Cancer Immunology Research

T-cell–based Immunotherapy: Adoptive Cell Transfer and Checkpoint Inhibition

Roch Houot[1,2], Liora Michal Schultz[3], Aurélien Marabelle[4], and Holbrook Kohrt[5]

Abstract

Tumor immunotherapy has had demonstrable efficacy in patients with cancer. The most promising results have been with T-cell–based therapies. These include adoptive cell transfer of tumor-infiltrating lymphocytes, genetically engineered T cells, and immune checkpoint inhibitor antibodies. In this review, we describe the different T-cell–based strategies currently in clinical trials and put their applications, present and future, into perspective. *Cancer Immunol Res; 3(10); 1115–22. ©2015 AACR.*

Introduction

T cells are believed to play a major role in immunosurveillance and tumor eradication. On the basis of this paradigm, over the past quarter century, therapies have been developed to generate, educate, and/or enhance T cells against tumors (Fig. 1). Although the initial demonstration that T cells had the potential to treat cancer comes from allogeneic stem cell transplantation, here we focus on autologous T cells, which can be manipulated in two ways to treat cancer: either *ex vivo* using adoptive cell transfer (ACT) or *in vivo* using T-cell targeting antibodies.

The first adoptive transfer approach consists of the infusion of autologous lymphocytes with antitumor properties. These lymphocytes can be derived from unmodified (i.e., naturally occurring) T cells isolated from resected tumors (tumor-infiltrating lymphocytes, TIL) or genetically engineered T cells recognizing tumor antigens [T-cell receptors (TCR) or chimeric antigen receptors (CAR)]. The second approach consists of direct *in vivo* stimulation of lymphocytes in patients using antibodies, including checkpoint inhibitors and bispecifics (Fig. 2). Each of these strategies has its own characteristics (Table 1): the identification of a tumor antigen (Ag), whether it is needed or not, the type of tumor Ag that is targeted (surface vs. intracellular), the nature of the T-cell response that is generated against the tumor (monoclonal vs. polyclonal), the durability of immune protection (short term/passive vs. long lasting/active), and the methods of production of the "drug" (off-the-shelf vs. customized, personalized vs. "universal"). On the basis of these characteristics, some T-cell therapies may be more applicable than others, depending on the type of tumor. Here we also compare and contrast the potential benefit and toxicities of monotherapy versus combinatorial approaches (adoptive T cells and checkpoint inhibitor antibodies; Table 2).

[1]CHU Rennes, Service Hématologie Clinique, Rennes, France. [2]INSERM U917, Rennes, France. [3]Department of Pediatrics, Division of Stem Cell Transplantation and Regenerative Medicine, Lucile Packard Children's Hospital and Stanford University, Stanford, California. [4]Gustave Roussy Cancer Center, Drug Development Department, INSERM U1015, Villejuif, France. [5]Department of Medicine, Division of Oncology, Stanford University, Stanford, California.

Note: All the authors contributed equally to this article.

Corresponding Author: Holbrook Kohrt, Stanford University Medical Center, Division of Oncology, 269 Campus Drive, CCSR 1105, Stanford, CA 94305. Phone: 650-736-1083; Fax: 650-736-1454; E-mail: Kohrt@stanford.edu

doi: 10.1158/2326-6066.CIR-15-0190

Adoptive T-cell Transfer

Adoptive T-cell Transfer offers several advantages. Antitumor T cells with high-avidity recognition of tumor antigens can be expanded *in vitro* in large numbers, genetically engineered, and/or activated *ex vivo* to acquire antitumor functions. In addition, the host can be manipulated before cell transfer to eliminate suppressor cells (such as T-regulatory lymphocytes and myeloid-derived suppressor cells) and promote *in vivo* expansion of transferred lymphocytes (through "homeostatic expansion") by eliminating endogenous lymphocytes that can behave like a cytokine sink that competes for the same survival and stimulatory factors (notably cytokines such as IL7 and IL15). The lymphocytes infused during ACT function as "living drugs" that can induce long-term protection.

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes are a heterogeneous cell population found within neoplastic lesions and are mainly composed of T cells. A fraction of TILs express TCRs directed against unique or shared tumor-associated antigens and exert cytotoxic effects against malignant cells. These TILs can be isolated from resected tumors, selected and expanded *ex vivo*.

The initial studies, published in 1988, consisted of the administration of TILs to patients with metastatic melanomas (1). However, these cells were unable to persist *in vivo*, despite concomitant administration of IL2, a T-cell growth factor. This approach was significantly improved by the administration of a lymphodepleting preparative regimen consisting of chemotherapy (usually cyclophosphamide and fludarabine) with or without total-body irradiation (TBI). In recent protocols, overall response rates and complete response rates for metastatic melanoma were around 50% and 20%, respectively (2, 3). Responses were seen in all visceral sites including brain. Most importantly, these responses appeared durable. Among patients who achieve a complete tumor regression (22% of the patients, $n = 20$), 95% of them have ongoing complete regressions beyond 5 years and may be cured (3).

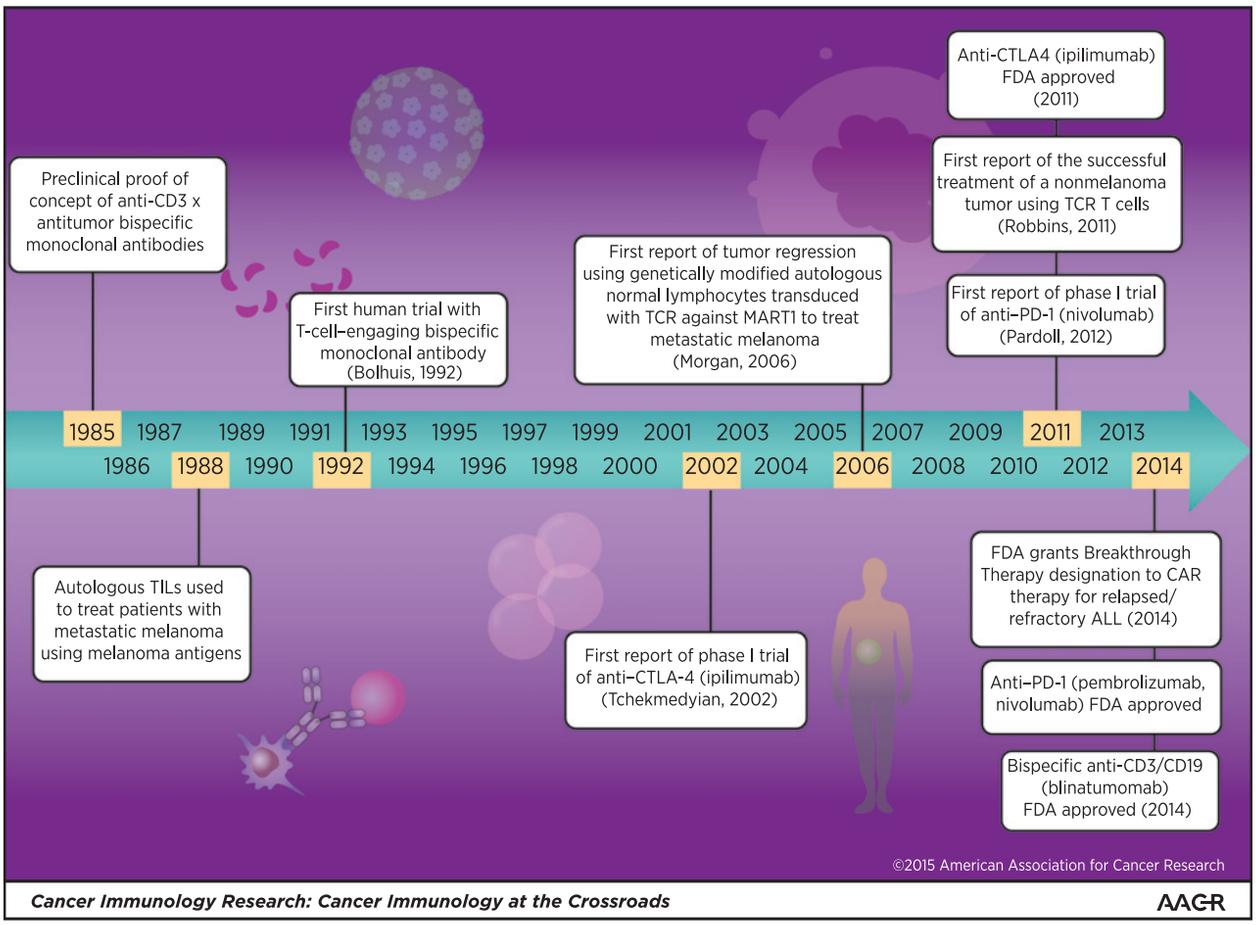

Figure 1.
History of T-cell therapy in cancer. This timeline presents a historical perspective on important developments in the field. See refs. 12, 15, 34, 40, and 41.

Although this strategy allows expansion and persistence of a large number of polyclonal antitumor T cells, there are several limitations to this approach. The first limitation relates to feasibility. The tumor needs to be resected for isolation and expansion of TILs. The patient needs to be "suitable" to tolerate lymphodepletion and IL2-based treatments and remain suitable during the several-week period required to expand the cells *in vitro*. The laboratories producing TILs need to be specialized and have highly trained personnel. The procedure is expensive. Most significantly, this approach may not be applicable in tumors other than melanoma. Indeed, the efficacy of naturally occurring TILs seems to be primarily restricted to melanoma, for reasons that are not fully understood. Melanoma is unusual among cancers in that it naturally gives rise to high numbers of antitumor T cells infiltrating into tumors. Although TILs can be isolated from many cancers, to date, only those from melanomas consistently possess specific cytolysis against the tumors from which they were generated (4). This may be due to the strong immunogenicity of melanoma resulting from the high frequency of mutational events (5). Nevertheless, some tumors might be responsive to TIL therapy, especially those expressing potentially immunogenic mutational epitopes (high frequency of somatic mutations) and those sensitive to checkpoint blockade. Unmodified T cells can also be used to treat virally associated cancers [e.g., Epstein-Barr virus (EBV)– or human papillomavirus (HPV)–associated tumors] that express viral foreign antigens. EBV-associated tumors include Burkitt lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders, some Hodgkin and non–Hodgkin lymphoma, and gastric carcinoma. EBV expresses latency genes in transformed cells, and the viral proteins can be targeted by T cells. EBV-specific T cells can be isolated and expanded from peripheral blood. Donor and third-party (i.e., allogeneic) EBV-specific T cells have been successfully used to treat posttransplant lymphoproliferative disorders (6). Autologous EBV–specific T cells also demonstrated promising efficacy in EBV-associated tumors such as nasopharyngeal carcinoma (7, 8) and Hodgkin disease (9, 10).

Genetically modified T cells (TCRs, CARs)

As mentioned previously, a major limitation in the more widespread application of TIL therapy is the difficulty in identifying antigen-specific T cells in other cancer types. Although naturally occurring TILs have demonstrated efficacy in melanoma, all tumor types with identifiable tumor-associated antigens can be lysed by T cells engineered to recognize tumor

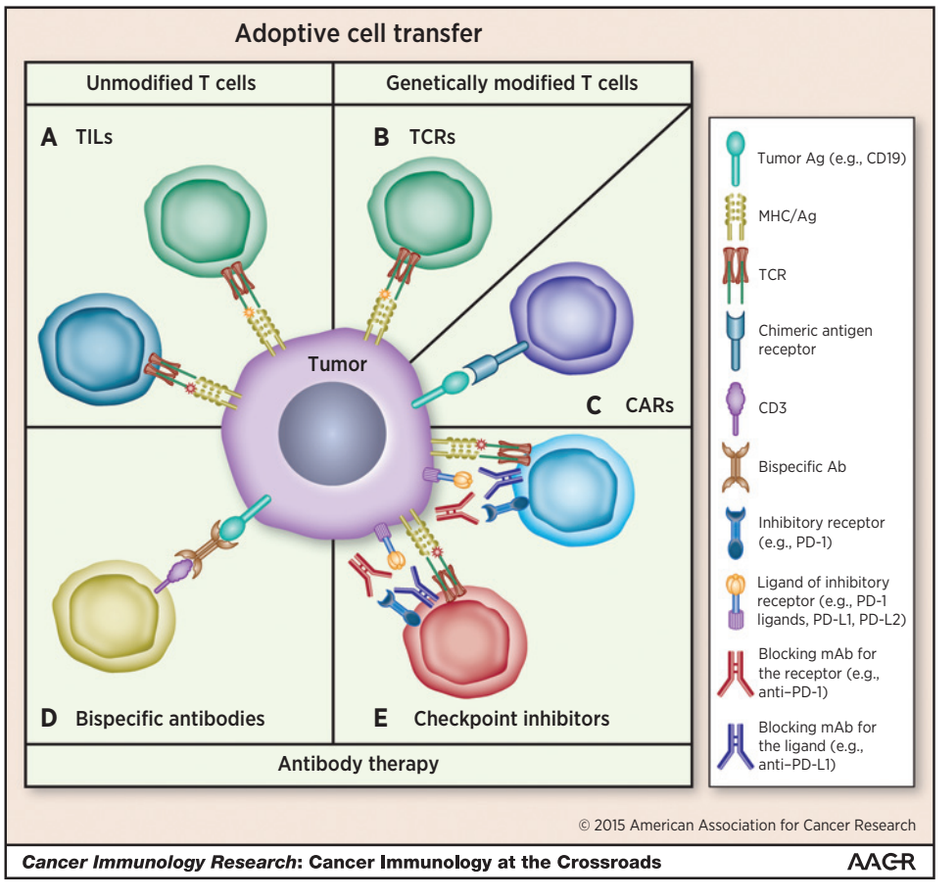

Figure 2.
Strategies for use of T-cell therapies for cancer. Anticancer T-cell–based therapy can be performed (A, D) by *ex vivo* manipulation of T cells through ACT of unmodified (TILs) or genetically modified T cells (TCRs, CARs) and (B, D) by *in vivo* manipulation of T cells using antibodies (bispecific and checkpoint inhibitors). These approaches may induce monoclonal (TCRs, CARs, bispecific antibodies) or polyclonal (TILs, checkpoint inhibitors) antitumor T cells. Ag, antigen.

antigens. Moreover, TIL therapy makes it necessary for each patient to have a surgical resection and requires the ability to consistently generate and expand T cells while preserving antitumor function. To overcome these obstacles, investigators have developed approaches on the basis of genetic modification of normal peripheral-blood T cells. These modifications allow T cells to be specifically redirected to tumor antigens and trigger T-cell activation and tumor eradication upon antigen binding.

There are two common approaches for redirecting T-cell specificity: (i) gene modification with TCRs directed against tumor-associated antigens and (ii) introduction of a CAR. Different types of antigens can theoretically be used to redirect autologous T cells against tumor cells: tissue-specific differentiation antigens [such as melanocyte differentiation antigens (MDA); e.g., gp100 or MART1, in melanoma and CD19 in B-cell malignancies], cancer testis (germ cell) antigens (such as NY-ESO-1), which are detected in many tumors but not in normal adult tissues, with the exception of the testis (11), overexpressed self-proteins (such as HER2), mutational antigens (such as BRAF-V600E), and viral antigens (such as EBV in Hodgkin disease and HPV in cervical cancer).

Genetically modified T cells (TCRs or CARs) are transfected using virus vectors (retroviruses or lentiviruses) or a transposon system (Sleeping Beauty). Following transfection, genetically modified T cells are expanded and transferred into patients treated with preconditioning lymphodepletion similar to that used with TIL protocols.

TCR T cells. TCR T cells are T cells cloned with TCRs in which variable α- and β-chains with specificity against a tumor antigen (either from a patient or from humanized mice immunized with tumor antigens). Such T cells recognize processed peptide antigens expressed in the context of MHC.

The first report of tumor regression after administration of autologous TCRs described the treatment of metastatic melanoma with a TCR with specificity for MART1 (12). Since then, other trials have tested TCRs against other antigens, including gp100 in melanoma (13), carcinoembryonic antigen (CEA) in colorectal cancer (14), and NY-ESO-1 (15) and MAGE-A3 (16) in melanoma and synovial sarcoma. Clinical responses were observed across trials.

Some of the advantages of TCR T cells arise from the fact that these cells can be produced from peripheral blood T cells (unlike TILs) and can "see" intracellular antigens (unlike CARs). However, many of the TCR T-cell trials were accompanied by on- or off-target toxicity. TCRs recognizing tumor-associated antigens expressed on healthy tissues induced on-target toxicity. MDAs such as MART1 and gp100 are expressed by normal melanocytes present in the skin, retina, and inner ear. TCR gene therapy against MART1 and gp100 resulted in skin rash, uveitis, and hearing loss (14). Similarly, patients with colorectal adenocarcinoma who were treated with CEA TCRs experienced dose-limiting severe colitis due to the expression of CEA on normal epithelial cells throughout the gastrointestinal tract (14). The use of T cells with MAGE-A3–specific TCRs, a cancer testis (CT) antigen, also resulted in on-target toxicity. These patients experienced neurologic

Table 1. Characteristics of different T-cell therapies for cancer

	TILs	TCRs	CARs	Bispecific antibodies	Checkpoint inhibitors
Type of therapy	ACT	ACT	ACT	Antibody	Antibody
Drug	Unmodified (naturally occurring) T cells isolated form tumors and expanded *ex vivo*	T cells genetically engineered *ex vivo* from peripheral T cells to express recombinant TCR against a tumor Ag	T cells genetically engineered *ex vivo* from peripheral T cells to express a CAR (scFv with TCR signaling molecules) against a tumor surface Ag	T-cell engaging bispecific antibodies directed against a tumor surface Ag and CD3	Antibodies blocking inhibitory molecules on T cells to enhance their function
Requirement for tumor Ag identification	No	Yes	Yes	Yes	No
Specificity against tumor cells	Polyclonal	Monoclonal	Monoclonal	Monoclonal	Polyclonal
Origin of Ag targeted	Intracellular and extracellular	Intracellular (MHC-I–restricted T cells)	Surface	Surface	Intracellular and extracellular
MHC-restricted	Yes[a]	Yes[a]	No	No	No
Long-lasting protection	Yes	Yes	Yes	No	Yes
Off-the-shelf	No	No	No	Yes	Yes
Personalized therapy	++++[b]	+++	++	+	None
Main limitations	Feasibility Mostly restricted to melanoma	On-target/off-tumor toxicity	On-target/off-tumor toxicity CRS Tumor escape (loss of surface Ag)	CNS toxicity (with CD19/CD3 antibody)	Autoimmune manifestations

Abbreviations: Ag, antigen; CRS, cytogen release syndrome.
[a]HLA-negative tumors are not "seen," but HLA presentation allows recognition of intracellular antigens.
[b]Plus signs indicate degree of personalization.

toxicity due to MAGE expression in the central nervous system (CNS; ref. 16). In addition, 2 patients, one with melanoma and the other with multiple myeloma, experienced off-target toxicity when treated with MAGE-A3–specific TCRs. Both patients had fatal cardiac toxicity due to unexpected recognition by the MAGE-A3 TCR of titin, a cardiac peptide antigen (17). No on-target toxicity was observed with the CT antigen NY-ESO-1 TCR T cells because of lack of NY-ESO-1 on normal tissues (15).

CAR T cells. The use of TCR T cells is limited by the fact that this therapy can only be proposed to MHC-compatible patients. In addition, tumors frequently lose antigen expression through downregulation of MHC. To overcome these limitations, CAR technology has been developed. CAR T cells are constructed by fusing an antibody-derived single-chain variable fragment (scFv) to T-cell intracellular signaling domains. Such T cells recognize cell surface antigens in a non–MHC-restricted manner. They do not depend on antigen processing and presentation. The first-generation CARs consisted of an scFv linked to the intracellular signaling domain of CD3ξ. To improve persistence and proliferation of infused T cells, second- and third-generation CARs were developed that incorporate the intracellular domains of one or multiple costimulatory molecules, such as CD28, OX40, and 4-1BB (which reproduce the "second signal") within the endodomain.

In early-phase clinical trials, the most promising results of CARs have been obtained in B-cell malignancies with CAR T cells with specificity for CD19. In lymphoma, anti-CD19 CAR T cells were promisingly efficacious (18). Specifically, anti-CD19 CAR T cells induced four complete responses (CR; three of them still ongoing at 9 and 22 months), two partial responses (PR), and one stable disease (SD) out of seven patients with chemotherapy-refractory diffuse large B-cell lymphoma (DLBCL). In chronic lymphocytic leukemia (CLL), of 23 patients treated with CD19 CAR T cells, 5 (22%) achieved a CR and 4 (17%) achieved a PR, for an overall response rate of 39% (19). However, the most spectacular results have been observed in acute lymphoblastic leukemia (ALL). By infusing CD19-targeting CARs in 30 patients (25 children and 5 adults) with relapsed or refractory ALL, Maude and colleagues reported a 90% complete remission (20). CAR T cells were also able to eradicate leukemia in the cerebrospinal fluid. Sixty-seven percent of the patients remained in remission at 6 months. Prolonged persistence of CD19 CARs (for years) has been observed. In July 2014, FDA granted breakthrough therapy designation to CD19 CARs for relapsed/refractory ALL. In solid tumors in general, the clinical results have been disappointing so far, due to either lack of efficacy or limiting toxicities. Nevertheless, promising results were achieved by Pule and colleagues with CARs directed to GD2 in neuroblastoma. Three of 11 patients with active neuroblastoma achieved CR (21, 22). CARs have also been developed that recognize many other targets, including HER2 for colorectal cancer (23), folate receptor-α for ovarian cancer (24), and carbonic anhydrase IX (CAIX) for renal cell carcinoma (25), but failed to show significant antitumor effect. CARs to a multitude of different antigens are currently under development.

CAR technology offers many advantages. Similar to TCRs, CARs can be produced from peripheral blood T cells. However, in contrast to TCR T cells that are restricted to a single type of MHC molecule, CARs can be used to treat all patients in an HLA-unrestricted manner. In addition, they can target tumors that have downregulated HLA class I molecules or fail to process or present proteins.

Despite promising efficacy, extensive use of CAR therapy may be limited by a number of issues. The first issue relates to on-target/off-tumor toxicity. To date, most CAR trials have targeted overexpressed antigens, nonmutated self-proteins

Table 2. Comparison of ACT, checkpoint blockade, and combinatorial adoptive T-cell and checkpoint blockade therapy

	Adoptive T-cell therapy (ACT)	Checkpoint blockade	Impact of combination therapy
Efficacy	Most experience and efficacy demonstrated with hematologic malignancies (CD19-expressing tumors)	Most experience and efficacy demonstrated with solid tumors (malignant melanoma, RCC, NSCLC)	Potential for additive or synergistic antitumor efficacy in tumor types where ACT or checkpoint blockade alone has suboptimal efficacy
Toxicity	On-target/off-tumor effect CRS	IrAE: Nonspecific autoreactive T-cell activity	Risk of additive toxicity
Effector cell source	*CAR/TCR:* Autologous: (Pre–stem cell transplant: patient-derived T cells; post–stem cell transplant: patient-derived engrafted donor T cells that have been tolerized) Allogeneic third-party T cells: Require HLA matching TIL: Tumor-derived	Recruits patients' own T cells without need for apharesis and *ex vivo* cell manipulation Relies on patient having endogenous tumor-specific T cells	Requires same T-cell source as would be required in ACT alone (refer to adoptive T-cell therapy) In contrast to checkpoint inhibition alone, combination therapy does not rely on patient having endogenous tumor-specific T cells
Need for antigen specificity	Requires T-cell specificity to cancer-associated antigen: CARs: Different constructs for different tumor types TILs: Patient specific	Effective across tumor types despite varying antigen expression profile	Antigen specificity required for ACT (refer to adoptive T-cell therapy)
Scalability	Complicated *ex vivo* cell manipulation: TIL: TIL isolation and expansion CAR: Apharesis, T-cell transduction (retroviral, lentiviral, sleeping beauty, gene editing via CRISPER) and expansion	Off-the-shelf	Cell manipulation required for ACT preparation (refer to adoptive T-cell therapy)
Time for treatment preparation	Time lag introduced to allow for *ex vivo* cell manipulation and expansion	Readily available	Time lag introduced for ACT preparation (refer to adoptive T-cell therapy)

Abbreviations: CRS, cytokine release syndrome; IrAE, immune-related adverse event.

expressed more highly on malignant than normal tissues. Similar to TCR T cells, some on-target toxicities have been observed with CAR T cells. For instance, anti-CAIX CARs have been tested in patients with renal cell carcinoma (RCC). CAIX is strongly expressed in RCC but is also present in normal tissues including liver (biliary epithelium), small intestine, and gastric mucosa. These patients experienced liver toxicity and no tumor regression (25). CARs have also been developed against HER2, an EGF receptor family member overexpressed in common malignancies including subsets of breast, colon, ovarian, gastric, and kidney cancers and melanoma. HER2 is also expressed in normal tissues including heart, lung, gastrointestinal tract, and kidney. Anti-HER2 CAR T cells (using the scFv from trastuzumab, the antibody to HER2 used in the treatment of breast cancer) were tested in one patient with metastatic colon cancer (23). This patient rapidly developed respiratory distress, hemodynamic instability, and cytokine release syndrome. His death 5 days after adoptive transfer appeared to be triggered by the recognition of low antigen expression on lung epithelial cells. Anti-HER2 CARs were found in the lung on postmortem examination. Another example of the on-target/off-tumor effect is B-cell aplasia resulting from CD19 CAR T cells targeting normal B cells that express CD19. These examples highlight the difficulty of targeting antigens expressed by normal tissues. Another CAR-mediated toxicity is cytokine release syndrome (CRS). CRS is a frequent and sometimes life-threatening complication of CAR therapy. Manifestations of CRS include fever, increased cytokine concentrations (IL6, IFNγ), hypotension, hypoxia, and neurologic symptoms. It usually correlates with tumor burden, T-cell proliferation, and clinical response. CRS can be effectively treated with glucocorticoids and/or, preferentially, antibody to IL6 receptor (tocilizumab). Another issue with CAR therapy is tumor escape by loss of target expression. Indeed, T-cell–driven selective pressure allows emergence of CD19-negative populations leading to tumor escape. This has been observed in several patients with ALL treated with CD19 CARs.

Although very promising, engineered T cells are facing several limitations including toxicity, applicability, and sometimes limited efficacy. Several approaches are trying to obviate these limitations. Regarding safety, toxicities may be limited by careful choice of target antigens to avoid off-tumor but on-target adverse effects. Ideal targets should be tumor-specific antigens. In case of antigens shared with normal tissues, these should not be present on tissues of vital organs. The identification of appropriate tumor antigens is perhaps the greatest challenge now facing researchers in the field of ACT. Toxicities may also be limited through on-demand cell destruction of engineered T cells by incorporation of suicide genes. These strategies include Herpes simplex virus thymidine kinase (HSV-TK) and inducible caspase-9 (26). Regarding applicability of engineered T cells, strategies are being developed to generate "universal" T cells that could be manufactured from one donor and administered to multiple patients. For instance, this could be done by eliminating the expression of endogenous TCR to generate T cells devoid of alloreactive TCRs (27). Finally, ACT efficacy may be improved by generating and/or selecting T cells with enhanced proliferative and antitumor capacity. For instance, preclinical data show that intrinsic properties related to the differentiation state of the adoptively transferred T-cell populations may be crucial to the success of ACT-based approaches (28). Efficacy may also be enhanced by combining ACT with other immunotherapeutic approaches, including checkpoint inhibitors (below).

Checkpoint Blockade

Multiple inhibitory mechanisms restrain endogenous TILs and modified T cells used for adoptive therapy from effectively

eradicating tumor. Beyond well-documented inhibitory cells including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC) that are upregulated in the tumor microenvironment, T cells have intrinsic regulatory mechanisms that abrogate their effector function. Activated T cells upregulate surface inhibitory receptors including cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed death ligand 1 (PD-1), and upon binding to their respective ligands, they transmit inhibitory signals to the T cells and attenuate T-cell activation. These receptors act as checkpoints that protect the host from unopposed T-cell activation. Many tumor types take advantage of these inhibitory pathways to circumvent T-cell–mediated destruction. Disrupting these receptor–ligand interactions using blocking antibodies removes this inhibition and enables enhanced T-cell–mediated antitumor activity. This approach to immune therapy is referred to as checkpoint blockade.

One benefit of checkpoint blockade as compared with T-cell therapy is the ease of generalizability to a spectrum of malignancies. Unlike adoptive T-cell therapy that relies upon the presence of cancer-specific antigens and requires *ex vivo* manipulations, checkpoint blockade can be achieved with the use of an off-the-shelf monoclonal antibody that can be applied across tumor types.

CTLA-4 blockade

CTLA-4 is a member of the CD28:B7 immunoglobulin family and acts as a surface inhibitory receptor found on effector T lymphocytes and Tregs (29). Antibodies blocking CTLA-4 receptor–ligand engagement were the first checkpoint blocking antibodies to be approved for clinical use in melanoma in 2011. Long-term follow-up of earlier studies show that 15% of treated patients survive 5 years, indicating that responses can be durable (30).

The survival improvement seen with antibodies to CTLA-4 in melanoma stimulated further investigation of CTLA-4 blockade in other solid and hematologic malignancies, including prostate cancer, non–small cell lung cancer (NSCLC), RCC, pancreatic cancer, ovarian cancer, glioblastoma, acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS) with encouraging preliminary results. CTLA-4 blockade is also being explored in children with refractory malignancies, including Wilm tumor, bone and soft-tissue sarcomas, neuroblastoma, and lymphomas. Currently, more than 100 trials investigating CTLA4 blockade are ongoing or in the pipeline (clinicaltrials.gov).

The effect of CTLA4 blockade, unfortunately, is not restricted to tumor-specific T cells. The appropriate inhibition of autoreactive T cells can be disrupted, leading to unwanted immune-mediated toxicities, including immune-related adverse events (irAE) most commonly affecting skin (rash, pruritus, vitiligo), bowel (diarrhea, colitis), liver (hepatitis, transaminitis), and endocrine glands (hormonal imbalance; refs. 31, 32).

PD-1 blockade

The PD-1 receptor is expressed on the cell surface membrane of activated T cells. Interaction with ligands PD-L1 (often upregulated on tumor cells including urothelial, ovarian, breast, cervical, colon, pancreatic, gastric, melanoma, glioblastoma, and NSCLC) and PD-L2 (found on antigen-presenting cells including macrophages, mast cells, and dendritic cells; ref. 33) results in an inhibitory signal to the T cell. Preclinical data demonstrating PD-1 expression by TILs, PD-L1 upregulation on certain tumor cells, and mouse models that showed enhanced antitumor immunity through PD1 blockade (34) led to clinical trials of PD-1 blocking agents in 2007 and to FDA approval in 2014 for use in treating melanoma (pembrolizumab, nivolumab).

PD-1 blockade has since been shown to affect RCC, NSCLC, Hodgkin disease, and uroepithelial bladder cancer. The responsiveness of NSCLC was notable, as unlike melanoma and RCC, NSCLC has not previously demonstrated responsiveness to immune therapy. A recent trial in relapsed/refractory Hodgkin disease showed remarkable effects of PD-1 blockade with objective responses in 87% of patients ($n = 23$; ref. 35). In contrast to most successes with PD-1 blockade, which have been in solid tumors, these results showed an effect of PD-1 blockade in hematologic malignancies.

More than 100 trials are investigating the use of PD-1 blockade agents as monotherapy or in combination with chemotherapeutic agents, targeted therapies, or alternate immunotherapy modalities for multiple tumor types (clinicaltrials.gov). To date, more than 7,000 patients, with multiple indications, including NCSLC, RCC, and malignant melanoma have participated in trials of PD-1 blockade and have tolerated the agent well. To date, the toxicity profile of PD-1 blockade has been less severe than CTLA-4–mediated irAE, consistent with PD-1 knockout mice having a milder autoimmune phenotype than CTLA-4 knockout mice (36).

Conclusions and Future Directions

Adoptive cell transfer and checkpoint blockade have both had striking success to date, but both treatment modalities are in their infancy, with much untapped potential. Methods to further optimize outcomes are in order. There is a strong rationale to combine checkpoint inhibition with adoptive T-cell therapy. Adoptive T cells require a permissive environment to allow for maximal antitumor effect. Checkpoint blocking antibodies remove T-cell inhibition; however, their effectiveness relies upon the presence of functional tumor-specific T cells. Providing both tumor-specific T cells and removing T-cell inhibitory stimuli through checkpoint blockade may offer outcomes superior to those achieved with either agent alone (Table 2).

The antitumor efficacy of combinatorial therapy with CAR T cells and checkpoint blockade using PD-1 blocking antibodies was investigated preclinically using a transgenic Her2 mouse model treated with Her2-specific CAR T cells alone, mAb to PD-1 alone, or a combined approach. Her2-specific CAR engagement with tumor Her2 antigen has triggered PD-1 upregulation on CAR T cell (37). PD-1 blockade enhanced proliferative and functional capacity of Her2-specific T cells and enhanced regression of established tumor when compared with arms treated with anti-Her2–specific CARs alone (37). An alternative approach to merging CAR technology with checkpoint blockade is being explored preclinically, in which a chimeric protein is generated that fuses an extracellular and transmembrane domain of CTLA-4 or PD-1 to a cytoplasmic-activating signal domain (such as CD28). This innovative chimera, upon binding ligand, transforms the inhibitory CTLA-4 or PD-1 signal into a positive stimulatory signal. CTLA-4/CD28 chimera maximized T-cell antitumor function in preclinical lymphoma and melanoma syngenic mouse models (38). T cells modified to express PD-1/CD28 chimeric molecules exhibited enhanced cytokine secretion, upregulation of activation markers, and

improved proliferation upon coculture with tumor cells *in vitro* when compared with control cells, and superior *in vivo* antitumor function in two xenograft models of human melanoma tumors (39). These innovative chimeric proteins can be cotransduced with tumor-targeting CARs to combine tumor T-cell specificity, T-cell activation, and abrogation of checkpoint inhibition in a single genetically modified T-cell.

Although preclinical data are encouraging, currently no clinical trials in practice are offering combinatorial therapy with CARs and checkpoint blockade. Studies combining genetically modified T cells specific for the NY-ESO-1 antigen expressed on some solid tumors in combination with ipilimumab are in the pipeline. The extent of possible antitumor effects and toxicities of such combinations has yet to be fully explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online October 5, 2015.

References

1. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med 1988;319:1676–80.
2. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 2008;26:5233–9.
3. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17:4550–7.
4. Yannelli JR, Hyatt C, McConnell S, Hines K, Jacknin L, Parker L, et al. Growth of tumor-infiltrating lymphocytes from human solid cancers: summary of a 5-year experience. Int J Cancer 1996;65:413–21.
5. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499:214–8.
6. Bollard CM, Rooney CM, Heslop HE. T-cell therapy in the treatment of post-transplant lymphoproliferative disease. Nat Rev Clin Oncol 2012; 9:510–9.
7. Louis CU, Straathof K, Bollard CM, Ennamuri S, Gerken C, Lopez TT, et al. Adoptive transfer of EBV-specific T cells results in sustained clinical responses in patients with locoregional nasopharyngeal carcinoma. J Immunother 2010;33:983–90.
8. Comoli P, Pedrazzoli P, Maccario R, Basso S, Carminati O, Labirio M, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. J Clin Oncol 2005; 23:8942–9.
9. Bollard CM, Aguilar L, Straathof KC, Gahn B, Huls MH, Rousseau A, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. J Exp Med 2004;200:1623–33.
10. Bollard CM, Gottschalk S, Torrano V, Diouf O, Ku S, Hazrat Y, et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. J Clin Oncol 2014;32: 798–808.
11. Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, et al. Genome-wide analysis of cancer/testis gene expression. Proc Natl Acad Sci U S A 2008;105:20422–7.
12. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126–9.
13. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009;114:535–46.
14. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther 2011;19:620–6.
15. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 2011;29:917–24.
16. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 2013;36:133–51.
17. Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 2013; 122:863–71.
18. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 2015;33:540–9.
19. Porter DL, Frey NV, Melenhorst JJ, et al. Randomized, phase II dose optimization study of chimeric antigen receptor modified T cells directed against CD19 (CTL019) in patients with relapsed, refractory CLL. Blood 2014;124:1982. [cited 2015 Feb 13]. Available from: http://www.bloodjournal.org/content/124/21/1982.abstract.
20. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507–17.
21. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 2011;118:6050–6.
22. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med 2008;14:1264–70.
23. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther 2010;18:843–51.
24. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 2006;12:6106–15.
25. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther 2013;21:904–12.
26. Di Stasi A, Tey S-K, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med 2011;365:1673–83.
27. Torikai H, Reik A, Soldner F, Warren EH, Yuen C, Zhou Y, et al. Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. Blood 2013;122:1341–9.
28. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 2012;12:269–81.
29. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol 2002;2:116–26.
30. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol 2014;11:24–37.
31. Gelao L, Criscitiello C, Esposito A, Goldhirsch A, Curigliano G. Immune checkpoint blockade in cancer treatment: a double-edged sword cross-targeting the host as an "Innocent Bystander." Toxins (Basel) 2014;6: 914–33.

32. Callahan MK, Wolchok JD. At the Bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol 2013;94:41–53.
33. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy—inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res 2012;18:6580–7.
34. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
35. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015;372:311–9.
36. Quezada SA, Peggs KS. Exploiting CTLA-4, PD-1 and PD-L1 to reactivate the host immune response against cancer. Br J Cancer 2013;108:1560–5.
37. John LB, Devaud C, Duong CPM, Yong CS, Beavis PA, Haynes NM, et al. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res 2013;19:5636–46.
38. Shin JH, Park HB, Oh YM, Lim DP, Lee JE, Seo HH, et al. Positive conversion of negative signaling of CTLA4 potentiates antitumor efficacy of adoptive T-cell therapy in murine tumor models. Blood 2012; 119:5678–87.
39. Ankri C, Shamalov K, Horovitz-Fried M, Mauer S, Cohen CJ. Human T cells engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity. J Immunol 2013;191: 4121–9.
40. Bolhuis RL, Lamers CH, Goey SH, Eggermont AM, Trimbos JB, Stoter G, et al. Adoptive immunotherapy of ovarian carcinoma with bs-MAb-targeted lymphocytes: a multicenter study. Int J Cancer 1992;Supplement 7:78–81.
41. Tchekmedyian S, Glaspy J, Korman A, Keler T, Deo Y, Davis T MDX-010 (human anti-CTLA4): a phase I trial in malignant melanoma. Proc Am Soc Clin Oncol (Abstract 56) 2002.