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Blockade of TGF-β Signaling Greatly Enhances the Efficacy of TCR Gene Therapy of Cancer

Gavin M. Bendle,*1 Carsten Linnemann,*1 Laura Bies,* Ji-Ying Song, † and Ton N. M. Schumacher*

TCR gene therapy is a promising approach for the treatment of various human malignancies. However, the tumoricidal activity of TCR-modified T cells may be limited by local immunosuppressive mechanisms within the tumor environment. In particular, many malignancies induce T cell suppression in their microenvironment by TGF-β secretion. In this study, we evaluate whether blockade of TGF-β signaling in TCR-modified T cells enhances TCR gene therapy efficacy in an autochthonous mouse tumor model. Treatment of mice with advanced prostate cancer with T cells genetically engineered to express a tumor-reactive TCR and a dominant-negative TGF-β receptor II induces complete and sustained tumor regression, enhances survival, and leads to restored differentiation of prostate epithelium. These data demonstrate the potential to tailor the activity of TCR-modified T cells by additional genetic modification and provide a strong rationale for the clinical testing of TGF-β signaling blockade to enhance TCR gene therapy against advanced cancers. The Journal of Immunology, 2013, 191: 3232–3239.

The therapeutic potential of adoptive T cell transfer as a treatment for human cancer has first been demonstrated in patients receiving T cell–replete allogeneic hematopoietic stem cell transplants or donor lymphocyte infusions for hematological malignancies (1, 2). Whereas these approaches have highlighted the potential of tumor-reactive T cell infusion, the significant morbidity/mortality associated with these nondirected forms of T cell therapy provides a clear rationale for the development of alternatives that deliver a greater level of tumor specificity.

Toward this goal, genetic modification of T cells with defined tumor-reactive TCR genes—an approach called TCR gene therapy—has been developed, first in mouse models (3, 4) and more recently also in clinical trials (5–7). These clinical trials, primarily in patients with metastatic melanoma and synovial sarcoma, have demonstrated the feasibility of this approach, and in particular the targeting of the NY-ESO-1 Ag has shown encouraging clinical responses (7). In parallel, a large engineering effort has been made to develop TCR gene therapy into a broadly applicable clinical approach. First, a number of strategies has been developed to isolate TCR genes of potential clinical interest from patient material (8, 9) or from nontolerized T cell repertoires (10–14), and the library of tumor-reactive TCR genes that will be required to target a variety of human malignancies will most likely be available within years. Second, a number of modified TCR gene formats has been developed that yield increased expression of the introduced transgenes and that reduce the formation of mixed dimers consisting of endogenous and exogenous TCR chains (15–19). Third, recent developments in directed gene inactivation may even make it feasible to produce TCR-edited T cell products for clinical use, in which the original TCR has been replaced by a TCR of interest (20). Collectively, these technological advances have made it feasible to test the value of TCR-directed T cells in diverse clinical settings, and trials that will use TCR-engineered T cells for the treatment of a variety of hematological and nonhematological malignancies are planned for the coming years.

The infusion of TCR-modified T cells solves the issue that in many cancer patients the frequency of tumor-reactive T cells is low. However, it does not guarantee the functional activity of these cells, in particular at the tumor site where immunosuppressive mechanisms may impede the reactivity of the infused cells. For this reason, it seems attractive to supply patient T cells not only with tumor-reactive TCR(s) of choice, but also engineer the cells such that they are insensitive to these local inhibitory circuits.

TGF-β is a key regulator of immune homeostasis (21–23), and support for the notion that this cytokine plays a key role in tumor-mediated immune suppression in humans comes from several studies. First, in vitro experiments have demonstrated that human T cells are susceptible to TGF-β–mediated immune suppression (24). Second, elevated levels of TGF-β are observed in a variety of human malignancies (25, 26), including multiple myeloma, Hodgkin lymphoma, prostate cancer, colorectal cancer, hepatocellular carcinoma, and melanoma. Third, TGF-β production is particularly evident in advanced cancer and is correlated with poor prognosis, for instance, in the case of prostate carcinoma (27).

On the basis of the above data, blockade of TGF-β signaling in tumor-reactive T cells appears an attractive approach to enhance the clinical efficacy of adoptive T cell therapy. Prior studies have described TGF-β–mediated immune suppression in mouse tumor models (28–31), and studies have shown that T cells engineered to...
be insensitive to TGF-β signaling display enhanced antitumor efficacy in mouse transplantable tumor models (32–34) and inhibit tumor development in autochthonous mouse tumor models (35). In contrast though, short-term experiments at a clinically more relevant time point in autochthonous mouse tumor models have not yielded encouraging data (36).

In this study we provide, to our knowledge, the first analysis of the long-term effects of joint genetic modification of T cells with a tumor-reactive TCR and a dominant-negative TGF-β receptor-II (dnTGFβRII) on invasive prostate carcinoma in an autochthonous mouse tumor model.

Materials and Methods

Mice

Transgenic adenocarcinoma of mouse prostate (TRAMP) mice (37) were obtained from the Experimental Animal Department of The Netherlands Cancer Institute. For all experiments, F1 offspring of B6 × TRAMP mice was used. All animal experiments were performed in accordance with institutional and national guidelines and were approved by the Experimental Animal Committee of The Netherlands Cancer Institute.

Retroviral constructs, retroviral transduction, and adoptive transfer of T cells

pMX-SV40IV-Cy5-β2-P2A-SV40IV-Cy5-α (19) and pMX-dnTGFβRII-IRESC-GFP (19) retroviral vectors were used to transfect Phoenix-E packaging cells to generate retrovirus (38). Splenocytes from male TRAMP mice were modified by retroviral transduction with the indicated vectors, as described previously (3).

Six to 24 h prior to adoptive cell transfer, irradiation-induced host conditioning was achieved by 5 Gy total body irradiation (TBI) with a radiobiology constant potential x-ray unit (Pantak HF-320; Pantak). The following day, mice received an adoptive cell transfer of the activated and retrovirally modified splenocytes containing either 1 × 10⁶ SV40IV–TCR–transduced CD8+ T cells, 1 × 10⁶ dnTGFβRII-transduced CD8+ T cells, 1 × 10⁶ SV40IV–TCR–transduced CD8+ T cells that had been cotransduced with the dnTGFβRII, or equivalent numbers of nontransduced CD8+ T cells.

Flow cytometry

Cell surface TCR expression and expression of the dnTGFβRII were measured 24 h posttransduction by flow cytometry. Cells were stained with fluorescently labeled anti-TCR Vβ9 mAb (eBiosciences), or with K5-SV40O44-411 multimers, in combination with PE- or allophycocyanin-conjugated anti-CD8 mAb (BD Biosciences). To measure dnTGFβRII expression, GFP expression was used as a surrogate marker as the dnTGFβRII receptor-II protein (Sigma-Aldrich) was used as a substrate-chromagen, and slides were counterstained with hematoxylin. Images were acquired using an Axioscam HR digital camera and processed with Axiovision 4 software (Carl Zeiss Vision). The extent of expression of SV40 large T Ag, Ki67, TGF-β1, and probasin in the prostate was defined semi-quantitatively as no expression (grade 0), expressed in ≤25% of cells (grade 1), expressed in ≥25% and <50% of cells (grade 2), expressed in ≥50% and <75% of cells (grade 3), expressed in ≥75% and <100% of cells (grade 4), and expressed in 100% of cells (grade 5).

Statistical analysis

Histopathological scores were compared using a nonpaired two-tailed t test in Microsoft Excel software. Survival curves were analyzed using a log-rank (Mantel–Cox) test in GraphPad Prism software. The p values < 0.05 were considered significant.

Results

Expression of TGF-β in advanced, invasive prostate tumors in TRAMP mice

To develop an autochthonous tumor model in which the effect of manipulation of T cell sensitivity to tumor-derived TGF-β could be analyzed, we evaluated prostatic tissue of TRAMP mice (37). TRAMP mice express the transforming protein SV40 large T Ag (SV40 large T) under the control of the rat probasin promoter, and prostate epithelial cells in these mice undergo pathological changes that lead to the development of invasive prostate carcinoma by 24 wk of age (37). Histopathological analysis of advanced TRAMP tumors shows that these tumors are characterized by increased cellularity within the prostate glands, loss of gland architecture, and invasion of the tumor cells into the stroma. The neoplastically transformed cells display an increased nucleus/cytoplasm ratio, enhanced intensity of nuclear staining, and increased mitotic activity (Fig. 1 and data not shown). Furthermore, as documented previously (40), development of prostate carcinoma is characterized by impaired differentiation of the secretory prostate epithelium, as demonstrated by the loss of probasin expression in TRAMP mice with invasive carcinoma of the prostate (Fig. 1, Supplemental Fig. 1A). Notably, in line with the findings from previous studies (41, 42), the development of prostate carcinoma in the TRAMP mice is also accompanied by increased expression of TGF-β1 in the prostate (Fig. 1, Supplemental Fig. 1B), with TGF-β1 usually detected at both the apical and lateral domains of cell membranes.

In addition to the expression of TGF-β in advanced tumors, the autochthonous tumor model used in this study bears other important similarities to the clinical setting in which TCR gene therapy of cancer is being tested. First, analogous to the situation for most human tumor-associated self-Ags targeted with TCR gene therapy, the endogenous T cell repertoire of TRAMP mice has been shown to be tolerant toward SV40 (43, 44). Therefore, in the context of the TRAMP model, the SV40 large T Ag should be viewed as a tumor-associated self-Ag rather than a foreign Ag. Second, lymphodepleting preconditioning regimens are routinely used prior to adoptive cell transfer in the clinical setting (5–7), and the mice in this study also receive a lymphodepleting preconditioning regimen prior to the adoptive transfer of T cells. Third, analogous to the clinical use of high-affinity TCRs isolated from nontolerant T cell repertoires (6, 7), a high-affinity TCR isolated from a nontolerant T cell repertoire is used in this study.
Combination of TCR gene therapy and blockade of TGF-β signaling can lead to complete regression of advanced, invasive prostate tumors in TRAMP mice

We have previously reported that TCR gene therapy using T cells modified with a SV40 epitope IV–specific TCR (SV40IV-TCR) at 10 wk of age leads to a marked delay in tumor development in TRAMP mice (43). However, the same TCR gene therapy regimen fails to show a substantial benefit when T cell infusion is performed at 24 wk of age when neoplastic lesions are fully developed (G. Bendle, unpublished observations; see also below). Furthermore, as described above, high levels of TGF-β1 in the prostate are observed in ∼24-wk-old TRAMP mice (Supplemental Fig. 1B). On the basis of these data, we hypothesized that the elevated levels of TGF-β expression observed in advanced prostate tumors may suppress the antitumor reactivity of TCR-modified T cells within the prostate. To test this hypothesis, we generated a retroviral vector that encodes a dnTGFβRII (19). Subsequently, the ability to control advanced prostate cancer of T cells that had been modified with only the dnTGFβRII, only the SV40IV-TCR, or the combination of the two transgenes was compared. The 24-wk-old TRAMP mice received a preconditioning regimen of nonmyeloablative TBI (5 Gy), followed by the adoptive transfer of either $1 \times 10^6$ SV40IV-TCR–transduced CD8+ T cells, $1 \times 10^6$ dnTGFβRII-transduced CD8+ T cells, or $1 \times 10^6$ SV40IV-TCR–transduced CD8+ T cells that had been cotransduced with the dnTGFβRII. As a control, a cohort of mice received equivalent numbers of nonmodified CD8+ T cells (Supplemental Fig. 2A, 2B). Four weeks after adoptive cell transfer (ACT), mice were sacrificed to analyze the short-term effects of the different gene-modified T cell populations on tumor development by histopathology. In all experiments, histopathological analysis was carried out by an animal pathologist who was blinded with respect to the treatment group to which samples belonged. In 100% of mice that received either nontransduced T cells or T cells transduced with the dnTGFβRII alone, large invasive prostate carcinomas were found. In line with this, widespread expression of the SV40 large T oncogene, the Ki67 proliferation marker, and TGF-β was observed in the prostate of these mice (Figs. 1, 2A). In mice receiving T cells modified with only the SV40IV-TCR, areas of local regression were sporadically encountered in the prostate tumors. Such areas were characterized by reduced cellularity of epithelial cells, morphological changes in epithelial cells (cuboidal shape and low nucleus/cytoplasm ratio), the local elimination of SV40 expression, and reduced Ki67 and TGF-β expression (Figs. 1, 2A). Nevertheless, many clusters of transformed cells were invariably found, and only of either 1 × 10⁶ SV40IV-TCR–transduced CD8⁺ T cells, 1 × 10⁶ dnTGFβRII-transduced CD8⁺ T cells, or 1 × 10⁶ SV40IV-TCR–transduced CD8⁺ T cells that had been cotransduced with the dnTGFβRII. As a control, a cohort of mice received equivalent numbers of nonmodified CD8⁺ T cells (Supplemental Fig. 2A, 2B). Four weeks after adoptive cell transfer (ACT), mice were sacrificed to analyze the short-term effects of the different gene-modified T cell populations on tumor development by histopathology. In all experiments, histopathological analysis was carried out by an animal pathologist who was blinded with respect to the treatment group to which samples belonged. In 100% of mice that received either nontransduced T cells or T cells transduced with the dnTGFβRII alone, large invasive prostate carcinomas were found. In line with this, widespread expression of the SV40 large T oncogene, the Ki67 proliferation marker, and TGF-β was observed in the prostate of these mice (Figs. 1, 2A). In mice receiving T cells modified with only the SV40IV-TCR, areas of local regression were sporadically encountered in the prostate tumors. Such areas were characterized by reduced cellularity of epithelial cells, morphological changes in epithelial cells (cuboidal shape and low nucleus/cytoplasm ratio), the local elimination of SV40 expression, and reduced Ki67 and TGF-β expression (Figs. 1, 2A). Nevertheless, many clusters of transformed cells were invariably found, and only...
To this end, 24-wk-old TRAMP mice received TBI (5 Gy) restricted to the apical domain of cell membranes (Figs. 1, 2A). Likewise, the expression of TGF-β was found to be minimal or fully absent (Fig. 1). In line with these histological changes, SV40 large T and Ki67 expression was found to be minimal or fully absent (<10% of cells) (Figs. 1, 2A). Likewise, the expression of TGF-β in the prostate was also reduced, with small clusters of positive staining that was largely restricted to the apical domain of cell membranes (Figs. 1, 2A). Furthermore, total body necropsy of these TRAMP mice revealed that treatment with SV40Large T-CR/dnTGFβRII–cotransduced T cells was not associated with any toxicological pathology. Taken together, these findings demonstrate that infusion of dual-modified T cells, which express both a tumor-reactive TCR and a dnTGFβRII, can lead to microscopically complete regression of established prostate carcinomas (Fig. 2B).

Complete tumor regression of TRAMP prostate tumors induced by combination of TCR gene therapy and blockade of TGF-β signaling is sustained long-term

Next, we addressed whether the observed tumor regression in TRAMP mice 4 wk after treatment with TCR-modified T cells that are cotransduced with the dnTGFβRII was sustained long-term. To this end, 24-wk-old TRAMP mice received TBI (5 Gy) and ACT with either $1 \times 10^{6}$ SV40Large T-CR–transduced CD8⁺ T cells, $1 \times 10^{6}$ SV40Large T-CR–transduced CD8⁺ T cells that had been cotransduced with the dnTGFβRII, or equivalent numbers of nontransduced CD8⁺ T cells (Fig. 5A). TRAMP mice infused with nontransduced T cells had a median survival of 32 wk post-ACT before mice had to be euthanized (7 of 7 mice; with 1 censored event due to lymphoma in the absence of regression of TRAMP lesions) (Fig. 5B). Histopathological analysis of the prostate in these mice after sacrifice revealed large tumor masses, macroscopically apparent especially in the seminal vesicles (data not shown). As expected, no signs of regression of TRAMP lesions in the prostate glands were observed (data not shown). TRAMP mice treated with SV40Large T-CR–transduced T cells had a comparable median survival of 31 wk post-ART. From this cohort, 100% (4 of 4 mice; with 1 censored event due to hepatocarcinoma in the absence of regression of TRAMP lesions) of animals had to be euthanized as a result of progressive prostate carcinoma tumor growth (Fig. 5B), and survival within this group was not significantly increased compared with mice receiving nontransduced T cells ($p = 0.98$).

At a median follow-up of 57 wk post-ART/81 wk of age TRAMP mice receiving SV40Large T-CR–dnTGFβRII double-modified T cells were sacrificed and examined histologically (3 of 5 censored events due to lymphoma). As at 40 wk of age, we again observed that the majority of treated mice displayed complete regression of their prostate neoplastic lesions (4 of 5 mice; the fifth mouse displayed regression in >75% of its prostatic neoplastic lesions) (Fig. 5B). Importantly, contrary to mice treated with SV40Large T-CR–modified T cells, the survival of mice receiving SV40Large T-CR–dnTGFβRII double-modified T cells was significantly higher than that of both mice receiving nontransduced T cells ($p = 0.001$) and mice treated with the SV40Large T-CR single-modified T cells ($p = 0.025$).

Probasin is a marker of differentiated secretory prostate epithelium in mice, and it has previously been demonstrated that the expression of this differentiation marker is lost in TRAMP mice.
with advanced prostate carcinoma (40). To evaluate whether successful TCR gene therapy could lead to restored differentiation of the prostate epithelium, we analyzed prostate tissue of the different treatment groups at various time points posttherapy. Strikingly, we observed clear expression of probasin within the prostate epithelial cells in the majority of mice following treatment with SV40IV-TCR and dnTGFβRII-cotransduced T cells at both 4 wk (28 wk of age) (Figs. 1, 2A) and 16 wk (40 wk of age) (Figs. 3, 4A) after ACT. In contrast, no expression of probasin was observed in TRAMP mice in which no tumor regression was observed. Thus, treatment of mice with advanced prostate cancer with TCR-modified cells that have been rendered insensitive to TGF-β signaling not only leads to long-term cancer regression, but also to the recovery of normal prostate gland function in TRAMP mice.

Discussion

In this study, we demonstrate that the blockade of TGF-β signaling can greatly enhance the efficacy of TCR gene therapy in an autochthonous mouse model of prostate carcinoma. This combination treatment not only leads to the sustained regression of advanced and invasive prostate carcinoma, but can also be shown to result in prolonged survival of treated mice. To our knowledge, this is the first example of long-term tumor control by TCR-modified T cells in an autochthonous tumor model. Strikingly, the complete tumor regression observed in these mice is accompanied by the restoration of probasin expression in prostate epithelial cells. Probasin is a marker of differentiated secretory prostate epithelium in rodents (40), and our data therefore suggest that treatment with dual-engineered T cells also leads to the recovery of normal prostate gland function. The molecular mechanism responsible for the recovery of normal prostate gland function following treatment with dual-engineered T cells has not been elucidated. However, the adult murine prostate is known to contain a population of stem cells capable of mediating the regeneration of a functional prostate following repeated cycles of androgen deprivation and replacement therapy (45–47). Therefore, it seems feasible that the prostate epithelial cells expressing probasin we observe following treatment with dual-engineered T cells are derived from this population of prostatic stem cells.

In contrast to the profound antitumor effect of adoptive cell transfer and TGF-β blockade in this study, Chou et al. (36) have recently observed that adoptively transferred T cells derived from OT-I TGFβRII knockout mice display only limited antitumor effects and are rendered tolerant in a mouse model of autochthonous prostate.
cancer. One clear difference between these studies is that in our study a cancer-driving Ag was the target of adoptively transferred T cells, whereas Chou et al. (36) targeted an Ag that did not play a role in tumor development or the maintenance of the malignant phenotype. Therefore, the likelihood of the emergence of tumor Ag loss variants would have been greater in the study by Chou et al. (36), but whether tumor Ag loss variants occurred in their study was not directly addressed. Another major difference between these studies is that the mice in our study received a lymphodepleting preconditioning regimen prior to the adoptive transfer of T cells, whereas no such regimen was used in the study by Chou et al. (36). Lymphodepleting preconditioning regimens are routinely used prior to adoptive cell transfer in the clinical setting (5–7), and studies in mice have demonstrated the crucial role this process plays in promoting the in vivo function of adoptively transferred T cells (48–50). In line with this, we have previously shown that, in the absence of the in vivo T cell activation provided by either lymphodepleting preconditioning or concomitant viral vaccination, adoptively transferred SV40 TCR-transduced T cells fail to prevent tumor development in 10-wk-old treated TRAMP mice (43).

Based on these data, we suggest that the marked effect of TGF-β signaling in T cells in the absence of TGF-β blockade observed in this study can only manifest itself under conditions in which the TCR-modified T cells are activated in vivo, either by vaccination, or by infusion into a lymphodepleted host.

A recent clinical study using TCR gene therapy targeting the cancer germ line Ag NY-ESO-1 to treat patients with metastatic melanoma and synovial cell carcinoma has reported very encouraging clinical response rates (7). However, the majority of these clinical responses were partial and not durable. It is tempting to speculate that, among other factors, the in vivo efficacy of TCR-modified T cells in these patients was hampered by suppression within the tumor microenvironment. In support of this notion are studies demonstrating that immune-suppressive mechanisms can be operational in melanoma (51, 52) as well as the findings reported in this work that demonstrate that tumor regression of advanced, invasive prostate tumors is transient and does not prolong survival for the majority of TRAMP mice when treated with TCR-modified T cells in the absence of TGF-β blockade.

The TGF-β signaling pathway in T cells (and tumor cells) is amenable to pharmacological blockade using small molecule inhibitors (53). However, given the central role of TGF-β in many biological processes, including immune homeostasis (21, 22), systemic blockade of TGF-β signaling may potentially lead to a variety of side effects. Therefore, the specific blockade of TGF-β signaling in TCR-transduced T cells, that is, with a dnTGFβRII, seems preferable. This study shows the feasibility, safety, and efficacy of such a cell–targeted approach and demonstrates that it can be used to promote successful TCR gene therapy of advanced, immunosuppressive cancers. Given the observation that a variety of human malignancies has been reported to express elevated levels of TGF-β (25, 26), blockade of TGF-β signaling in TCR-modified T cells can be viewed as a generally applicable approach to enhance the therapeutic efficacy of TCR gene therapy. However, one note of caution is that in transgenic mouse models in which a

### FIGURE 4

Durable regression of invasive prostate carcinoma observed in TRAMP mice by combined TCR gene therapy and blockade of TGF-β signaling is sustained. (A) Immunohistochemistry scoring showing reduced SV40 large T, Ki67, and TGF-β1 expression in the prostate of recipients of SV40IV-TCR/dnTGFβRII–cotransduced T cells at 40 wk of age. Symbols represent individual mice; bars indicate group averages. *p < 0.05; **p < 0.01. (B) Grade of tumor regression in 40-wk-old TRAMP mice that were recipients of either non-Td T cells, SV40IV-TCR–transduced T cells, or SV40IV-TCR/dnTGFβRII–cotransduced T cells. Symbols represent individual mice; filled triangles represent mice receiving non-Td T cells, and open triangles represent mice that did not receive an adoptive transfer of T cells.

### FIGURE 5

Durable regression of invasive prostate carcinoma observed in TRAMP mice receiving a combination of TCR gene therapy and blockade of TGF-β signaling results in enhanced survival. (A) Characterization of non-Td T cells, SV40IV-TCR–transduced T cells, and SV40IV-TCR/dnTGFβRII–cotransduced T cells before adoptive transfer. Dot plots show live-gated lymphocytes. Histograms show live-gated CD8+Kb-SV40404–411 multimer+ cells. (B) Kaplan–Meier survival plot for recipients of non-Td T cells (n = 7; 1 censored event due to lymphoma), SV40IV-TCR–transduced T cells (n = 4; 1 censored event due to hepatocarcinoma in the absence of signs for regression of TRAMP lesions), and SV40IV-TCR/dnTGFβRII–cotransduced T cells (n = 5; 3 censored events due to lymphoma; 4 of 5 mice showed complete regression of TRAMP lesions). SV40IV-TCR/dnTGFβRII–cotransduced versus non-Td, p = 0.001. SV40IV-TCR/dnTGFβRII–cotransduced versus SV40IV-TCR–transduced T, p = 0.025. SV40IV-TCR–transduced versus non-Td, p = 0.98.
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dominant-negative TGF-β receptor transgene is expressed under control of the CD2 or CD4 promoter, development of CD8+ T cell lymphomas has been observed (54–56). Although in these models expression of the dominant-negative TGF-β receptor already initiates during T cell development, on the basis of these data it may be prudent to design clinically applicable dominant-negative TGF-β receptor formats in which the duration of suppression of TGF-β signaling can be controlled.

On a more general note, the targeted manipulation of the activity of TCR-modified T cells by additional genetic modification appears an attractive strategy to promote the efficacy of TCR gene therapy. First, such additional modifications can easily be accommodated in current clinical protocols (57). Second, such modifications may be tailored allowing, for example, the control of gene expression using NFAT-promoter elements (57, 58), the use of small interfering RNA instead of genes (59), or the use of gene-editing strategies to permanently silence specific genes (20). Third, and probably most importantly, such a targeted approach is likely to avoid deleterious side effects associated with systemic adjuvant therapies such as cytokine supplementation (60, 61) or blockade of T cell checkpoints (62). Thus, the further exploration of combined genetic modifications to enhance the therapeutic efficacy of TCR gene therapy for the treatment of advanced cancers seems warranted.

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Disclosures

The authors have no financial conflicts of interest.

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