Review

Cancer Research

Trafficking of T Cells into Tumors

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Abstract

T cells are a crucial component of the immune response to infection and cancer. In addition to coordinating immunity in lymphoid tissue, T cells play a vital role at the disease site, which relies on their efficient and specific trafficking capabilities. The process of T-cell trafficking is highly dynamic, involving a series of distinct processes, which include rolling, adhesion, extravasation, and chemotaxis. Trafficking of T cells to the tumor microenvironment is critical for the success of cancer immunotherapies such as adoptive cellular transfer. Although this approach has achieved some remarkable responses in patients with advanced melanoma and hematologic malignancy, the success against solid cancers has been more moderate. One of the major challenges for adoptive immunotherapy is to be able to effectively target a higher frequency of T cells to the tumor microenvironment, overcoming hurdles associated with immunosuppression and aberrant vasculature. This review summarizes recent advances in our understanding of T-cell migration in solid cancer and immunotherapy based on the adoptive transfer of natural or genetically engineered tumor-specific T cells and discusses new strategies that may enhance the trafficking of these cells, leading to effective eradication of solid cancer and metastases. *Cancer Res; 74(24); 7168–74. ©2014 AACR.*

Introduction

Trafficking of $CD8^+$ T cells to the site of disease is a critical step for a successful immune response against pathogens and cancer. This is a highly regulated and dynamic process that has been well characterized in both inflammatory and infectious disease settings (1). In the cancer setting, the presence of tumor-infiltrating lymphocytes (TIL) has been reported to correlate well with positive clinical outcomes (2–5). $CD8^+$ T cells are recruited from the circulation to the site of infection by a series of distinct processes, involving attachment/adhesion, rolling/tethering, chemotaxis, and extravasation (1, 6). In general, T-cell trafficking to the tumor microenvironment is markedly reduced compared with an infectious disease setting due to both intrinsic and extrinsic factors (7). Despite this, immunotherapies using checkpoint inhibitors or adoptively transferred T cells have generated some remarkable responses against cancer particularly in patients with advanced metastatic melanoma (8–10). In the future, one of the major challenges for broadening immunotherapy for other malignancies will be to overcome the present barriers that restrict targeting of T cells to the tumor site. In the following review, we briefly discuss T-cell trafficking in normal and diseased conditions, and then focus on the migration of T cells following adoptive transfer of natural or genetically modified tumor-specific $CD8^+$ T cells. We discuss recent advances in our understanding of how adoptively transferred T cells infiltrate into primary tumors and metastases and describe how this information may help design better T-cell therapies for promoting tumor eradication.

Key Events in Lymphocyte Trafficking

T cells are a key component for generating an effective immune response. They have the capacity to move through tissues to scan for MHC-peptides that are specific for their T-cell receptors. The trafficking of T cells involves complex interactions between T cells and endothelial cells (EC). This process requires an initial transitory attachment to the endothelium, followed by rolling, firm adhesion, and T-cell activation on the endothelial surface, and finally extravasation through the blood vessel wall to the site of infection (1). Chemokine receptors, selectins, and integrins all play key roles in these steps.

When naïve T cells are primed and become effector T cells, they undergo a dramatic shift in the expression of surface proteins and inflammation-specific receptors. Effector T cells lose expression of CD62L and CCR7, thereby losing their ability to access lymph nodes through the high endothelial venules. Instead, activated T cells gain expression of a cohort of homing molecules that enable them to migrate to diseased tissues. This includes ligands for E- and P-selectin, which enable rolling of T cells on endothelium, and chemokine receptors such as CXCR3 that bind inflammatory chemokines CXCL9 and CXCL10

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secreted by infected tissues. The binding of chemokine receptors causes activation of LFA-1 and newly expressed integrins, such as very late antigen 4 (VLA-4), which bind to ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1), respectively, expressed on infected tissue, which enables adhesion. Further expression of chemokines by diseased tissue facilitates extravasation of T cells into the tissue to exert contact-dependent functions, resulting in resolution of the disease threat (1, 6).

T Cells Trafficking to Solid Cancers and Metastases

The tumor stroma consists of a variety of cell types that include EC, fibroblasts, pericytes, and immune subtypes such as lymphocytes, granulocytes, and macrophages. The profile of the TILs present within the tumor microenvironment reflects the diversity in tumor biology and host–tumor interactions. In various solid cancer settings, the frequency and type of TILs have been reported to correlate with outcomes in some patients (2–5), although this may vary according to tumor type (11). Nevertheless, improved antitumor responses have been shown to positively correlate with increased cytotoxic T lymphocyte (CTL) infiltration in various cancers, including colorectal (2), breast (3), cervical cancers (4), and glioblastoma (5).

Naturally primed CTLs have the capacity to identify and eradicate malignant cells through recognition of tumor-associated antigens presented by MHCI. However, only a small number of CTLs are generally able to infiltrate the tumor site (7), which contrasts the inflammatory or infectious disease setting. Notably, these tumor-specific $CD8^+$ cells can also traffic indiscriminately to multiple organs (12), which can cause potential pathology, and this is an important consideration for development of immunotherapy. The reasons for the poor homing of tumor-specific T cells to the tumor site are becoming clear. As discussed in the previous section, CTL trafficking is a tightly controlled process, and factors such as mismatching of chemokine–chemokine receptor pairs, downregulation of adhesion molecules, and aberrant vasculature may all contribute to the poor homing of these cells.

Chemokine/chemokine receptor mismatching

Mismatching of chemokine receptors on T cells and tumor-secreted chemokines has been shown to account for the suboptimal trafficking of T cells into the tumor microenvironment (Fig. 1A, 1). Several chemokines have been reported to regulate the migration of CTLs into the tumor site. CXCR3 is one of the major chemokine receptors expressed by activated TILs in melanoma (13), colorectal (14), and breast (15) cancers, highlighting its importance for CTL trafficking to the tumor microenvironment. Previously it has been reported that efficient trafficking of CTL to metastatic sites in patients with melanoma correlated well with the expression of chemokines CXCL9 and CXCL10 (ligands for CXCR3), where CXCR3 was found to be unregulated on the effector T cells (13). Consistently, in murine models of solid cancer, increased expression of CXCL9 and CXCL10 by tumor cells resulted in increased infiltration of $CXCR3^+$ CTL that was accompanied with an enhanced antitumor response (16–18). However, not all tumors express sufficient levels of the ligands for CXCR3 (13, 15), which may lead to inefficient recruitment of effector and memory $CD8^+$ T cells. Interestingly, the three ligands for CXCR3 (CXCL9, CXCL10, and CXCL11) are all IFNγ-inducible ligands (19) and IFNγ is known to be one of the key effector molecules for the antitumor function of CTL (8). Thus, IFNγ production within the tumor microenvironment by CTLs may enhance the CXCR3-mediated T-cell recruitment to the tumor site (Fig. 1B). Another important chemokine receptor is CXCR6 (receptor for CXCL16). This chemokine receptor is expressed at very low levels on naïve T cells and is upregulated upon activation. It has been shown that mice lacking CXCR6 demonstrated reduced infiltration of T cells in mammary tumors and impaired tumor regression (20). Thus, expression of certain chemokines in tumor is a good correlate with the presence of TILs within the tumor microenvironment and these may serve as useful prognostic markers.

Aberrant vasculature and endothelial anergy

One important vascular cell type is pericytes, a population of contractile cells supporting EC of capillaries and venules and is important in vessel maintenance and remodeling by direct physical contact and paracrine signaling. In tumors, pericytes can be absent or loosely attached and this causes the tumor vessels to become leakier than normal (Fig. 1A, 2; ref. 7). The leakiness of the vessel can promote irregular blood flow causing inefficient trafficking of T cells within the tumor bed (7). An elegant study by Hamzah and colleagues reported the role of the regulator of G-protein signaling 5 (RGS5) as a master gene in pericyte maturation accounting for abnormal vascular morphology. In RGS5-deficient mice, tumor-resident pericytes demonstrated a normal mature phenotype and the tumors vessels were normalized resembling blood vessels in normal tissue. This resulted in a reduction in tumor hypoxia and vessel leakiness, and enhanced influx of effector cells, which augmented survival of mice (21). This finding has opened up new therapeutic possibilities by targeting the RGS5 pathway for reversing angiogenesis. Furthermore, tumor endothelium has been reported to work as a barrier that prevents CTL infiltration (22). It was previously demonstrated in a mouse model of ovarian cancer, that the endothelin B receptor (ET_BR) was expressed at higher levels by EC from tumors lacking TILs than those with TILs present. The ligand for ET_BR, endothelin-1 can be produced at high levels by ovarian cancer cells. In this study, the interaction of endothelin-1 and ET_BR was found to suppress T-cell adhesion to the tumor vasculature by inhibiting the expression and clustering of the trafficking molecule ICAM-1 on EC, thus inhibiting T-cell adhesion (Fig. 1A, 3). Specific ET_BR blockade increased T-cell infiltration to tumors (22), highlighting the potential of manipulating tumor endothelial barrier to enhance the efficacy of T-cell immunotherapies.

Surprisingly, overproduction of angiogenic factors such as VEGF can also cause tumor EC to downregulate the expression of adhesion molecules such as ICAM-1 and 2, VCAM-1 and CD34 (Fig. 1A, 3). This phenomenon is called "EC anergy" and results in the inhibition of effector T-cell adherence to the EC and extravasation to the tumor site (7). Several strategies for

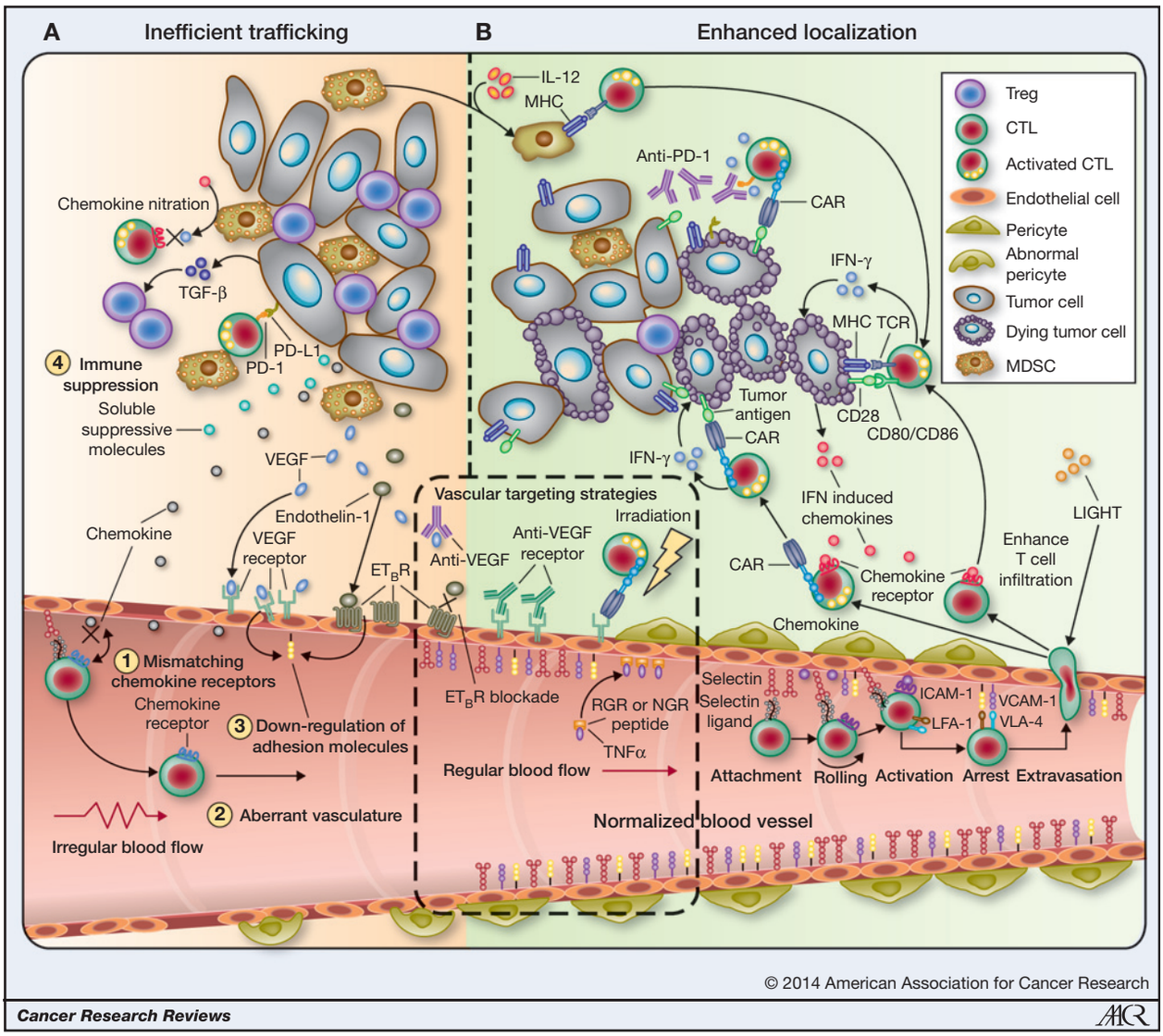

Figure 1. Factors controlling infiltration of T cells into solid tumors. A, inefficient trafficking of CTL into the tumor microenvironment may be due to several factors, including mismatching of chemokine receptors on T cells and tumor-secreted chemokines (1); aberrant vasculature (2); downregulation of adhesion molecules on endothelial cells (3); and tumor immune suppression mechanisms that are mediated by a cohort of suppressor cells, soluble molecules, and checkpoint pathways (4). B, enhanced localization of CTL to the tumor site. A number of strategies have been shown to enhance T-cell infiltration to solid tumors. These include the use of checkpoint inhibitors such as anti–PD-1, genetically engineering CTL or CAR T cells to express certain chemokine receptors, administration of IL12 to enhance antigen-presentation, and delivery of LIGHT. Several strategies for tumor vasculature targeting (boxed area) have also demonstrated great promise for improving T-cell infiltration into the tumor site.

normalizing tumor vasculature and inducing reexpression of adhesion molecules have been investigated (Fig. 1, box area). These include using antibodies targeting VEGF and its receptors that have been shown to transiently overcome EC anergy and enhance T-cell trafficking to the tumor site (7, 23, 24). In addition, other strategies for normalizing tumor vasculature include irradiation (25) or direct targeting of cytokines such as TNF to the blood vessel using tumor vasculature homing peptides NGR and RGR (7, 26). These approaches have demonstrated enhanced T-cell infiltration into the tumor site, and some are currently under clinical investigation. In summary, tumors are able to create cellular and molecular barriers that restrict efficient entry of T cells into intratumoral regions, and fully determining the mechanisms underlying these processes may lead to more effective therapies.

Tumor immunosuppression

Although some CTLs can be found within the tumor microenvironment, malignant cells still often persist and metastasize. The immunosuppressive tumor microenvironment can effectively suppress CTL function thereby causing tumor escape (Fig. 1A, ④). It has been reported in patients and preclinical mouse models that a number of inhibitory checkpoint pathways, such as programmed cell death protein 1

(PD-1), cytotoxic T lymphocyte antigen 4 (CTLA-4), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3), lymphocyte activation gene 3 protein (LAG3; ref. 8), and CD73 (27), can shut down antitumor CTL responses. The use of checkpoint inhibitors targeting these pathways such as anti–PD-1 and anti–CTLA-4 has been demonstrated to enhance T-cell responses in patients, resulting in promising clinical activity against some cancers (9, 10). In addition, there is an enrichment of suppressive immune cells within the tumor microenvironment, such as regulatory T cells, myeloid-derived suppressor cells (MDSC), and suppressive molecules such as TGFβ, nitric oxide, and indoleamine-2,3-dioxygenase (28–30). This suppressive landscape can prevent efficient T-cell response and infiltration of CTLs into the tumor site. Interestingly, MDSCs have been demonstrated to be capable of altering T-cell trafficking by downregulation of L-selectin on T cells (31) or producing reactive nitrogen species that induce CCL2 nitration and reduce the chemoattractant effect of this chemokine on $CD8^+$ T cells (32). Strategies to reprogram MDSCs, such as the local delivery of IL12, to decrease immunosuppression, and enhance antigen presentation, have been confirmed to greatly enhance T-cell infiltration and local proliferation within the tumor (33).

Anatomical location

The anatomical location of immune effector cells in the tumor is an important determinant for whether a tumor may or may not regress. The center of the tumor is a highly hostile environment marked by the presence of suppressive cytokines, molecules, chemicals, and other suppressor cells. Galon and colleagues investigated the location of immune cells within tumors and determined whether this correlated with the clinical outcomes of patients with colorectal carcinoma. They found that patients with a high density of effector and memory T cells in both the center and the invasive margins of the tumor had the best-predicted survival outcome (2). Other studies have reported that infiltration of CTLs into the tumor stroma is important for antitumor efficacy (34).

A number of mechanisms seem to play a role in T-cell–mediated tumor regression. Indeed, elegant studies using microscopy have demonstrated that, besides CTL-mediated direct killing of tumor cells (35, 36), CTL can also be involved in destruction of stromal elements, including endothelial cells, leading to tumor necrosis (36). Interestingly, this can result in effects against antigen-negative cancer cell variants within tumors. Furthermore, stromal elements can play a role in antigen presentation to enhance T-cell activity against tumors (36, 37).

In some cases, the tumor microenvironment can facilitate the formation of tertiary lymphoid structures (TLS), which have been identified in lung, colorectal, and breast cancers, and their presence has been linked with a positive prognosis for patients with cancer (38). The relative location of CTLs to TLS may be important for CTLs receiving help from T helper cells for generation of an effective and durable antitumor immune response. Interestingly, it has been reported that LIGHT, a TNF superfamily member and a ligand for the lymphotoxin β receptor, facilitates the generation of TLS (39, 40) and recruits naïve T cells into the tumor (41). Expression of LIGHT in tumors has been shown to enhance chemokine secretion within the tumor, favoring the infiltration and expansion of functional $CD8^+$ T cells (42). However, it remains unclear what role TLS plays in the LIGHT-mediated T-cell recruitment to tumor.

Adoptive T-cell Transfer and Trafficking

Adoptive cellular transfer (ACT) involving a transfusion of tumor-specific T cells has emerged as a powerful treatment for cancer particularly in patients with advanced metastatic melanoma (8). The *ex vivo* expanded TILs are infused back into a patient to infiltrate the tumor and mediate its destruction. ACT has yielded some dramatic results with >50% objective responses reported in patients with melanoma (43). The efficiency of adoptively transferred T cells infiltrating the tumor site and the persistence of these cells have been found to correlate well with clinical responses and outcomes in patients (44). In mouse studies, it has been confirmed that the concentration of adoptively transferred $CD8^+$ T cells within the tumor microenvironment was an important parameter, for whether these cells could effectively kill established cognate antigen-expressing tumors *in vivo* (45). However, of the large number of *ex vivo* expanded T cells, only a small fraction of these transferred T cells eventually reach the tumor tissue in both humans (44, 46) and mice (18, 47).

The past decade has seen the emergence of a novel form of adoptive cellular immunotherapy in patients involving the genetic modification of CTL-expressing chimeric antigen receptors (CAR; ref. 48). CAR T-cell therapy is a personalized treatment involving genetic modification of a patient's autologous CTLs, enabling specific recognition and targeting of tumor-associated antigen expressed by the tumor. The CAR consists of an extracellular tumor-specific antibody-derived domain fused with T-cell signaling domains that redirect the effector function of CTLs against tumor cells. CAR T cells have several advantages over naturally derived T cells. This includes high-affinity interaction with the tumor antigen and recognition of tumors in an MHC-independent manner (48). The first CARs to be developed, termed "first generation," contained only one signaling domain comprising either CD3ξ or FcRγ chains. However, these first-generation CARs failed to induce optimal cytokine production and T-cell expansion *in vivo*. Subsequently, second-generation CARs were generated that incorporated additional costimulatory domains that included either CD28, 4-1BB, CD27, OX40, or ICOS that conferred stronger signaling and persistence to the T cells. More recently, third-generation CARs have been developed that contain three stimulatory domains (48). However, these types of CARs require further validation in syngeneic mouse models. CAR T–cell therapy has recently shown some striking results in patients with hematologic cancers with some durable responses (48). However, results in the solid tumor setting have been less convincing to date where new strategies are required to enhance the trafficking of these gene-modified T cells to the tumor microenvironment.

Genetic modification technology allows the modification of T cells for potentially improving their capability to more effectively home to tumor sites. Kershaw and colleagues demonstrated for the first time that engineering the chemokine receptor CXCR2 (receptor for CXCL1 secreted by tumor cells) into T cells enabled T cells to effectively migrate toward tumor cells *in vitro* (49). Following this, effort has been directed for enhancing the trafficking of T cells to tumor by the modification of other chemokine receptors into T cells. Di Stasi and colleagues demonstrated that expression of CCR4 on CAR-CD30 T cells improved the migration of these cells toward $CD30^+$ Hodgkin lymphoma that secreted CCL17, the ligand for CCR4. Furthermore, adoptive transfer of CCR4-CAR-CD30 T cells resulted in stronger antilymphoma activity in a xenograft mouse model due to enhanced trafficking of CAR T cells to the tumor site (50). Tumor types can vary in their secretion of chemokines, and successful redirection of T-cell migration depends on matching the chemokine with its appropriate chemokine receptor. For example, a number of tumors, including glioma, can secrete CCL2. It was shown that glioma-derived production of CCL2 (ligand for CCR2) could attract adoptively transferred human $CD8^+$ T cells genetically modified to express CCR2, the receptor for CCL2 (51). Moon and colleagues demonstrated that enhanced CCR2b expression on mesothelin-reactive CAR T cells led to a more than 12.5-fold increase in CAR T-cell homing to $mesothelin^+$ malignant pleural mesothelioma in mice, resulting in enhanced antitumor effects (52). A separate study reported that expression of CCR2b on CAR-GD2 T cells produced a greater than 10-fold increase in migration of CAR T cells toward CCL2 produced by neuroblastoma cells (53). Another candidate chemokine receptor that may be used to enhance ACT efficacy is CXCR3. An interesting study reported that adoptive transfer of T cells expressing a NKG2D-based CAR could recruit and activate endogenous antigen-specific $CD4^+$ and $CD8^+$ T cells at the tumor site in a CXCR3-dependent manner to achieve optimal eradication of ID8 ovarian cancer (54). Potential treatments such as PD-1 blockade (55) and chemotherapy (18) have been shown to enhance recruitment of adoptively transferred T cells to the tumor site by elevating the level of CXCR3 ligands. Thus, careful selection or generation of T cells with a chemokine receptor profile appropriate for chemokines secreted by individual tumor types could facilitate T-cell infiltration into tumors and enhance the efficacy of CAR T cell antitumor effects.

To overcome the need for penetration into tumor, constructions of CARs targeting EC components, such as $\alpha_v\beta3$ integrin (56) and VEGF receptor-2 overexpressed on tumor vasculature (57) have also been reported. In this way, CAR-mediated targeting of EC may be sufficient to destroy tumor vasculature and thereby mediate tumor regression with a reduced need for T cells to penetrate tumors. However, although these approaches have been shown to be effective in mouse models, the engineering of chemokine receptor transgenes and EC-targeting CARs into T cells for ACT has yet to be tested in humans.

The limited persistence and trafficking of adoptively transferred T cells *in vivo* highlight the need to find new methods of optimizing T-cell homing. It will also be important to identify chemokines that specifically attract T cells but not suppressor cells such as MDSCs. Nevertheless, these studies suggest that combination treatments may enhance the efficacy and homing capabilities of adoptively transferred T cells in patients in the future.

Conclusions

Efficient trafficking of T cells to the tumor site is a critical step for success of cancer immunotherapy. As localization of T cells to the tumor microenvironment is a major barrier for a successful antitumor immune response, a number of new strategies for increasing trafficking of T cells that have been tested in mouse models will need to be validated in the patient setting in the future. ACT utilizing *ex vivo* expanded TILs or gene-modified T cells represents a promising immunotherapeutic approach that has achieved some remarkable antitumor responses in some patients with cancer. However, the broad application of this approach will require additional interventions to enhance the trafficking and persistence of T cells to the tumor site. The genetic modification of T cells with chemokine receptors has shown promise for enhancing trafficking of adoptively transferred T cells in mouse models. Combination therapies using checkpoint inhibitors to overcome the immunosuppressive environment or antiangiogenic agents such as anti-VEGF antibody (24) or peptide-cytokine conjugates to normalize blood vessels have been shown to enhance the trafficking of adoptively transferred T cells into the tumor site, resulting in enhanced antitumor efficacy. These new combination approaches require testing in phase I clinical trials. Further insight and understanding of the mechanisms that restrict the trafficking of T cells to the tumor microenvironment may help in the development of future strategies to further increase localization and persistence of T cells and reveal the full potential of adoptive immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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