INVITED REVIEW

Antigen-specific T cell therapies for cancer

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Abstract

Adoptively transferred antigen-specific T cells that recognize tumor antigens through their native receptors have many potential benefits as treatment for virus-associated diseases and malignancies, due to their ability to selectively recognize tumor antigens, expand and persist to provide long-term protection. Infusions of T cells targeting Epstein–Barr virus (EBV) antigens have shown encouraging response rates in patients with post-transplant lymphoproliferative disease as well as EBV-positive lymphomas and nasopharyngeal cancer, although a recent study also showed that human papilloma virus-reactive T cells can induce complete regression of metastatic cervical cancer. This strategy is also being evaluated to target non-viral tumor-associated antigens. Targeting these less immunogenic antigens is more challenging, as tumor antigens are generally weak, and high avidity T cells specific for self-antigens are deleted in the thymus, but tumor responses have been reported. Current research focuses on defining factors that promote in vivo persistence of transferred cells and ameliorate the immunosuppressive microenvironment. To this end, investigators are evaluating the effects of combining adoptive transfer of antigen-specific T cells with other immunotherapy moieties such as checkpoint inhibitors. Genetic modification of infused T cells may also be used to overcome tumor evasion mechanisms, and vaccines may be used to promote in vivo proliferation.

Introduction

Over the last few years, there has been increasing interest in cellular immunotherapy as a strategy to harness the immune system to fight tumors. One approach is to use T cells genetically modified with chimeric antigen receptors (CARs) that comprise immunoglobulin variable regions recognizing tumor antigens fused to the cytotoxic signaling domains from the T cell receptor (TCR ζ chain) and to costimulatory endodomains. CARs have produced outstanding clinical results in B cell leukemias and are moving toward definitive licensing studies (1–3). The CAR strategy targets tumors without a requirement for major histocompatibility complex (MHC) matching; however, targeting a single epitope on a single antigen may lead to immune escape, and identifying suitable tumor-specific target antigens has been challenging. T cells targeting antigens through their native receptors have also been used extensively and successfully, particularly when directed to viral antigens in the hematopoietic stem cell transplant (HSCT) setting. Virus-specific T cells (VSTs) generated from the transplant donors have been shown to prevent and treat viral infections and Epstein–Barr virus (EBV)-associated lymphoproliferative disease (PTLD) (4–6). Autologous VSTs that recognize EBV have also shown activity in patients with less immunogenic EBV-associated malignancies occurring outside the HSCT setting, including EBV-associated Hodgkin lymphoma, NK-T lymphoma and nasopharyngeal carcinoma (7–10). Recent studies have also validated Human papilloma virus (HPV) antigens as targets in HPV-associated malignancies (11). For tumors not associated with viruses, several classes of tumor-associated antigens (TAAs) may be targeted. These include antigens overexpressed on tumors relative to normal tissues, antigens expressed only during fetal development or in immune-privileged sites such as testis and neoantigens generated by gene rearrangements or mutations. In this review, we will focus on T cell immunotherapy approaches that target antigen through the native TCR and discuss how to augment these cells by genetic transfer to render them resistant to tumor evasion mechanisms. (Fig. 1)
discuss the potential benefits of combining T cell therapy with checkpoint inhibition, small molecules and oncolytic viruses (OVs) (12,13).

**Virus-specific T cells**

**Epstein–Barr virus**

EBV is associated with a diverse array of malignancies, all associated with the viral latent cycle in which up to nine latency-associated antigens are expressed. There are three broad patterns of latent gene expression, each associated with specific tumors: type 3 latency, in which all nine latency proteins including six nuclear antigens (EBNAs), two membrane proteins (LMPs) and the secreted BARF1 gene product are expressed, is seen in the highly immunogenic lymphomas that develop in immunocompromised patients such as recipients of HSCT or solid organ transplantation. Tumors expressing EBV type 2 latency, such as nasopharyngeal cancer and lymphomas arising in immunocompetent individuals, express a more limited array of antigens including LMP1, LMP2, EBNA1 and BARF1. Finally, type 1 latency in which only EBNA1 is expressed is seen in Burkitt’s lymphoma and gastric carcinoma. However, variations on these latency types have been described (14,15).

The presence of EBV antigens in these tumors prompted exploration of EBV-specific T cells as treatment. In the setting of allogeneic HSCT, T cells generated from the healthy stem cell donor have been successful as treatment of viral reactivation or disease (4). More than 70% of the patients infused with donor-derived EBV-specific T cells for the treatment of EBV-associated PTLD showed complete responses (CRs) resulting in durable remissions with minimal toxicity. In early studies, VSTs were generated using EBV-transformed B-lymphoblastoid cell lines (EBV-LCLs) as antigen-presenting cells (5,6). Although these VST preparations produced impressive response rates, the manufacturing time was at least 12 weeks, including 6 weeks for EBV-LCL production, limiting broader application. More recent studies have tested rapid manufacturing techniques that selected VSTs from donor peripheral blood or apheresis products (18). VSTs could be directly selected using peptide-human leukocyte antigen (HLA) complexes (HLA multimers) or by capture of T cells that secrete interferon gamma (IFN-γ) after stimulation with viral antigens (16,17,19). These strategies produce small numbers of highly specific T cells that can expand exponentially after infusion in patients with viral reactivation producing clinical responses and reduction of viral titer. However, a drawback of these approaches is the large volume of blood required, especially when the donor has a low frequency of T cells reactive to the viral antigens.

We have also clinically evaluated T cells produced after a short ex vivo stimulation with overlapping pools of 15-mer peptide libraries that incorporate viral antigens in the presence of potent prosurvival cytokines. Although these VST preparations produced impressive response rates, the manufacturing time was at least 12 weeks, including 6 weeks for EBV-LCL production, limiting broader application. More recent studies have tested rapid manufacturing techniques that selected VSTs from donor peripheral blood or apheresis products (18). VSTs could be directly selected using peptide-human leukocyte antigen (HLA) complexes (HLA multimers) or by capture of T cells that secrete interferon gamma (IFN-γ) after stimulation with viral antigens (16,17,19). These strategies produce small numbers of highly specific T cells that can expand exponentially after infusion in patients with viral reactivation producing clinical responses and reduction of viral titer. However, a drawback of these approaches is the large volume of blood required, especially when the donor has a low frequency of T cells reactive to the viral antigens.

We have also clinically evaluated T cells produced after a short ex vivo stimulation with overlapping pools of 15-mer peptides overlapping by 11 amino acids (pepmixes) spanning the entire protein sequences of immunogenic viral antigens. One initial concern was whether the shorter culture period would carry a higher risk of alloreactivity, but this has not been observed and response rates have been similar to those observed with LCL-induced EBV-specific T cells (17,20,21). Papadopoulou et al. (22) evaluated pepmix-activated VSTs that recognized 12 immunogenic antigens from five viruses (EBV, adenovirus, CMV, BKV and HPV6) that frequently cause disease in immunocompromised patients. We observed activity against all five viruses, including a complete remission in one case of EBV lymphoma.

The success of VST therapy in treating the immunogenic EBV type 3 latency tumors that develop after transplant led to interest in exploring the potential benefits of combining T cell therapy with checkpoint inhibition, small molecules and oncolytic viruses (OVs) (12,13).
in treating the type II latency expressing EBV-associated malignancies of the immunocompetent host. These tumors present a more challenging target, as T cells specific for type 2 latency antigens are usually low in frequency and functionally anergic in patients (23). To overcome these obstacles, we activated and expanded LMP-specific T cells using mature dendritic cells (DCs) transduced with an adenoviral vector (Ad5/35) expressing LMP antigens for the first stimulation and EBV-transformed LCLs transduced with the same vector for the second and subsequent stimulations (7,24). Twenty-eight of 29 patients with high-risk or multiply relapsed disease who received LMP-specific T cells as adjuvant therapy remained in remission at a median of 3.1 years after infusion. Twenty-one patients with active disease received LMP-VSTs and 13 had clinical responses, including 11 CRs. Of note, T cells specific for LMP antigens could be detected in the blood after VST infusion. In some responding patients, T cells reactive with non-viral TAAs (epitope spreading) also became detectable (7). Similar results were recently reported in a study from Korea in which LMP1- and LMP2-specific T cells re-activated by stimulation with LMP1/2a RNA-transfected DCs were infused in 10 patients with NK-T lymphoma after induction therapy. They reported 4-year overall survival and progression-free survival of 100 and 90%, respectively (25).

We also initiated trials in nasopharyngeal cancer in which >95% of the tumors express EBV type 2 latency. Among patients with active disease, 48% had a CR/CRu (33%) or PR (15%) with a 2-year disease-free survival of >60 versus the expected 5–20% in published studies (10,26). This approach has proved exportable in a collaborative study in Singapore showing the same 60% 2-year survival in the CTL treatment group, compared with patients receiving chemotherapy alone (9). Notably, in both studies, responses and survival were significantly greater in subjects whose T cell infusions contained subsets of T cells with specificity for LMP2.

Human papilloma viruses

HPV is associated with genital and oropharyngeal carcinomas. The strains carrying the highest risk for malignancy are HPVs 16, 18, 31 and 45, and integrated fragments encoding the E6 and E7 oncoproteins found in tumor cells provide targets for T cells. Although vaccines targeting the major capsid antigen of HPV (L1) prevent primary infection with HPV, they have no effect in preventing established disease, even in early stages when L1 is still expressed. The first clinical trial of an adoptive immunotherapy approach targeting HPV was recently reported by Stevanovic et al. (11), who expanded tumor-infiltrating lymphocytes (TILs) from tumor biopsies obtained from patients with cervical cancer. The TILs were infused after lymphodepleting chemotherapy to nine patients with metastatic cervical cancer. Three recipients had objective tumor responses, including two CRs that had persisted for more than 15 months (11). In responders, reactivity against the HPV E6 and E7 peptides was detected in the infused TILs, and HPV-specific T cells were detected in the peripheral blood for several months. The TIL manufacturing process is complex and requires a tumor biopsy. Ramos et al. (27) have shown that it is also possible to expand polyclonal E6-/E7-specific T cells from the peripheral blood of patients with cervical and oropharyngeal cancer by stimulating T cells with E6 and E7 peptide-pulsed monocyte-derived DCs. The drawback of this strategy is that the infused T cells will have specificity only for the known viral antigens, whereas TILs likely also contain T cells specific for other tumor antigens including tumor-specific mutations that are abundant in cervical carcinoma (11).

Third-party VSTs

Even the most rapid strategies to generate T cells require time to coordinate blood collection, especially from unrelated donors. In some cases, VSTs fail to grow from the blood of patients who have received aggressive lymphotoxic chemotherapy or have immunosuppressive tumors. Nor is it easy to grow VSTs from sero-negative HSCT donors (28). An alternative strategy for these patients is to use highly characterized, banked VSTs, which were initially selected on the basis of best HLA antigen match. However, as the specificity of most EBV-specific T cell lines is restricted by only a few HLA alleles, it is better to match on the basis of the known HLA restriction of each T cell line, as performed in subsequent studies (29,30). The first clinical study used banked EBV-specific T cells to treat post-transplant lymphomas that were persistent, despite standard therapy after solid organ transplant or HSCT. The overall response rate was 52% at 6 months (31). The Memorial Sloan Kettering group also used LCL-induced EBV-specific T cells to treat 49 patients with rituximab-resistant PTLD and observed overall response rates (CR + PR) of 65% in recipients of HSCT and 62% in recipients of solid organ transplant (29). We used third-party T cells specific for EBV, cytomegalovirus and adenovirus to treat patients with refractory viral infections in a multicenter study. Nine of the 50 patients treated had EBV lymphoma. For these patients, the overall cumulative incidence of CR or PR by day 42 post-infusion was 66.7%. The response rates in these studies, therefore, range from 52 to 67%, which is promising but slightly less than that observed with donor-derived VSTs. The major concern with this strategy is that infused allogeneic T cells would be rejected by recipient alloreactive T cells. Indeed, increases in the frequency of VSTs in patient blood were transient, compared with the prolonged expansions observed after infusion of VSTs from the stem cell donor (30,31). Nevertheless, the clinical and virologic responses observed suggest that this is a clinically relevant strategy.

Remaining questions about banked T cells include whether there will be equivalent activity outside the transplant setting in cancer patients who are relatively immunocompetent and more likely to reject allogeneic third-party cells and the number of VSTs that will be required to provide coverage for patients with diverse genetic backgrounds. It is encouraging that a recent preliminary report from the Scottish bank found that as few as 25 lines allowed products to be identified for the majority of searches (32). Twelve lines had been infused at the time of reporting, and eight of 10 patients with lymphoproliferative disease attained complete remission. However, two patients with EBV-associated non-hematopoietic sarcoma failed to respond (32).

T cell therapy for non-viral TAAs

As antigen-specific T cells have shown significant activity against virus-associated tumors, the strategy is also being explored for the treatment of non-viral cancers. A major challenge in this setting is the identification of suitable TAAs. The ideal target would be selectively expressed on tumor cells and crucial for tumorigenicity. Many potential candidates are also expressed on normal tissues, resulting in immune tolerance through central and peripheral mechanisms. TAAs that are being evaluated in the clinic can be classified into the following groups.

(i) Tumor-specific mutations. Transcriptome analysis has demonstrated that many tumors express multiple neoantigens created by mutagenesis. Such antigens are targets of melanoma-derived TILs that can produce clinical responses
Cancer/testis antigens including MAGE, BAGE, GAGE, antigen presentation machinery, lack of costimulatory ligands, and the generation of specific recombinant TCRs.

Tumor-specific antigens that are overexpressed in tumors but are not expressed or are expressed at very low levels in normal cells. Such antigens include hTERT and survivin (35,36). In a study in which a multipeptide vaccine derived from these antigens was administered with costimulated T cells after autologous stem cell transplantation for myeloma, faster cellular and humoral immune reconstitution was observed (37). There are also several trials in the clinic administering T cells that target survivin (38).

Lineage-specific antigens expressed on tumor cells as well as on their normal counterpart such as the melanoma-associated antigens MART and gp100. T cells specific for these antigens have predictable off-target effects on normal cells, but this may be an acceptable toxicity for patients with relapsed malignancy. For example, infusion MART-specific T cells can produce clinical activity (39), but can also induce viliogto due to destruction of normal melanocytes. WT1 is another popular target that is selectively expressed on myeloid leukemia. Donor-derived WT1-specific cytotoxic T cell clones have produced anti-leukemic activity in two patients with relapsed AML after HSCT. IFN donors were well tolerated without evidence of damage to normal tissues such as kidney and muscle that express WT1 (40).

Cancer/testis antigens including MAGE, BAGE, GAGE, NY-ESO-1 and SSX are expressed on immune-privileged germline tissues, but are also upregulated in a variety of malignancies. T cells specific for these antigens can be activated and expanded from patients with lymphoma and other malignancies for subsequent adoptive transfer (38) and clinical efficacy has been reported when NY-ESO is targeted (41). These antigens have also been targeted with T cells genetically modified to express affinity-enhanced TCRs. In a recent trial of T cells genetically engineered with an NY-ESO1-reactive TCR, 11 of the 18 patients with NY-ESO-positive synovial sarcomas and 11 of the 20 patients with NY-ESO-positive melanomas demonstrated objective clinical response (42). Although no toxicities were attributed to the NY-ESO construct, lethal off-target effects produced by two different enhanced MAGE A3 TCRs (43,44) highlight the need to better define the specificity of engineered TCRs for a safe clinical application, but as the novel epitopes recognized may bear little resemblance to the targeted epitope, this may be very challenging. One strategy to reduce the risk of such unexpected target recognition is to mutate only TCR sequences in the CDR1 or CDR2 regions that enhance MHC but not peptide binding (45).

Overcoming tumor evasion mechanisms

Another challenge to any T cell therapy is that tumors have evolved numerous mechanisms to evade both innate and adaptive immunity. These mechanisms include downregulation of antigen presentation machinery, lack of costimulatory ligands, upregulation of co-inhibitory receptors and production of inhibitory factors such as transforming growth factor (TGF)-β, interleukin (IL)-10 and the tryptophan-depleting enzyme, indoleamine 2,3-dioxygenase. Further, many tumors recruit a variety of immunosuppressive cell types such as T regulatory cells and myeloid-derived suppressor cells.

Several strategies have been employed to generate a more favorable microenvironment for T cell expansion and function. Many adoptive immunotherapy regimens use cytotoxic lymphodepletion both to eliminate immunosuppressive cells and their associated inhibitory cytokines and ligands and to decrease competition for homeostatic cytokines such as IL-7 and IL-15. T cells can also be genetically modified to provide resistance to immunosuppressive molecules such as IL-4, TGF-β and PD-L1 produced both by tumors and their immunosuppressive infiltrates. For example, the T cell inhibitory effects of secreted TGF-β can be overcome by a dominant-negative TGF-β receptor type II (TGF-β-DNR) modified to delete its cytoplasmic signaling domains (46). Not only does the TGF-β-DNR abrogate TGF-β signaling, but also acts as a cytokine sink, enhancing the expansion and function of bystander T cells. We have tested this strategy in a clinical trial in patients with relapsed/resistant EBV-associated lymphoma who received LMP1/LMP2-specific T cells genetically modified with the TGF-β-DNR. We observed clinical benefit, including CRs in one patient who had only a partial response to unmodified LMP1/LMP2-specific T cells. (Bollard et al., unpublished data) To overcome the suppressive effects of IL-4, Leen et al. (47) expressed a novel chimeric cytokine receptor (4/7R) that comprises the extracellular domain of the IL-4 receptor fused with the endodomain of the IL-7 receptor in T cells. These T cells proliferated and retained their cytolytic function in the presence of tumor-derived IL-4, which is usually used to support tumor growth and to suppress effector T cell.

Combination therapies

There is considerable interest and activity in combining immunotherapy approaches with other treatment modalities to increase antitumor activity. Major advances have recently been made in the clinical translation of immunological checkpoint blockade using antibodies that target cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 pathway (PD-1/PD-L1). These antibodies block inhibitory receptors or their ligands on T cells and immune inhibitory cells, respectively, releasing tumor-specific T cells from immune paralysis. Studies using these checkpoint blockades showed encouraging clinical activity in a variety of malignancies, particularly those with T cell infiltrates (48-50). Ipilimumab (CTLA-4), nivolumab (PD-1) and pembrolizumab (PD-1) are now approved by the FDA for the treatment of advanced melanoma, and additional regulatory approvals are expected for other indications, raising the possibility of combining adoptive T cell transfer and checkpoint inhibition to enhance the activity of both.

OVs provide another promising approach to modify the cytokine and antigen milieu of the tumor microenvironment. OVs, which are potent immunogens that activate both innate and adaptive immunity, can be modified to express both TAA and immune stimulatory cytokines to modify the tumor microenvironment and to favor antigen presentation. OVs could be combined with tumor-specific T cells to encourage tumor infiltration and to promote T cell expansion and function (51). Finally, epigenetic drugs can increase the expression of tumor antigens, inhibit immunosuppressive elements of the tumor microenvironment and overcome T cell anergy by removing
methylation from the IL-2 promoter. HDAC inhibitors are generally very toxic to T cells, but used in sequence it may be possible to combine them to improve the antitumor efficacy of T cells (52).

Conflict of Interest statement. C.M.R. and H.E.H. have a licensing agreement with Cell Medica for EBV VSTs in lymphoma and nasopharyngeal cancer. The authors’ Center has a collaborative research agreement with Celgene for the clinical use of genetically modified T cells.

Funding

This work was supported in part by NIH grants P50CA126752 and P01 CA94237 and a Specialized Center of Research Award from the Leukemia Lymphoma Society. We also appreciate the support of shared resources by the Dan L Duncan Cancer Center support grant P30CA125123.

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