of T helper 1 (Th1) or T helper 2 (Th2) cells (57). One important advantage of this bead-based system is that it does not cross-react with CTLA-4 and therefore provides unopposed CD28 stimulation for more efficient expansion of T cells. Another, unanticipated discovery was that cross-linking of CD3 and CD28 with bead-immobilized antibody renders CD4+ T lymphocytes highly resistant to HIV infection. This is due to the downregulation of CCR5, a necessary coreceptor for the internalization of HIV, as well as the induction of high levels of β-chemokines, the natural ligands...
Apheresis or solid tumor excision  
Cell purification and enrichment  
Gene modification, transgenesis/editing  
Activation, expansion, and ex vivo culture  
Cryopreserve cell product  
Pass release testing  
Infusion  
Endpoints: Safety, efficacy, and follow-up

Clinical application of gene-modified cell therapies. Cells of interest are isolated from the whole blood of a patient, followed by enrichment, activation, and expansion. At the time of activation, the lentiviral vector is added. On the final day of culture, cells are harvested and cryopreserved in an infusible media. The patient is infused with gene-modified cells, and endpoint assays are conducted at designated time intervals. At the conclusion of active monitoring, in the United States, the patient is transferred according to a destination protocol for long-term follow-up as per FDA guidelines.

for CCR5 (58–60). Thus, CD4+ T cells may be efficiently cultured from HIV-infected study subjects. Ex vivo expansion may also indirectly enhance T cell activity by removing T cells from the tumor-induced immunosuppressive milieu (61–64). In contrast to previous methods, exogenous growth factors or feeder cells are also not needed to enable T cell stimulation and expansion.

Cell-Based Artificial Antigen-Presenting Cells

Cell-based aAPC lines have been derived from the CML line K562 (65–67). K562 cells do not express MHC or T costimulatory ligands, but they may represent a DC precursor that retains many other attributes that make DCs such effective APCs, such as cytokine production, adhesion molecule expression, and macropinocytosis. These cells have been transduced with a library of lentiviral vectors, thus allowing researchers to customize the expression of stimulatory and costimulatory molecules that can be used to activate and expand different subsets of T cells and that can be further modified to amplify antigen-specific T cells in culture. These aAPCs also express molecules other than CD3 and CD28 on their surface. The K562 aAPCs have been transduced with vector to express the antibody Fc-binding receptor and the costimulatory molecule 4-1BB. Expression of CD64, the high-affinity Fc receptor, on K562 aAPCs also allows researchers to load antibodies directed against T cell surface receptors. CD3 and CD28 antibodies are added to the cells and are bound by the Fc receptor to yield a cell that binds to CD3, CD28, and 4-1BB. Compared with magnetic bead–based aAPCs, K562 aAPCs are more efficient at activating and expanding T cells, especially CD8+ and antigen-specific T cells (65–67). In addition, these cells can efficiently stimulate CD4+ T cells.

Thus, K562 cells may represent ideal cell scaffolds to which the desired MHC molecules, costimulatory ligands, and cytokines can be introduced to establish a DC-like aAPC. Mimicking the advantages of natural DCs, this artificial DC platform demonstrates high levels of MHC expression, a wide array of costimulatory ligands, and the ability to engage in cytokine cross talk with the T cell. In addition, the DC-like aAPC does not display several recognized disadvantages, including the need to derive natural DCs either from CD34+ cells mobilized by granulocyte colony-stimulating factor or from monocytes, patient-specific differentiation, a limited life span,
and limited replicative capacity. Moreover, these cells have been injected into humans as part of a tumor vaccine (69), signifying that these cells can be used in accordance with Good Manufacturing Practices. To date, our laboratory and our collaborators have developed either bead- or cell-based aAPCs optimized for Th2 cells (57, 70) and for regulatory T cells (71).

**Manufacturing Process**

Independent of which of the above aAPC is used, the manufacturing procedure remains similar, starting with an apheresis product. Alternatively, T cells can be derived from a blood draw, bone marrow, ascites, or TILs. The apheresis product may be washed out of the collection buffer in a Haemonetics Cell Saver\textsuperscript{⃝} 5+ or other automated cell-washing device. It could also be directly loaded in the Terumo Elutra\textsuperscript{TM} Cell Separation System to deplete the monocytes and isolate the lymphocytes. The depletion of CD4\textsuperscript{+}, CD8\textsuperscript{+}, or CD25\textsuperscript{+} T cells can be accomplished using a Miltenyi CliniMACS\textsuperscript{⃝}, an electromechanical device for isolating certain cell subsets via large-scale magnetic cell selection in a closed and sterile system. Before selection, the washed cells from an apheresis product are magnetically labeled using particles conjugated with anti-CD4, anti-CD8, or anti-CD25 monoclonal antibody. A single-use tubing set, including separation columns, is then attached to the CliniMACS and the cell-preparation bag containing the labeled cells. After the selection program has begun, the system automatically applies the cell sample to the separation column, performs a series of washing steps depending on the chosen program, and elutes the purified target cells.

The lymphocyte fraction from the Elutra system, or enriched T cells, is cultured in a nutrient media. Then, addition of antibody-coated magnetic beads or of irradiated and antibody-preloaded K562 aAPCs (see descriptions above) stimulates the cells to divide and grow. Gene transduction of anti-CD3/anti-CD28 aAPC–stimulated T cells with retroviral, lentiviral, or adenoviral vectors is very efficient (72–74). The whole mixture of cells, growth media, vector, and aAPC is added to a gas-permeable plastic bag or alternative culture vessel. Tubing leads on the bags and a variety of connecting devices allow the cells to be grown in a closed system with minimal risk of contamination. After gene-vector washout, if needed, on the Baxter CytoMate and during log-phase cell growth, cultures are transferred to the WAVE Bioreactor, where cell concentrations may reach $1 \times 10^7$ cells/ml or higher. The advantage of the WAVE is that T cells can be grown at higher densities, which saves labor on processing and during cell harvest. Cultures are maintained for 9–11 days prior to being harvested and prepared for reinfusion, or they may be cryopreserved for later infusion. At harvest, the magnetic bead–based aAPCs are removed, and the cells are washed, resuspended, and cryopreserved in an infusible solution. If cells are to be infused when fresh, in-process samples are taken for microbiological testing, viability, and cell phenotyping via flow cytometry for release. Testing is repeated on the final product, although results for some tests are not available until after the cells are infused.

**GENETIC ENGINEERING PLATFORMS**

T lymphocytes can be modified by gene-transfer methods to permanently or transiently express therapeutic genes to enhance and expand the therapies. Importantly, genetic engineering can also be used to endow lymphocytes with several other features, including increased proliferative potential (75), prolonged in vivo persistence (76), improved capacity to migrate to tumor tissues (77), or the ability to recognize an entirely new antigen. Redirection of antigen specificity is usually based on either a TCR of known specificity (78) or a synthetic receptor such as CAR,
which recognizes antigens through antibody-derived complementarity-determining regions and signals through TCR-associated molecules (79).

Current clinical trials of permanently modified T cells employ viral- and nonviral-based approaches. Retroviral (gammaretroviral and lentiviral) vectors can be used to transduce cells without producing any immunogenic viral proteins, with the transgene becoming a permanent part of the host-cell genome. Retrovirus-based gene delivery is an extremely useful tool in gene therapy research and is commonly used in trials of T cell therapies. Nonviral DNA transfection or transposons are also used for permanent gene expression in gene-modified T cell–based therapies. Gene delivery via adenoviral vector or RNA transfection enables expression of the transgene for up to 1 week; these approaches may be useful in scenarios where transient transgene expression is desirable (Figure 5).

**Gammaretroviral Vectors**

Currently, most retroviral vectors are derived from murine or avian retroviruses. The Moloney murine leukemia retrovirus (gammaretrovirus) has been extensively studied as a vector and can
package up to 8 kb of genetic material. Vectors that are derived from gammaretrovirus have been most useful for long-term gene expression because they can integrate into the host genome, which results in permanent expression of the transgene with low intrinsic immunogenicity (80). The first human trial of immunotherapy with gene-modified T cells was reported in 1990 for patients with advanced melanoma. In this case, TILs were modified via retroviral gene transduction (81); the retrovirus was then used to encode neomycin resistance and tracked only the fate of the infused T cells. Also in 1990, two girls suffering from adenosine deaminase severe combined immunodeficiency (ADA-SCID) were treated with T lymphocytes transduced with a gammaretrovirus expressing the ADA gene; incorporation of those gene-corrected T cells led to the reconstitution of the patients' immune systems (82). Since then, retroviral vectors have been the major tool for permanent transgene expression and have been widely used for gene therapy as a vehicle to deliver genes into different cell types, including T lymphocytes (83, 84).

HSCs, which have the potential to self-renew and differentiate into all blood lineages, were initially thought to be the most desirable targets for retroviral gene modification to treat genetic disorders and other diseases (85–91). Although initial results were encouraging, adverse events were observed in trials for SCID-X1 and X-chromosome-linked chronic granulomatous disease (X-CGD) as a result of vector integrations in the vicinity of well-characterized proto-oncogenes (87, 92, 93). Because the target cells most vulnerable to insertional-mediated transformation may be primitive progenitor cells, more mature cells may be less prone to this event (94–97).

By contrast, T lymphocytes remain major targets for retroviral-based gene modification. Not only can they be used to deliver therapeutic genes, but they can also be redirected for specific tumor-associated antigens (3, 78, 98–100). Unlike HSCs, T lymphocytes are less susceptible to transformation (101). Rarely, insertional mutagenesis can contribute to transformation, but this is a rare event and appears to be the result of a synergistic effect between the activation of a proto-oncogene, such as LMO2, and robust signaling through T cell homeostatic cytokines, such as IL-2 or IL-15 (102, 103). Whether a stimulating signal generated via a transduced TCR or CAR can synergize with activation of a proto-oncogene (i.e., as a result of retroviral insertional mutagenesis) to cause transformation of transduced T cells remains to be elucidated. Malignant transformation has not been observed thus far in clinical trials of retroviral-based gene transfer into mature T cells (3, 5, 72, 104).

**Lentiviral Vectors**

Lentiviral gene transfer is a relatively new process and has many features found in the retroviral system. Lentiviruses are distinct members of the retrovirus family. Lentiviral vectors have been constructed from several types of lentiviruses, but the most commonly used is HIV because its molecular biology has been extensively studied (83, 105).

Similar to gammaretroviral vectors, lentiviral vectors stably integrate into the target cell’s genome, resulting in persistent expression of the gene of interest. They can also accommodate up to 10 kb of transgene material, and the immunogenicity of the vector is low. However, in contrast to gammaretroviral vectors, lentiviral vectors transduce nondividing cells (106), have broader tissue tropisms, and have a potentially safer integration site profile (107, 108). Furthermore, lentiviral vectors are less susceptible to gene silencing by host restriction factors (109). These distinctive features broaden the possible applications of lentiviral vectors, especially in settings where gammaretroviral vectors are not suitable. Lentiviral vectors have been safely used in human clinical trials to engineer HSCs and T lymphocytes, and no oncogenic events have been observed (73, 89, 90, 110, 111). However, clonal expansion and dominance of hematopoietic progenitors were reported in a clinical trial in which HSCs were genetically modified with a lentiviral vector.
that expressed the beta-globin gene for the treatment of thalassemia (90). Therefore, genotoxicity of insertional mutagenesis is still a potential safety concern when working with lentiviral vectors.

From a manufacturing perspective, stable packaging cell lines are easily established for gammaretroviral vectors, whereas doing so for lentiviral vectors is still challenging owing to the toxicity of envelope proteins; this limitation forces researchers to generate vectors from inefficient, transient multiplasmid transfections. In addition to the risk of insertional mutagenesis, another potential safety issue applicable to both gammaretroviral and lentiviral vectors is the possibility of generating replication-competent retroviruses (RCR). Although new generations of vectors have been designed to reduce the production of RCR, these additional steps have resulted in decreased efficiency of vector production.

**Adenovirus Vectors**

Adenovirus vectors transduce both dividing and quiescent cells; they can accommodate relatively large transgenes, production of high-titer vector stocks is relatively easy, and the vectors are nononcogenic owing to their lack of integration into the host genome. Adenovirus vectors are widely used in clinical trials, especially for cancer-targeted gene therapies. Application of adenovirus vectors in T cell–based therapy is limited by transient transgene expression and the immunogenicity of the vector. Chimeric adenoviral vectors Ad5/F35 mediate gene transfer in up to 10% of resting T cells and 30–45% of T cells after activation with phytohaemagglutinin (112). Ad5/F35 vectors could result in gene transfer in more than 90% of T cells after activation by CD3- and CD28-specific antibodies (8, 74). Adenovirus vectors may be used as vehicles to deliver genes to T cells in clinical situations where duration of transgene expression of less than a week is required and there is no foreseeable need for repeated cell infusions. Examples of such situations can be envisioned for gene-editing strategies such as ZFNs or transcription activator-like effector nucleases (TALENs) or for clustered regularly interspaced short palindromic repeat (CRISPR)-mediated specific gene silencing (8).

**DNA Transfection and Transposon-Based Gene Delivery**

Nonviral-based DNA transfection methods remain popular as vectors for gene therapy owing to their low immunogenicity and low risk of insertional mutagenesis. The first clinical trial testing the adoptive transfer of T cells engineered using electroporation was recently reported (113). Although this approach was safe, the cells were short-lived after transfer, probably owing to the long-term culture of the cells that was required to select sufficient numbers of permanently integrated T cell clones for treatment.

Transposon-based systems can integrate transgenes much more efficiently than can plasmids that do not contain an integrating element (114, 115). *Sleeping Beauty* (SB), for example, provides efficient and stable gene transfer as well as sustained transgene expression in primary cell types, including human hematopoietic progenitors, mesenchymal stem cells, muscle stem/progenitor cells (myoblasts), pluripotent stem cells, and T cells (116). Various transposon-based systems are now entering clinical trials to test the safety and feasibility of this approach to engineer T cells (117). Nonviral vectors have several advantages over viral vectors as a modality for engineering cells, including lower costs and perceived safety benefits. However, the safety profile of these approaches is still uncertain, given that the relative genotoxicity of transposons is unknown. Approaches to achieve site-specific integration and DNA editing are under development, and if these prove to be efficient, they should allay concerns regarding the use of non-site-specific integration approaches within lymphocyte engineering.
RNA Transfection

Thus far, we have discussed viral transduction or plasmid DNA transfection of T cells, which can result in stable genomic integration, allowing for constitutive, permanent expression of the transgenes. Safety concerns, such as genotoxicity, potential generation of RCR, and the difficulty of predicting off-tumor toxicities, may limit the widespread use of gene-modified T cells, particularly as a first approach in the clinical setting. Moreover, for some clinical situations, multiple infusions of engineered T cells may be required (i.e., to overcome the lack of persistence or the immunosuppressive influence of the tumor microenvironment), making the manufacture of a clinical dose of T cell product difficult and expensive. When transient expression of the transgene is desired, such as to identify potential off-tumor toxicities, or when recursive infusions are planned, RNA transfection of T cells is an attractive approach because it is relatively inexpensive and fast and the transfection efficiencies can easily approach 100%. RNA-based electroporation of human T lymphocytes using in vitro–transcribed mRNA mediates transient expression of proteins for approximately 1 week. The self-limiting transgene expression can provide a safety check for off-tumor, on-target, or off-target toxicities as well as for other unwanted side effects, as the engineered T cells are essentially a “biodegradable” product. Furthermore, there is no genotoxicity concern because the introduced mRNA does not integrate into the host genome. RNA electroporation has been used to deliver messages for TCR or CAR, chemokine receptors, or cytokines (118–121). In one study, T cells modified by CAR RNA were evaluated in a side-by-side comparison with retrovirus-modified T cells: RNA engineering was at least as efficient as retroviral gene transfer (122). Alternatively, for transposon-based gene delivery systems, transposase enzymes can be delivered as mRNA, thereby avoiding the possibility of genomic integration (123). Gene-editing strategies based on ZFN, TALENs, or CRISPR can be also delivered by RNA transfections. In preclinical animal studies, multiple injections of CAR RNA–modified T cells mediated regression of disseminated tumors (124–126). Clinical trials in which solid tumors are treated with RNA-electroporated CAR T cells have been initiated by several groups (33), and the safety and efficacy results will provide valuable information for future cancer treatments using genetically modified T cells.

T CELLS REDIRECTED WITH SPECIFIC T CELL RECEPTORS

Transduction of T cells with a specific TCR has the advantage of redirecting the T cell to an intracellular antigen. Given that most oncogenic proteins are intracellular, development of a panel of TCRs specific to oncogenic driver proteins has great appeal. However, a library of MHC-restricted antigen-specific TCR reagents needs to be characterized and available to treat patients who have diverse HLA alleles. Furthermore, the low affinity of most tumor-directed TCRs is thought to impact their efficacy significantly. As such, most peptide-cancer vaccines alone or in combination with adjuvants or professional APCs have produced underwhelming clinical responses, despite in vitro evidence of tumor-directed T cell responses (127–129).

Several groups have explored retroviral transduction of native TCRs with the goal of redirecting polyclonal T cells to an intracellular antigen. Potentially significant obstacles were hypothesized because four potential TCRs can be expressed at the cell surface when a T cell transcribes the chains for two different TCRs: native α/β, transduced α/β, and native/transduced “mispaired” heterodimers. This is problematic for two crucial reasons: (a) The native/transduced heterodimers have unknown specificity and potential autoimmune consequences, which have been found in some mouse models (130); and (b) there is dilution of the signal transduction apparatus, because the availability of CD3 complex molecules is limiting. Early studies of

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HIV-directed TCRs encountered low levels of expression of the transduced TCR as well as mispairing; this combination resulted in decreased efficacy in vitro and heterogeneous populations of T cells (131). Several groups have described methods to favor pairing of the transduced TCRs by engineering the transduced TCR chains, including (a) partial murinization of the constant regions (132–134), (b) the addition of disulfide bonds (130, 135–138), (c) altering the knob-in-hole directional interaction of the endogenous TCR constant regions (139), and (d) adding signaling domains to the intracellular portions of the transduced TCR (140). Another interesting approach is to knock down the endogenous TCR with gene editing or shRNA (141); a third-party bank of T cells that also have endogenous HLA knocked down provides yet another possible approach, which has the further advantage of bypassing full HLA matching (142). Most of these modified TCR designs are still in preclinical development (Figure 6).

Nevertheless, trials of native TCR-transduced T cells have been reported, and though some have resulted in significant antitumor responses (99), others have noted significant on- (78, 143) and off-target toxicity (144), particularly when the TCR has relatively high affinity for its cognate antigen. In one clinical study of a high-affinity transduced TCR to the MART-1 melanocyte antigen, investigators observed off-tumor toxicity in the form of significant uveitis and otitis, resulting from the destruction of pigmented cells in the eye and inner ear, respectively (78). In a second example of off-tumor on-target toxicity, a TCR specific for the MAGE-A3 cancer testis antigen cross-reacted with an epitope derived from the related antigen MAGE-A12. In clinical trials, investigators observed neurologic toxicity caused by previously unrecognized expression of MAGE-A12 in the brain (143). The dose-limiting toxicity of TCRs directed to carcinomaembryonic antigen was colitis (145). Although testing for on-target toxicity can be relatively straightforward, for example, by reverse-transcription polymerase chain reaction (PCR) from archived normal tissues, testing for potential off-target toxicities of TCRs is significantly more challenging. Typically, the starting point for identifying off-target toxicity requires that the new TCR be tested against a panel of live cultured cells that are representative of human tissues to serve as targets. In one case, despite extensive preclinical testing on panels of cell lines, cardiac toxicity of a MAGE-A3-directed TCR could not be replicated in vitro until beating cardiac myocytes derived from induced pluripotent cells were used as targets (144, 146); the cause of “off-target” toxicity was the result of TCR cross-reactivity to an unrelated peptide derived from titin (144, 146). Interestingly, the effects of TCR-transduced T cells that have been encountered clinically have not been a result of the predicted effects of mispairing and poor signaling; rather, the toxicities have been related to TCR affinity and specificity, and they demonstrate the high potency of TCR-transduced T cell products. In hematologic malignancies and solid tumors, clinical trials with native-TCR- and engineered-TCR-transduced T cells directed to a number of HLA-restricted antigens are under way (Table 3), with results showing early promise (147).

T CELLS REDIRECTED WITH CHIMERIC ANTIGEN RECEPTORS

The first generation of CARs were engineered receptors comprising a single-chain variable fragment (where the variable portions of the light and heavy chains of a high-affinity antibody are connected by a linker sequence), a transmembrane domain, and the signaling domain of CD3ζ (79, 148). Second-generation CARs have included the costimulatory domains derived from CD28, 4-1BB, or OX40 to optimize T cell activation, and these have improved function in vivo particularly against more aggressive tumors that do not express costimulatory molecules (149). Third-generation CARs also include the signaling domains of a third molecule such as TNF-receptor family members including 4-1BB or OX40; these have less potent cytotoxic activity but persist longer in vivo (150–152). Because CARs are antibody based, however, high-affinity single-chain
Strategies to improve the function of transgenic TCRs. Expression of a transgenic TCR may be improved by preventing the formation of mixed dimers between endogenous and engineered TCR chains. These strategies include the alteration of amino acid interactions, the creation of TCR-CD3ζ fusions, scTCRs, and TCR chimeras (“murinization”) as well as the introduction of additional disulfide bonds or glycosylation. Mutating amino acids in the CDR3 and knocking down/out endogenous TCR expression may also increase transgenic TCR activity. Abbreviations: CDR3, complementarity-determining region 3; CRISPR, clustered regularly interspaced short palindromic repeat; sc, single chain; TALEN, transcription activator-like effector nuclease; TCR, T cell receptor.

variable fragments derived from antibody sequences typically have been directed to native surface antigens, and this restricts suitable targets to proteins or epitopes displayed on the surface of the target cell (Figure 7).

The exact mechanisms of how CARs function are still unknown (153), but CARs appear to homodimerize independently of the TCR and become part of the CD3 complex only if the transmembrane domain selected is that of CD3ζ (154). This is not entirely surprising given that
Figure 7

T cells can be redirected to possess specificity for tumors. (a) Endogenous T cells express a single TCR. (b) Alternatively, these genes can be engineered to express CARs that target surface antigens in an MHC-independent manner. CARs are fusion proteins composed of an extracellular portion that is usually derived from an antibody and intracellular signaling modules derived from T cell signaling proteins. First-generation CARs contain CD3ζ, whereas second-generation CARs possess a costimulatory endodomain (e.g., CD28 or 4-1BB) fused to CD3ζ. Third-generation CARs consist of two costimulatory endodomains linked to CD3ζ. (Abbreviations: CAR, chimeric antigen receptor; MHC, major histocompatibility complex; scFv, single-chain variable fragment; TCR, T cell receptor.)

the transmembrane or intracellular signaling domains typically dimerize (CD8α, CD28), although some of them (4-1BB) may form trimers in their native conformation. Some evidence also suggests that the spacing and conformation of binding to the epitope between the target and the CAR T cell are important in optimizing CAR-induced signaling (155), whereas affinity appears to play a smaller role in CAR T cells (particularly compared with TCR-directed T cells).

Similarly to TCRs, CAR-directed T cells seem to maintain an exquisite sensitivity to low levels of the cognate antigen. This may be surprising because the signaling domains of CARs are not expected to amplify signal to the same degree as TCR triggering of the entire CD3 receptor complex. Indeed, in vitro data suggest that CAR T cells have a threshold for signaling at approximately 50 molecules per target, whereas native T cells need to engage only 1–10 molecules for TCR triggering (156, 157). Recent results suggest that the TCR has multiple modes of downstream signaling that regulate discrete functional events and that the number of ITAMs (immune-receptor tyrosine-based activation motifs) recruited to the synapse regulate these distinct signaling pathways (158). Clinical investigations of CAR T cells have confirmed this low threshold for activation: In trials of CAR T cells directed to the tumor antigen Her2/neu or carbonic anhydrase IX, subjects have experienced severe toxicity based on low-level expression of the target antigen in the lung or the biliary tract, respectively (159, 160).

Currently, most preclinical investigations of new forms of CAR T cells involve xenogeneic immunodeficient models, where a human tumor is implanted either subcutaneously or orthotopically, and human T cells are injected either into the tumor or intravenously, either simultaneously or after tumor engraftment. However, these models are limited: The tumor microenvironment is not replicated in the animal, the remaining arms of the immune system are absent or debilitated,
and there is generally no possibility of evaluating off-tumor expression of the target. For CD19, for example, preclinical models did not predict that CAR T cells would cause the degree of cytokine release or macrophage activation that has been observed clinically (32). Although syngeneic models (161, 162) may overcome some of the limitations of the xenogeneic models, the active cell under evaluation is the engineered human T cell. In addition, mouse and human T cells exhibit significant mechanistic differences that affect the evaluation of the engineered CAR molecule; for example, mouse T cells are much more dependent on CD28 signaling, and 4-1BB signaling has modest effects at most (163, 164).

A number of clinical trials of CAR T cells directed to a variety of antigens are under way (Tables 1 and 2) (165). Several centers have focused some effort to studies of CD19-directed CAR T cells (Table 1) and to other B cell markers such as immunoglobulin light chains and CD20 (Table 2), in part because hematopoietic cells have been extensively characterized and the expression of their surface molecules is often lineage-dependent. Multiple reviews of the CD19 CAR T cell trials are available elsewhere (166–169) and are discussed only in aggregate here. One issue that has complicated the interpretation of the CD19 CAR T cell trials is that each center has developed its own CAR, with different single-chain variable fragments to effect antigen binding, different signaling domains, different modes of introduction of the CAR gene into T cells, different conditioning regimens, and different interventions post–CAR T cell infusion. However, some B cell malignancies are more consistently clinically responsive to CD19 CAR T cells; for example, trials in chronic lymphocytic leukemia (CLL) have yielded very mixed clinical results (111, 170–173), whereas trials in acute lymphoblastic leukemia (ALL) have yielded impressive responses in multiple centers (32, 100, 174). In one case of pediatric ALL, a patient relapsed with CD19-negative disease, indicating that the CD19 CAR T cells exerted very strong selective pressure on cells expressing the CD19 target (32). In contrast, tumor cells from all the nonresponding patients with CLL appear to retain CD19 expression. The fate and length of persistence of the CAR T cells seems to have a significant impact on the clinical responses, but the determinants of these variables are still unclear. It is likely that factors such as the input cell population and the tumor microenvironment play prominent roles in determining CAR T cell persistence and, therefore, in clinical efficacy, even when other variables (type of CAR T cell and manufacturing process) are controlled.

There is also great interest in developing CAR T cells against other hematologic malignancies. The carbohydrate antigen Lewis Y is being tested as a potential target in AML, myelodysplastic syndromes, and multiple myeloma patients (http://www.clinicaltrials.gov, NCT01716364). Specific targets for AML (175) and multiple myeloma (176) are in preclinical development.

Developing CARs for solid tumors has been challenging, in part because of the lack of extensive literature on specific surface markers expressed on malignant epithelial cells. Furthermore, it is not clear that many surface markers are exclusively expressed on tumor cells, and more often, targets with merely higher levels of expression on tumor than on normal tissue have been selected as CAR targets. Although this approach has safety concerns (160, 177), a therapeutic window may be found. For example, despite the death that rapidly ensued after administration of 10^10 dose of Her2/neu CAR T cells in a patient aggressively conditioned, new trials directed against the same antigen but starting with lower doses are under way (NCT00902044, NCT01109095).

T cell products that employ a safety-check mechanism, whether based on transient expression of the CAR [such as RNA electroporation (125)] or a suicide gene encoded into the transduced cells, are considered attractive methods to initiate clinical testing. This may be necessary, for example, for FAP-directed CAR T cells (178, 179), GD2-directed CAR T cells (180), or PSMA-directed CAR T cells (NCT01140373), where preclinical testing or antibody-based testing in the clinic indicates some concerning potential tissue-directed toxicities. The choice of safety-check
mechanism may also affect the function of the CAR T cells: Transient expression of the CAR may sensitize the patient to the CAR (33); incorporating transient viral vectors or viral proteins may also be immunogenic. The humanized caspase 9 suicide system is very attractive and has been clinically tested in the setting of DLIs after bone marrow transplant (181), but should the necessity arise, it is unclear whether all the transduced T cells can be completely eliminated. Alternatively, some lessons learned from nature may be applied to the synthetic biology of CAR T cells: For example, one way to increase specificity of the CAR T cells is to separate T cell signal 1 (antigen) from signal 2 (costimulation). This strategy has been successfully implemented in vitro and in mouse models, where the primary antigen-receptor had low affinity and delivered a weak signal (akin to a TCR signal), but a second engineered receptor (the chimeric costimulatory receptor) that engaged a separate antigen delivered the costimulatory signals (182). However, only engagement of both engineered receptors generated a sustained T cell response.

The field of T cell engineering is now entering adolescence, and creative solutions to many of the current limitations are sure to emerge (Figure 8). For example, one group of investigators hypothesized that, in GvHD following allogeneic bone marrow transplant, T cells could cause damage to the tissues only if they trafficked to those tissues: When tissue trafficking was reduced...
via administration of a drug blocking CCR5 (maraviroc), GvHD was ameliorated in early studies (183), and confirmatory studies are under way. The incorporation of chemokine receptors to alter tumor-specific T cell migration is also under investigation (77), as are mechanisms to improve T cell resistance to inhibitory signals and perhaps to avoid conditioning chemotherapy regimens (162).

The future of CAR therapies looks bright, in part because early studies have shown that CAR T cells are quite potent. Strategies to expedite the discovery of suitable surface antigens may make CAR T cells more widely applicable. The generation of single-chain variable fragments to perform rapid throughput testing on tumors and on normal tissues to identify potential off-tumor reactivity may also increase the applicability of CAR therapies.

BIOMARKERS FOR T CELL THERAPIES

The field of biomarkers has undergone a dramatic revolution over the past few years: Researchers have increasingly realized not only that relevant and meaningful measures of biological activity are unlikely to be generated simply on the basis of hypothesis testing but also that endeavors to interrogate for biological activity need to be supplemented by broader and hypothesis-generating studies (184). Such understanding is particularly relevant for strategies that seek to manipulate the immune system through therapies that affect immune modulation or immune activation, where the inherent multidimensional and integrated complexity of the immune system inevitably confounds scientific reductionism. A parallel concept is the need to implement uniform data-reporting guidelines to support more transparent and systematic analyses of data from T cell therapy trials (185).

In a general sense, biomarkers provide information about the bioactivity of the tested therapy. Beyond this point, in early-stage trials, the principal objectives of biomarker studies are to identify parameters that reveal specific information about the mechanism of action of the treatment and to provide proof that the tested principle is operative. In more advanced trials, the focused principal objective of biomarker studies is to identify and, eventually, to validate biomarkers that correlate with treatment efficacy and can potentially be pursued as surrogate endpoints for the treatment.

Unlike traditional strategies, where the treatment modality is an inert chemical compound, cell therapies are characterized by the fact that the “drug” is a biologically viable, dividing, and evolving entity that interacts with and responds to the myriad complexities of the host biology. As a result, biomarker strategies for cell therapies must focus on studying not only the impact of the infused cells on patient biology but also the infused cells (186). A fundamental starting point for these studies is a thorough understanding of the properties of the manufactured cell product, obtained through product release, potency, and characterization assays.

Arguably, the field of biomarker studies for T cell therapy trials was ushered in by the seminal reports from researchers at the National Cancer Institute that demonstrated cancer regression in melanoma patients following adoptive transfer of bulk TIL-derived cells (187) and, subsequently, of gene-engineered MART-1-specific T cells (98). These studies were among the first to examine persistence of infused cells, characterize their surface phenotype, and indirectly demonstrate in vivo functionality through cancer regression and autoimmunity. Perhaps predictably, persistence of infused cells correlates with cancer regression and durability of remission (188). Indeed, a major limitation for maximal efficacy of T cell therapy–based approaches may be the lack of robust long-term persistence of transferred cells, a limitation that now appears to have been overcome in at least some settings (32, 111). More controversy exists with regard to the phenotype and functional status of T cells needed for optimal antitumor immunity. Earlier work suggested that TIL cells cultured minimally were less differentiated, were more diverse phenotypically, and had superior efficacy
following transfer (189). Other work from the surgery branch of the National Cancer Institute has indicated that naive rather than memory cells are superior for adoptive transfer, as these cells demonstrated better transduction, more robust expansion, enhanced proliferative potential, and telomere length and were less susceptible to terminal differentiation (190). The same group has also shown that adoptive T cell treatment efficacy may be related to the persistence of T cells that either are or can convert in vivo to memory cells (191). More recently, work principally in primate models has suggested that central memory cells may be more effective in adoptive T cell transfer strategies (43). Even more recent and provocative work has suggested that there is a phenotypic plasticity within at least some naive and memory T cell subsets (192). Together, these disparate results highlight the potential folly of interrogating differentiation phenotypes of persisting cells as an essential element of biomarker studies. Direct assessment of the in vivo functionality of infused cells has been difficult to accomplish, at least in part owing to the aforementioned relatively poor persistence of infused cells, although recent studies have demonstrated directly ex vivo the ability of long-term persisting cells to recognize antigen-positive targets (111). Less direct measures of T cell bioactivity have included the measurement of systemic cytokine levels in patients post-T cell infusion. Initial efforts in this area have focused on specific cytokines directly associated with T cell effector functions; more recent and hypothesis-agnostic efforts considering potent clinical efficacy have revealed a broader profile of immune activation that may be important for the ultimate efficacy of T cell–based immunotherapy strategies (32, 100, 111, 173).

The principal mechanisms by which T cells effect antitumor activity are (a) direct engagement and cytolysis of target cells and (b) production and secretion of soluble cytokines and chemokines that directly impact tumor cells and orchestrate a more integrated antitumor inflammatory response. Accordingly, biomarker studies to interrogate T cell mechanisms of action focus on detecting the presence and effector functionality of infused T cells. Although accomplishing this objective is relatively straightforward in cases where cancers are predominant in peripheral blood or bone marrow, it is a considerably more difficult challenge for cancers where disease is not readily accessible. Indeed, recent clinical data have provided compelling evidence to support the need to evaluate the T cell function at the site of disease (193, 194). Data accumulated principally in the context of adverse events demonstrate that infused T cells do, in fact, traffic throughout the body and home to sites where target antigen is expressed (144, 146), thus highlighting how critical it is to develop innovative and sensitive approaches to enable the interrogation of tissues for T cell presence and functionality.

In most adoptive T cell immunotherapy studies, the total number of infused cells corresponds to a small fraction of total T cells, although lymphodepleting conditioning can skew this ratio at early time points post-infusion. Homeostatic and antigen-driven expansion drives high frequencies of infused T cells in patients at late times post-infusion (32, 98, 111). Both molecular- and flow-cytometry-based approaches have been developed to evaluate persistence and homing of infused T cells.

Quantitative PCR–based approaches have been developed in a number of cases to detect and quantify the persistence of infused T cells. Such approaches are feasible if the infused T cells have been genetically engineered prior to infusion. They also typically enable the detection of infused cells at frequencies as low as 0.01% of total cells, thus providing important information about persistence, trafficking, and homing of infused T cells in patients. Such approaches are being increasingly applied in clinical studies to monitor T cell persistence (see, for example, 32, 100, 111, 173) as well as to interrogate and demonstrate the contribution of infused cells to serious adverse events (144). Recent technological advances in high-throughput deep sequencing of CDR3 domains for TCR loci to detect and quantify individual TCR clonotypes in samples (195) expand the potential of obtaining molecular signatures for individual clonotypes that persist in patients and
of correlating this signature to the original infusion product. A considerable limitation of molecular approaches is that they generate data from bulk cell populations. The robust development of single-cell multiplexed digital PCR-based approaches (196) opens further exciting possibilities: Infused cells in samples may be detected with greater sensitivity, and the functionality of infused engineered cells may be interrogated at the single-cell level.

Flow-cytometry-based approaches depend on the availability of antibody reagents to detect gene-engineered and infused cells. Such reagents have included MHC class I multimers (dextramers, tetramers, pentamers) that have been employed in a large number of clinical trials. More recently, in CAR trials, idiotype-specific antibodies that recognize CAR-engineered cells have been employed to detect infused cells with high specificity (32, 100, 111). This approach typically requires that the frequency of antigen-specific cells be at least 0.2% of the total CD3 population. Reports of considerably more sensitive detection have been published (197), as have assays with higher-throughput combinatorial strategies that increase sensitivity and reduce sample usage (198). One advantage of flow-cytometry-based approaches is that they can be readily combined with stains for surface-phenotypic or functional markers, thus allowing for the generation of more integrated data sets.

Technical advancements in polychromatic flow cytometry combined with an increased understanding of T cell biology have precipitated a number of flow-cytometry-based approaches that interrogate T cell function (for a summary of specific markers and strategies, see 186). The continued development of mass-cytometry-based platforms and algorithm-driven hierarchical clustering approaches that enable simultaneous interrogation of very large numbers of T cell molecules including surface and intracellular proteins, phosphoproteins, and RNA species (199, 200) may render obsolete the traditional flow-cytometry-based approaches that interrogate T cell functionality as a stand-alone platform.

As discussed above, the integrated complexity of the immune system mandates that part of the evaluation of T cell therapy focus on the impact that treatment has on the broader immune system. Although this relatively new concept has not yet been broadly implemented in clinical trials, one approach that has been implemented with some success has been to evaluate systemic cytokine levels in patients during treatment. In studies targeting leukemias using CAR-engineered T cells, this strategy has revealed that engineered T cell activation and antitumor activity result in broad and potent cytokine-driven effects, including cytokine-release syndrome (32, 111), macrophage-activation syndrome, and hemophagocytic lymphohistiocytosis. Notably, the hypothesis-agnostic interrogation of cytokines in these trials unexpectedly identified IL-6 as a major cytokine induced by CAR therapy: As a result of this observation, the anti-IL-6 receptor antagonist antibody tocilizumab was successfully deployed to mitigate the observed cytokine-induced toxicity (32), a treatment now being applied more systematically to counteract cytokine-release syndrome (201).

BUILDING T CELL THERAPIES INTO A PILLAR OF MEDICINE

Now that adoptive T cell therapy is showing such dramatic effects, the question becomes, How can we move past offering this as boutique medicine in major medical centers and offer it in communities all over the world? There are several logistical issues that need to be addressed: shipping and tracking of autologous blood products, large-scale manufacturing of vectors and T cell products, and validation of processes and immunologic assays necessary for quality control. Resolving these issues is likely to require adaptation by a variety of fields outside the scope of most physicians and immunologists, ranging from blood banking, a field that has learned to manage cell collection and processing techniques in standardized ways at the international level, to robotic
manufacturing techniques used in high-throughput laboratories and systems such as automobile manufacturing (202).

Understandably, some scientists have raised concerns about the complexity of this type of therapy; even performing multicenter studies with T cell products has been challenging at the academic level. Also, most regulatory health agencies are set up to handle drug testing where drugs are manufactured centrally, the active ingredient is measured and controlled, and one lot treats many patients. In contrast, the active “ingredient” of a T cell product is challenging to define because it reflects a heterogeneous population of T cells: Typical, phase I dose-escalation trial design may not be the most appropriate method to test the safety of T cell products, given that the “dose” is neither static nor maximal immediately after infusion. Furthermore, each “lot” produced can be used for only one patient, and thus far, T cell products have been manufactured locally. As these therapies move to phase II or III studies to obtain an indication for use, it is not yet clear whether it will be possible to perform double-blind randomized controlled trials that are considered the gold standard in establishing a standard of care therapy.

Although these issues pose significant challenges, they are not necessarily insurmountable barriers; organ and bone marrow transplants are now considered fairly routine (203). As the manufacturing of cell products becomes more automated, and as scientists better define the key components of what makes the most bioactive, optimal T cell product, it will become an absolute necessity to develop large-scale, centralized processes that can generate standardized, quality-controlled T cell products. This endeavor will require scientists, physicians, and industry to work together to build the necessary infrastructure and adapt the current regulatory standards to reflect an entirely new pillar of medicine, one composed of personalized cell therapies (202).

**DISCLOSURE STATEMENT**

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**LITERATURE CITED**


55. Levine BL, Ueda Y, Craighead N, Huang ML, June CH. 1995. CD28 ligands CD80 (B7-1) and CD86 (B7-2) induce long-term autocrine growth of CD4+ T cells and induce similar patterns of cytokine secretion in vitro. Int. Immunol. 7:891–904


68. Deleted in proof


165. Melenhorst JJ, Levine BL. 2013. Innovation and opportunity for chimeric antigen receptor targeted T cells. *Cytotherapy* 15:1046–53


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