New Strategies in Engineering T-cell Receptor Gene-Modified T cells to More Effectively Target Malignancies

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Abstract

The immune system, T cells in particular, have the ability to target and destroy malignant cells. However, antitumor immune responses induced from the endogenous T-cell repertoire are often insufficient for the eradication of established tumors, as illustrated by the failure of cancer vaccination strategies or checkpoint blockade for most tumors. Genetic modification of T cells to express a defined T-cell receptor (TCR) can provide the means to rapidly generate large numbers of tumor-reactive T cells capable of targeting tumor cells in vivo. However, cell-intrinsic factors as well as immunosuppressive factors in the tumor microenvironment can limit the function of such gene-modified T cells. New strategies currently being developed are refining and enhancing this approach, resulting in cellular therapies that more effectively target tumors and that are less susceptible to tumor immune evasion. Clin Cancer Res; 21(23); 5191–7. ©2015 AACR.

Background

Adoptive transfer of tumor antigen-specific T cells isolated from patients that have been activated and expanded in vitro can in some cases lead to robust antitumor T-cell responses (1–3). However, many patients still fail to respond to such therapies, or only respond transiently. The ineffective immune recognition and/or eradication of tumor cells by adoptively transferred T cells can be attributed to several factors: (i) suboptimal affinity of tumor-specific T cells as a result of thymic deletion (negative selection) and/or peripheral tolerance of high-affinity tumor/self-specific T cells (4); (ii) the inability of ex vivo expanded T cells to persist following transfer in vivo (5, 6); (iii) heterogeneous expression of the antigen(s) targeted leading to outgrowth of antigen-negative variants (7); and (iv) various factors expressed by the tumor or present in the tumor microenvironment that induce T-cell dysfunction, including secreted factors such as TGFβ and inhibitory cells recruited to the tumor environment such as regulatory T cells (Tregs), suppressive myeloid cells, and cancer-associated fibroblasts (8, 9). Genetic modification of patient T cells can provide a means to overcome such obstacles by potentiating tumor cell killing, and enhancing the antitumor efficacy of adoptive T-cell therapy (Fig. 1). TCR gene therapy is of particular interest, as it provides a fairly straightforward “off-the-shelf” approach to redirect the antigen specificity of autologous T cells through gene transfer of a well-characterized tumor antigen-specific TCR, which has been selected based on recognition of an appropriate tumor antigen and possessing sufficient affinity to potentially mediate eradication of the tumor being targeted (10). TCR gene therapy provides significant flexibility, as specific T-cell subsets can be selected for TCR transduction based on defined phenotypic and functional characteristics to maximize in vivo persistence and tumor elimination (11, 12); and can serve as a platform for additional cellular engineering to enhance T-cell activity in the tumor microenvironment. For example, T cells modified to express engineered costimulatory receptors, chemokine receptors that enhance T-cell homing, or cytokines that improve function, and/or that have disrupted inhibitory pathways, may be better equipped to maintain robust T-cell activity within the potentially immunosuppressive tumor microenvironment.

Vector design

The ability of a TCR gene–modified T cell to efficiently recognize antigen-bearing tumor cells depends in part on the affinity of the TCR as well as the level of TCR surface expression on the transduced T cell. A normal TCR complex consists of the TCR chains (TCRα and TCRβ) in association with CD3γ, δ, ε, and ζ subunits (13), which are required for TCR surface expression but are produced in limiting amounts. Consequently, to achieve adequate surface expression the transgenic TCRβ must successfully compete with the endogenous TCRβ for association with the CD3 subunits (14). Furthermore, functional expression of the introduced TCR requires proper pairing of the introduced TCRβ chains as well as limited mispairing between transgenic and endogenous TCR chains, which could result in untested, potentially self-reactive TCRβ pairs (15). Therefore, the TCR gene therapy vectors currently being used in the clinic are engineered to achieve coordinated, high-level TCRβ transgene expression, by such means as promoter modifications and codon optimization, and commonly employ strategies to promote proper transgenic TCRβ pairing (16–18) to maximize the avidity of the T cell expressing the transferred TCR (10, 19). Alternatively, it is also...
feasible to directly prevent expression of the endogenous TCR, either by engineered gene disruption (20) or by constitutively expressing an shRNA that selectively targets the endogenous chain (21). However, although TCR mispairing can result in autoimmunity with fatal GVHD in mice (15), off-target immune recognition due to TCR mispairing has not yet been observed in any human TCR gene therapy trials, with or without modifications to promote transgenic TCR pairing.

TCR gene therapy in the clinic

In most human trials, TCR gene transfer has been accomplished through γ-retroviral or lentiviral transduction of substrate T cells, both of which result in highly efficient transduction and stable transgene integration into the host cell genome (22). One concern, associated with γ-retroviruses in particular, is the potential for insertional mutagenesis as a result of integration into the host genome near transcriptional start sites. Lentiviruses exhibit less integration bias, and current self-inactivating (SIN) lentiviral vectors that lack the U3 region of the viral long terminal repeat have proven to be particularly safe in this regard, with no reports of insertional mutagenesis from the many clinical studies that have utilized these vectors. Recently, transfectable transposon-based systems have been developed for therapeutic gene transfer as well. The sleeping beauty system in particular exhibits little insertional bias and minimal activation of endogenous genes (23). However, compared with virus-based approaches, transposon-based systems exhibit lower gene-transfer efficiency, requiring more extensive in vitro expansion of gene-modified T cells.

The choice of substrate cell clearly plays a critical role in the behavior of transferred T cells, and several studies have assessed the relative benefits of various CD8+ T-cell subsets as substrate cells for TCR gene transfer. Less terminally differentiated T-cell lineages, such as central memory T cells (TCM; ref. 11), stem cell memory (24), or naïve T cells (12), have been reported to exhibit enhanced persistence and effector function in vivo. However, the choice of substrate cell will likely be disease dependent: for example, in solid tumors with large persisting antigen burdens, TCM-derived T cells may be selectively lost (25). Indeed, for some malignancies multiple infusions of short-lived effector T cells may prove to be most effective.

Figure 1. Enhanced engineering of T cells for improved therapeutic targeting of malignancies. A, solid tumor environments commonly include complex mixtures of immunosuppressive cells including myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), suppressive dendritic cells (DC), cancer-associated fibroblasts (CAF), CD4+Foxp3+ regulatory T cells (Treg), endothelial cells (not shown), and tumor cells that are all embedded within a robust extracellular matrix (ECM). B, a selected list of the major obstacles to eliciting curative responses with engineered T cells and corresponding counteractive strategies (C) are depicted.
On the Horizon
Enhanced-affinity TCRs specific for tumor/self antigens

The TCR complex has evolved to recognize any transcribed protein that can be processed and presented on MHC with exquisite antigen sensitivity (26), and can not only distinguish cells expressing normal versus foreign antigens, but can also distinguish cells overexpressing tumor/self antigens from cells expressing physiologic levels (27). For this reason, a substantial number of tumor-associated or tumor-specific proteins can potentially be targeted by TCR gene therapy. A persistent challenge of adoptive T-cell therapy in general has been the identification of antigens that can be safely and effectively targeted, including unique target antigens expressed exclusively by tumor cells such as those derived from mutations or oncogenic viruses, or target antigens sufficiently overexpressed by tumors such that tumor cells and not normal tissues will be selectively recognized (28). Researchers have taken a variety of approaches to identify/generate therapeutic TCRs. One approach involves targeting self-proteins that contribute to the malignant phenotype and are overexpressed by tumor cells, but are not detected or are expressed at only low levels by normal self-tissues. These pro-oncogenic antigens often have the advantage of being shared between individuals with the same tumor type. Another benefit is that loss of the target antigen as an escape mechanism confers a growth disadvantage to the tumor. However, a disadvantage of this approach is that most T cells specific for these antigens that can be isolated are of lower affinity than, for example, antiviral TCRs due to peripheral tolerance and central tolerance (negative selection in the thymus) mechanisms. Therefore, efforts are being made to increase the affinity of self/tumor antigen-specific TCRs isolated from the normal repertoire through mutagenesis in vitro (29–31). This strategy can result in gene-modified T cells that mediate a robust antitumor response in the presence of lower levels of antigen (32). Modifications are generally targeted to the antigen-binding complementarity determining regions (CDR1, 2, and 3), which associate predominantly with either the peptide (CDR3) or the restricting MHC (CDR1/2). A risk of this approach is that modifications could potentially result in cross-reactivity with other self-antigens, leading to T-cell-mediated destruction of normal-self tissue. Modifications to CDR1 and 2 are particularly problematic, as this can become permissive for triggering by alternative peptides by stabilizing TCR interactions with invariant MHC contact points, as exemplified by a recent study in which a TCR modified with CDR2-targeted affinity-enhancing mutations exhibited unpredicted cross-reactivity with a self-antigen in the heart, resulting in fatal autoimmune toxicity (33). However, the risk of cross-reactivity is likely diminished when affinity-enhancing mutations are restricted to the peptide-associated CDR3 regions of the TCR, and this approach has resulted in effective TCR-based therapy without on-target or off-target toxicity (29, 34). An alternative strategy for acquiring enhanced-affinity TCRs is to bypass negative selection entirely by isolating tumor/self antigen-specific T cells from human HLA gene-transgenic mice that express the restricting HLA allele but do not express the selected target human tumor antigen/epitope (35). Recently, HLA-transgenic mice have been generated that express the entire human TCRα and TCRβ gene loci, making it possible to produce high-affinity, fully human TCRs to avoid the problems of immunogenicity of an expressed murine TCR and/or the requirement to humanize components of the murine TCR (36). However, the risk of this approach is that the resulting TCRs have been selected against only the human self-antigens that are conserved in the mouse, and therefore have a higher likelihood of exhibiting dangerous autoimmune cross-reactivity (37). Nonetheless, the method used to enhance TCR affinity, it is essential with this approach that rigorous safety testing be performed prior to clinical translation. Two recent trials targeting the cancer-testis antigen Mage-A3 with enhanced-affinity TCRs reported fatal autoimmune toxicities due to cross-reactivity with secondary targets on healthy tissue, which likely would have been identified with more thorough safety screening (33, 37). Therefore, in addition to assessing TCR-transduced T-cell reactivity against diverse antigen-negative cell lines, preclinical safety screens should include testing of all potential self-peptides predicted to bind the restricting MHC and to share critical TCR contact residues with the target antigen (38), and, if the target epitope is conserved in the mouse, HLA-transgenic mice can provide an additional assessment of in vivo safety.

Neoantigens

A second approach is to focus on targeting mutations that result in new tumor-specific epitopes (neoepitopes). These antigens should be recognized as foreign by the cellular immune system, provided that a peptide containing the mutation can be processed and effectively presented on MHC. Such targets bypass the problem of self-tolerance, thereby increasing the likelihood that a high-affinity antigen-specific T cell exists in the normal repertoire. Indeed, a strong correlation has been observed between the detection of endogenous intratumoral cytotoxic immune responses and the abundance of tumor neoantigens (39). Although most tumors have tens to hundreds of mutations in protein-encoding regions of genes, which might give rise to a targetable neoepitope (40), only a fraction of these mutations will actually result in epitopes presented by the patient’s MHC molecules. Moreover, these tumor-associated mutations, which generally reflect the genomic instability of a cancer cell, are usually random and associated with proteins not involved in the oncogenic phenotype, and are therefore termed "passenger" mutations, making them likely to be patient specific rather than detectable in a significant fraction of patients. Efforts have been made to identify driver mutations that are immunogenic, which would represent attractive targets for TCR gene therapy as these mutations are broadly shared among patients with a specific malignant disease (41). However, in many cases, these epitopes have proven to be poorly immunogenic (42). Therefore, a major drawback of the focus on neoantigens is that every individual patient would likely require first identifying targetable neoantigens and then generating unique antigen-specific TCRs, a practice that would exceed the practical efficiency of current technologies (43). Thus, with the exception of mutations in driver oncogenes, vaccine strategies to induce endogenous host responses may prove more adaptable for targeting neoantigens. The feasibility of targeting such neoantigens was initially demonstrated in mice, taking advantage of whole-exome sequencing to first identify expressed genetic variants in tumor versus normal tissue, then using algorithms to predict likely MHC-binding epitopes, and finally synthesizing the epitopes to identify and/or expand with a vaccine neoantigen-reactive T cells with antitumor activity (44, 45). More recently, this strategy has been validated in patients to identify neoantigen-specific T cells with apparent antitumor...
activity in melanoma patients following treatment with CTLA-4 blockade or within populations of tumor-infiltrating lymphocytes (TIL; refs. 46–48); also, PD-1 blockade has been shown to specifically release neoantigen-reactive T cells in melanoma and lung cancer (49, 50). Technological advances over the past several years have made whole-exome sequencing of individual tumors not only exponentially less expensive but also achievable in a short time frame. Vaccine strategies targeting identified neoantigens are already in the clinic and will provide valuable insights into pursuing these targets (51). Of note, as the great majority of random mutations do not provide a growth or survival advantage as compared with driver mutations, and occur stochastically in growing tumor cells, responses to multiple such neoantigens will likely be needed to achieve tumor eradication. Although one could envision a personalized TCR gene therapy approach in which a set of unique neoantigen-specific TCR gene therapy vectors are generated for each individual patient that would be highly tumoral specific without the toxicities associated with on-target off-tumor recognition of normal self tissues (43), the time required with current technologies for TCR identification, gene synthesis, and preclinical safety and efficacy testing precludes efficient deployment of this strategy (currently this takes at least 1 year), and personalized TCR gene therapy is likely on the distant horizon pending development of technical advances that can accelerate this process.

Third-party cell products

In an effort to make adoptive T-cell therapy more accessible in general, the use of third-party cell banks for tumor immunotherapy has been proposed. This approach has already been successfully applied to the treatment of viral infections following transplantation with banked virus-specific T-cell lines (52), and could potentially help broaden the applicability of TCR gene therapy. However, treating patients with gene-modified allogeneic T cells of otherwise unknown antigen specificity would pose a significant risk of GVHD, and such donor-derived T cells would likely be at risk of rejection by the patient’s immune system, diminishing the therapeutic potential of the treatment. These hurdles may eventually be overcome through ongoing efforts to develop universal donor cells using genome editing to eliminate expression of endogenous TCR genes (to mitigate GVHD), as well as HLA genes (to decrease alloreactivity); further genetic engineering would then be required to protect these HLA-deficient T cells from NK-mediated lysis (53). Although successful implementation of this approach would greatly increase the accessibility of TCR gene therapy, even the described efforts may be insufficient to allow persistence except in a very immunocompromised host, and further studies will be required to establish the practicality and safety of this approach prior to clinical translation.

CD4 T cells

Until recently, adoptive T-cell therapy approaches have focused almost entirely on cytotoxic CD8+ T cells. However, as we demonstrated decades ago (54, 55), CD4+ T cells can play an essential role in antitumor immunity (56–58). In addition to providing help to boost tumor-reactive CD8+ T-cell activation, CD4+ T cells can enhance antitumor immunity in the tumor microenvironment by producing IFNγ and other cytokines and chemokines that not only enhance the function of CD8+ T cells but can also recruit and activate innate inflammatory cells, such as tumoricidal NK cells and macrophages (59, 60). Furthermore, tumor-specific CD4+ T cells can contribute to tumor cell lysis through direct perforin- or granzyme-mediated killing as well as production of tumoricidal cytokines (61–63). In a murine model of Mesothelin-targeted CAR–T-cell therapy, early infiltration and activation of CAR-transduced CD4+ T cells was essential for enhanced antitumor activity, and could eliminate pleural malignancies even in the absence of CAR-transduced CD8+ T cells (64).

Therefore, strategies to redirect CD4+ T cells to target cancer through gene therapy are currently being pursued. The most straightforward approach is to genetically modify CD4+ T cells with tumor antigen-specific MHC–II–restricted TCRs. Although a few tumor antigen-specific MHC–II–restricted epitopes have been characterized (65), the practicality of this approach would be limited by the necessity that patients must express targetable MHC-I as well as MHC-II restricting alleles. More importantly, most tumor cells are Class II-negative, thereby requiring an APC to present the antigen. A more attractive alternative might be to transduce both CD4+ and CD8+ T cells with the same MHC-I–restricted TCR. Although most naturally occurring MHC–I–restricted TCRs require concomitant binding of CD8 to peptide/MHC for antigen recognition, affinity enhanced TCRs are generally capable of antigen recognition independent of CD8, making them potentially viable candidates for redirecting CD4+ T cells to recognize MHC-1–restricted antigens (66, 67). CD4+ T cells expressing an MHC–I–restricted TCR have been shown to reverse tumor/self-antigen–specific CD8 T-cell tolerance (68). However, CD4+ T cells may require for full function a more potent and/or complete signal than can be provided only through the TCR complex. Thus, as an alternative strategy, cotransfer of a native affinity MHC–I–restricted TCR with CD8 coreceptor genes is being pursued (69). Expression of CD8α alone, despite abundant surface expression of the CD8βα homodimer, failed to recapitulate MHC–I–restricted antigen recognition in CD4+ T cells in murine studies, whereas expression of the CD8αβ heterodimer was able to confer MHC–I–restricted antigen recognition and effector function to CD4+ T cells in vitro (69). In addition, maintenance of CD4+ T-cell function is generally dependent on reception of costimulatory signals, and thus strategies to incorporate such signals in addition to a Class I–restricted TCR to facilitate the recognition and targeting of tumor cells that do not express CD80/86 are being evaluated.

Further gene modifications to enhance TCR-transduced T-cell function

Tumors can employ multiple tactics to evade or misdirect tumor-specific immunity. In the context of persistent tumor antigen, T cells can become progressively less functional, typified by the loss of cytokine secretion and the inability to lyse target cells (70, 71). A variety of inhibitory receptors (PD-1, Tim3, Lag3, 2B4, Tigit) are upregulated on T cells following antigen encounter and may play a role in limiting the activity of adoptively transferred T cells (68). TCR gene–modified T cells can be further engineered to enhance specific T-cell functions, particularly in the setting of the targeted tumor environment. Expression of costimulatory ligands on T cells can provide autocrine and trans-costimulatory signals in the tumor microenvironment (72); and chimeric costimulatory receptors can co-opt tumor-inhibitory pathways by switching an immunosuppressive signal into an immunostimulatory signal. For example, the chimeric receptor PD-1/CD28 increases T-cell proliferation, cytokine secretion, and antitumor activity in preclinical models (73), and the chimeric cytokine receptor IL4-IL7R can also enhance T-cell proliferation and activation in cancer (74).
Likewise, disrupting inhibitory signaling pathways in T cells, such as Chib-b (75), Shp-1 (76), or TGFβ (77), can enhance T-cell expansion, survival, and function during T-cell therapy. Alternatively, introduction of cytokine genes, such as IL12 (78) in donor T cells can modulate the tumor environment to be more conducive to the activity of T cells, but does have the drawback of also increasing toxicity. Because of the numerous inhibitory molecules expressed by both tumor and stromal cells, multiple inhibitory pathways may need to be simultaneously targeted to sustain/optimize function. As different T-cell lineages may respond differently to inhibitory signals (79), determining how distinct T-cell subsets are in T-cell lineages may respond differently to inhibitory signals (79), determining how distinct T-cell subsets are in donor T cells can modulate the tumor environment to be more self-tolerant, and tumors, which can subvert the immune delicate interplay between the immune system, which is necessary how T cells "see" a tumor, which cell types are signaled to respond, and how those cells are influenced by immunosuppressive factors in the tumor microenvironment.

Conclusions

Advances in tumor immunology will continue to broaden our understanding of the antigenic landscape of tumors, and the delicate interplay between the immune system, which is necessarily self-tolerant, and tumors, which can subvert the immune system and masquerade as healthy self-tissue. TCR gene therapy allows scientists and physicians to precisely define how T cells recognize, and initiation of signaling. Cold Spring Harb Perspect Biol 2010;2:a005140.

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Disclosure of Potential Conflicts of Interest

P.D. Greenberg is a founder of, reports receiving commercial research support from, has ownership interest (including patents) in, and is a consultant/advisory board member for Juno Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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