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Genetic engineering with T cell receptors☆

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Abstract

In the past two decades, human gene transfer research has been translated from a laboratory technology to clinical evaluation. The success of adoptive transfer of tumor-reactive lymphocytes to treat the patients with metastatic melanoma has led to new strategies to redirect normal T cells to recognize tumor antigens by genetic engineering with tumor antigen-specific T cell receptor (TCR) genes. This new strategy can generate large numbers of defined antigen-specific cells for therapeutic application. Much progress has been made to TCR gene transfer systems by optimizing gene expression and gene transfer protocols. Vector and protein modifications have enabled excellent expression of introduced TCR chains in human lymphocytes with reduced mis-pairing between the introduced and endogenous TCR chains. Initial clinical studies have demonstrated that TCR gene-engineered T cells could mediate tumor regression *in vivo*. In this review, we discuss the progress and prospects of TCR gene-engineered T cells as a therapeutic strategy for treating patients with melanoma and other cancers.

Keywords

T cell receptor; Gene therapy; Cancer immunotherapy

1. Introduction

Adoptive cellular immunotherapy (ACT), the administration of autologous or allogenic tumor-reactive T cells into patients, is a promising cancer therapy. It has been successful in achieving tumor regression in transplant-related malignancies, leukemia, and melanoma. In melanoma, this process involves the identification of lymphocytes with high tumor recognition that can be expanded *in vitro*, and administered back to patients [1]. In pioneering clinical studies at the Surgery Branch, US National Cancer Institute, it was shown that using selected tumor-reactive infiltrating lymphocytes (TIL) mediated a 50–70% response rate in patients with metastatic melanoma when combined with lymphodepleting chemotherapy and IL-2 administration [2,3]. However, the generation of such patient-specific tumor-reactive lymphocytes is time-consuming and technically demanding, limiting the number of cancer patients that receive treatment.

The success of ACT for the treatment of metastatic melanoma patients laid the foundation for the current interest in genetic engineering with T cell receptors to redirect effector T cell reactivity. Genes encoding TCRs can be isolated from tumor-reactive T cell clones and introduce into circulating lymphocytes for therapeutic application. This approach has opened

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the possibility to treat patients with a variety of cancer types other than melanoma. In addition to the generation of anti-tumor effector cells, genetic engineering of T cell also offers a strategy to introduce molecules that can augment T cell function or overcome tumor evasion mechanisms, such as adding genes encoding homeostatic or pro-inflammatory cytokines, chemokine receptors and costimulatory factors, as well as elements to silence inhibitory molecules.

During the past decade, progresses have been made to improve the efficiency of TCR gene transfer and expression with subsequent enhanced function of the TCR gene-engineered cells. The aim of this review is to discuss the development of TCR gene transfer and update the clinical application of this cell-based drug delivery.

2. TCR as a therapeutic tool

The T cell antigen receptor is the protein expressed on the surface of T lymphocytes and mediates recognition of antigenic peptides presented by major histocompatability complex (MHC) proteins. Antigen specificity is determined by the TCR heterodimer, which is composed of two-chains, either αβ or γδ. Genes encoding the TCR α and β chains are molecularly cloned from highly reactive T cells that recognize and lyse target tumor cells and can be inserted into gene transfer vectors such as, retroviral or lentiviral vectors using recombinant DNA techniques. The genetic transfer of TCR α and β chains directed against specific tumor antigens can create antigen-specific T cells from any normal T cell.

The identification of large numbers of tumor antigens has been essential to the development of TCR based immunotherapy [4]. The most widely studied antigens recognized by tumor infiltrating lymphocytes in human melanoma include; melanocyte differentiation antigens, tumor-specific antigens, and normal proteins highly over-expressed in tumors [5]. Recently, many investigators have focused on cancer testis (CT) antigens as targets for therapeutic cancer vaccines and TCR based adoptive immunotherapy. More than 110 CT antigens have been identified and these are normally expressed only in the human germ line, but are also expressed in various tumor types [6,7]. Targeting T cells to tumor-associated CT antigens might selectively eliminate tumor cells and avoid or reduce toxicity to normal tissue. Identification of suitable target antigens for TCR gene therapy will be a high priority for the coming years.

The first TCR gene transfer to human peripheral blood lymphocytes conferring anti-tumor reactivity was reported in 1999 using a melanoma-antigen specific TCR [8]. Since then several reports demonstrated that transfer of a tumor antigen-specific TCR into T cells results in an antigen-specific T cell population [9–11]. This approach bypasses the need to isolate tumor-specific T effector cells from individual cancer patients as the TCR-transduced T cells display tumor-specific recognition. It has demonstrated in vitro that TCR gene-engineered T cells secrete immunostimulatory cytokines (IFN-γ, IL-2, and TNFα) upon encounter with antigen positive tumor cells, exhibit antigen-specific cytotoxicity, and are able to expand in response to the antigen stimulation [11,12]. In early clinical studies, circulating lymphocytes from cancer patients were engineering with TCRs against melanocyte differentiation antigens MART-1 and gp100 and the adoptive transfer of these cells into the lymphodepleted patients was shown to mediate cancer regression [13,14]. The clinical success of adoptive cell transfer therapy of TCR engineered T cells offers the possibility to treat patients who do not have natural tumor-reactive T cells.

2.1. Improving transgenic T cell receptor gene expression

It is known that TCR function depends on the expression level of the α and β TCR chains. Initial TCR gene transfer studies generated small numbers of effector T cells with poor

function most likely caused by low-level expression of the introduced TCR chains. Much progress has been made to improve the TCR gene transfer efficacy by optimizing the transduction protocol and by modifying TCR genes to improve specific chain pairing and expression (Fig. 1).

One of the potential problems in ectopic expression of tumor-specific TCR is the formation of mixed dimmers between endogenous and introduced TCR chains. Theoretically, co-expression of two α and two β chains could form four different TCRs: (i) the endogenous αβ heterodimer; (ii) the introduced αβ heterodimer; (iii) the introduced α chain paired with endogenous β chain; and (iv) the endogenous α chain paired with the introduced β chain. The mixed pairing of TCR chains will not target tumor antigens and, in theory, could result in T cells with novel reactivity that may be deleterious to the host. Though there is no evidence of toxicity or autoimmunity in reported clinical trials, several strategies have been developed to prevent mixed dimer formation while increasing TCR expression.

The first strategy to lessen mixed dimmer formation was the development of murine-human hybrid TCRs in which the constant region of the human TCR chains was replaced by their murine counterparts [15]. Compared with human TCR, the hybrid TCR exhibited enhanced expression leading to superior function of the engineered T cells including higher levels of cytokine release and cytolytic activity [15]. Most significantly, the chimeric TCR chains preferentially associated with themselves and not the endogenous TCR chains. Another approach to increase the specific pairing of TCR chains was to introduce additional cysteine residues within the constant region of the TCR α and β chains [16,17]. The additional disulfide bond formed by the extra cysteine residues was demonstrated to enhance TCR expression and therefore improve the functional activity. Alternatively, mutational inversions of the critical interacting amino acids in the TCR α and β chain constant regions favor the pairing of the introduced chains and also increase TCR reactivity [17]. As an alternative to protein engineering, Okamoto et al. have reported that small interfering RNA (siRNA) can be used to specifically down-regulate the endogenous TCR resulting in improved expression and reactivity of the transduced TCR [18]. Finally, a non-molecular approach to avoid mis-pairing is to use γδ T cells for αβ TCR gene engineering, however, the function and persistence of γδ T cells in adoptive cell therapy are not well studied.

The most critical property for a TCR used for engineering T cells for clinical application is the requirement for high affinity specific recognition of the tumor antigen peptide-HLA complex. Producing T cells with high functional avidity can be achieved by increasing the TCR affinity or increasing the expression of the TCR on the T cell surface. Several methods have been published recently to increase TCR affinity by modification of TCR genes. It is known that the third complementarity determining region (CDR3) of the TCR is critical for antigen recognition and binding. Single or dual amino acid substitutions in the CDR α or β chains have been shown to provide modest increase in TCR affinity that can enhance antigen-specific reactivity in T cells [12,19–21]. Some reports suggested that the removal of defined N-glycosylation motifs in the constant domains of TCR chains resulted in increased functional avidity and enhanced recognition of tumor cells [22]. At the level of mRNA, it has been demonstrated that improved expression of TCR genes on the T cell surface can be achieved by codon optimization [23]. In this approach rare codons used by the wild type human or murine derived TCR are replaced by those codons most frequently distributed in highly expressed human genes. During the optimization process cis-acting AT or GC rich sequence stretches, cryptic splicing and RNA instability motifs are also removed. An alternative biological strategy to generate high avidity TCRs is to immunize human-HLA-transgenic mice with human tumor antigen peptides. Murine CTL generated by this method is used to isolate murine TCR genes specific for HLA-restricted human peptides. This approach has been shown feasible for peptides from the human gp100 [13], p53 [24], CEA

[20] and MAGE-A3 [12]. It has been reported that some patients treated with murine TCR gene-engineered T cells developed antibodies directed to murine TCR variable regions. However, the development of patient immune response did not affect the clinical outcome [25]. One potential concern regarding the generation of very high affinity TCRs, is the observed lack of peptide-specificity in some ultra-high affinity TCRs and, it is not clear what is the affinity threshold for optimal TCR function [26].

2.2. TCR gene delivery systems

The success of TCR gene transfer is built on the gene delivery system. It has been demonstrated that getting high efficiency and stable gene transfer in T cell using traditional chemical methods is difficult. To date, most of the reported TCR gene transfer studies are based on viral vector-based expression system. Gamma-retroviral vectors have been used as gene transfer reagents in human application for more than 20 years, including MFG/SFG-, MP71/SF91-, and MSGV1-based vector systems [27–29]. These vectors have been used in several TCR clinical studies in which they were shown to have good transduction efficiency and no vector-associated toxicity has been reported to date. Alternatives to gamma-retroviral vectors are lentiviral vectors' expression systems. Lentiviral vectors can transduce non-dividing or minimal stimulated T cells, which may be beneficial as less-differentiated or naïve T cells have been reported to be superior for adoptive immunotherapy in animal models [30,31]. It is also reported that lentiviral vectors offer a larger gene insertion capacity and may be less prone to insertional mutagenesis due to their random integration unlike gamma-retroviral vectors that have preferential integration near the transcription start sites of genes [32]. Transposon-based non-viral gene delivery systems, such as Sleeping Beauty and PiggyBac, also have random integration profiles with acceptable gene transfer efficiency and are under development for potential clinical application [33–35]. It has been reported that electroporation/nucleofection yielded good gene transfer with RNA-based expression systems [36]. Although the short half-life of RNA expression post-transfer may limit its clinical application, RNA-based therapy would eliminate the safety concern of gene transfer caused by genome integration [37].

Design of the optimal TCR expression cassette is critical for efficient expression in viral vectors. In the early studies, TCR α and β chains were assembled in different configurations in retroviral vectors either alone or together. Initial bicistronic vectors were generated with dual promoters or the chains were linked with IRES sequences [9]. The gene inserted downstream of the IRES is expressed poorly compared to the gene positioned upstream, leading to suboptimal expression of the TCR chains and poor T cell reactivity. More recent vectors are constructed using picornavirus ribosomal skip peptides to link TCR α and β chain expression and this affords optimal stoichiometric expression of the TCR chains [13,30].

3. TCR gene transfer clinical studies

The first human clinical trial of melanoma TCR gene therapy was reported in 2006 from our group [14]. In this trial HLA-A2 positive metastatic melanoma patients were treated with retrovirally transduced autologous PBL expressing a TCR against the MART-1:27–35 epitope. Fifteen patients were treated with MART-1 TCR gene-engineered T cells plus IL-2 after nonmyeloablative chemotherapy and we observed durable tumor regression in 2 out of 15 patients [14]. This study provided the first proof-of-principle for this novel genetic immunotherapy involving TCR modified T cells. In an attempt to improve the efficacy of TCR based therapy, efforts were made to isolate higher avidity TCRs [13]. In a second reported TCR gene therapy trial targeting either MART-1 or gp100, objective cancer regression was observed in 6 of 20 (30%) and 3 of 16 (19%) patients respectively. However, patients also exhibited destruction of normal melanocytes in the skin, eye, and ear and

required local steroid administration. This trial revealed that T cells expressing highly reactive TCRs might target cognate-antigen expression on the normal cells and cause on-target/off-tumor toxicity [13].

The approach to redirect effector cells using genetically modified T cells with tumor-specific T cell receptors (TCR) has opened the possibility to treat patients with a variety of cancer types other than melanoma. The first clinical trial utilizing lymphocytes transduced with a TCR targeting carcinoembryonic antigen (CEA) to treat metastatic colorectal cancer was reported by Parkhurst et al. recently [38]. CEA is a 180-kDa tumor-associated glycoprotein over-expressed in many epithelial cancers, most notably in colorectal adenocarcinoma. As reported by Parkhurst et al., three patients with metastatic colorectal cancer were treated; all patients experienced a decrease in serum CEA levels (74–99%), and one experienced a measurable response. Severe transient colitis was also observed in the patients presumptively due to targeting CEA, which is also expressed in normal intestinal epithelial cells [38]. This is another example of how targeting self-antigens with a high affinity TCR can mediate cancer regression, but can also result in recognition and destruction of normal tissue(s), which may be a limitation to this treatment.

The observation of on-target/off-tumor toxicities suggests that ideally, future TCR gene therapies should choose antigens that are only expressed by the tumor or nonessential organs. Cancer testis (CT) antigens are expressed in a wide variety of epithelial cancers including melanoma, and carcinomas of the bladder, liver, and lung [6,7]. Their expression is restricted in normal adult tissues to the testes, whose cells do not express HLA molecules, and are thus not susceptible to recognition by a TCR. The first clinical trial using adoptive transfer of autologous lymphocytes genetically engineered to express a TCR against CT antigen-NY-ESO-1 has recently been conducted at Surgery Branch, NCI. In this trial reported by Robbins et al., there was a measurable response rate in synovial cell sarcoma patients of 66% (4/6) and in melanoma patients of 45% (5/11) with two melanoma patients being ongoing complete responders [39]. In contrast to the vigorous on-target/off-tumor toxicity seen using TCRs targeting melanoma differentiation antigens and in the CEA TCR trial, none of the patients who received NY-ESO-1 TCR gene-modified T cells experienced toxicity related to the infused cells. These objective regressions with the concomitant lack of toxicity exemplify the use of CT antigens as targets in adoptive cell therapy to treat established solid tumors without damage to normal tissues. Other CT antigens, such as MAGE-A3 [12], are currently under clinical investigation (Table 1).

These encouraging results point to the feasibility of transferring T cells engineered with TCR to treat solid tumors. However, the durable objective response rate of this approach is generally lower than the rate seen in TIL therapy. Several reasons could contribute to this including the fact that antigen targets may not be the same as TIL. Phenotype of the PBL for used TCR engineering may also be different from that of the TIL in terms of the expression of homing and other molecules necessary for the efficient tumor trafficking and/or effector function. Alternatively the immune response by the TIL against the tumor may be of polyclonal nature; targeting a variety of tumor antigens.

4. Strategies to improve TCR gene therapy

Though highly effective T cells can be generated against the desired tumor antigens *ex vivo*, the environment that these cells encounter at the tumor site can be a major determining factor in the outcome of therapy (Fig. 2). It has been shown in some cases that normal tumor growth proceeds despite high levels of circulating anti-tumor CD8 cells [40]. The mechanism of the local suppression of effector T cell function at the tumor is not fully understood. Tumors display multiple immune suppressive mechanisms, including the local

presence of inhibitory cytokines such as IL-10 [41], TGF-β [42–44], recruitment of regulatory T cells [45] and myeloid derived suppressor cell [46–48], or the expression of ligands for inhibitory molecules on T cells such as PD-1 [49,50] and CTLA-4 [51–53].

Since the first report of the use of TCR engineered lymphocytes in human in 2006, efforts have been made to improve treatment efficacy of adoptive cell therapy. Strategies to address some of these issues are already incorporated in the current clinical trials involving TCR modified T cells, for example, lymphodepletion of the host increases the effectiveness of cell transfer therapy by eliminating cellular elements like regulatory T cells and myeloid derived suppressor cells and also by removing the lymphocyte pool in the body that might compete for available growth factors or cytokines [54]. In an attempt to blunt the inhibitory molecules expressed on the tumor-active T cell, strategies like the expression of inhibitory RNAs designed to suppress the expression of PD-1 or CTLA-4 may potentially improve the functionality of TCR engineered lymphocytes [55,56]. As reported recently, anti-CTLA-4 antibody (ipilimumab), with or without a gp100 vaccine, improves survival in patients with metastatic melanoma [57]. T cells can undergo apoptosis after infusion due to the lack of growth factors or co-stimulation. Survival of tumor-specific T cells may be enhanced by engineering them to co-express anti-apoptotic genes like Bcl-2 [58,59]. To block TGF-b signaling, T cells were modified to express a dominant-negative or soluble form of the TGF-β receptor, which resulted in the improvement of TCR transfer therapy in several mouse models [60–62].

Cytokine interleukin 12 (IL-12) was recognized as an important regulator of cell-mediated immunity, potentially beneficial for the treatment of infectious and malignant diseases by enhancing the cytotoxic activity of NK cells and cytotoxic T lymphocytes (CTL) [63,64], and mediating the differentiation of naïve $CD4^+$ T cells to Th1 cells [65,66]. The anti-tumor activity of recombinant IL-12 was tested in a variety of murine tumor models where it caused tumor regression and prolonged the survival of tumor-bearing animals [67–70]. However, its clinical application has been hindered by the toxicity associated with its system administration. Kerkar et al. recently demonstrated that co-expression of IL-12 in TCR gene-engineered lymphocytes dramatically augmented tumor treatment efficacy of adoptive transfer in the B16 murine melanoma model without need the administration of IL-2 and vaccine [71]. To further limit potential toxicity related to constitutive express of IL-12, an NFAT responsive promoter was used to control human IL-12 expression at the tumor site. T cells genetically modified with a tumor-specific TCR and NFAT-IL12 demonstrated powerful therapeutic efficacy in mouse B16 tumor model [72]. Based on these results, a phase I/II clinical study using the administration of NFAT-IL12 vector-transduced TIL to patients with metastatic melanoma is in progress.

5. Summary

Recent TCR based clinical trials in melanoma patients have highlighted the promise as well as the challenges facing this therapy. Various factors will contribute towards the clinical efficacy of TCR gene therapy including, the affinity of the transgenic TCR, the maintenance of TCR gene expression over time, and the *in vivo* persistence of the TCR engineered T cells. Lessons learned from the clinical response of patients treated by TCR gene therapy demonstrated the importance of choice the tumor antigen in order to limit off-tumor/on-target toxicity. The identification of best subset of T cell to engineer and development of novel in vitro expansion protocols, such as using artificial APC, may benefit cell persistence *in vivo* [73]. These approaches to enhance TCR expression and recognition of tumor antigens may need to be coupled with mechanisms designed to overcome inhibitory elements within the tumor microenvironment. Alternative strategies to improve the adoptive cell therapy could be to co-administer TCR gene-engineered T cells with antibodies that

block T cell negative regulatory activity, such as anti-CTLA-4 or anti-PD-1. Based on preliminary clinical results, it is likely that this therapy will continue to improve and potentially be of benefit to patients with a variety of cancers.

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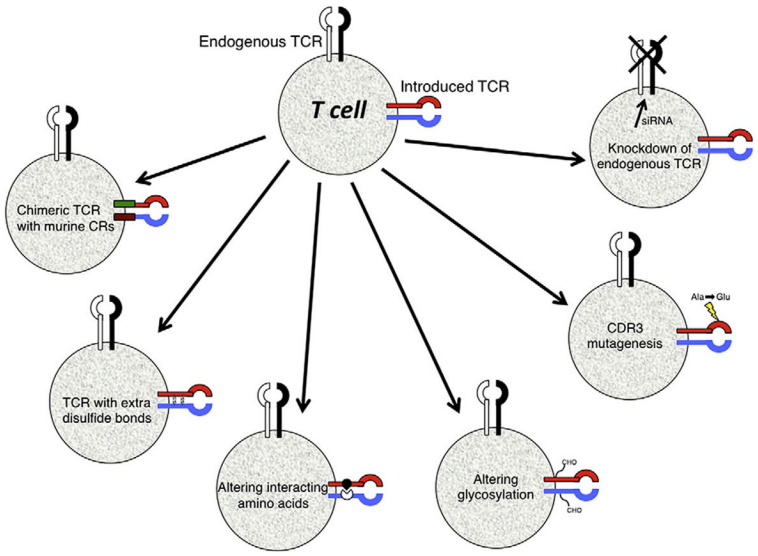

Fig. 1.

Strategies to improve TCR activity. Shown are the varieties of different strategies that can be used to potentially improve TCR activity (see text for details). These methods include creating hybrid molecules containing the constant region (CR) from murine TCR chains, as well as adding extra disulfide bonds, and altering amino acids or glycosylation sites within the TCR chains. Mutagenesis of amino acids in the complementarity determining region 3 (CDR3) and knocking down endogenous TCR gene expression using short interfering RNAs (siRNA) can also increase the overall reactivity of the introduced TCR.

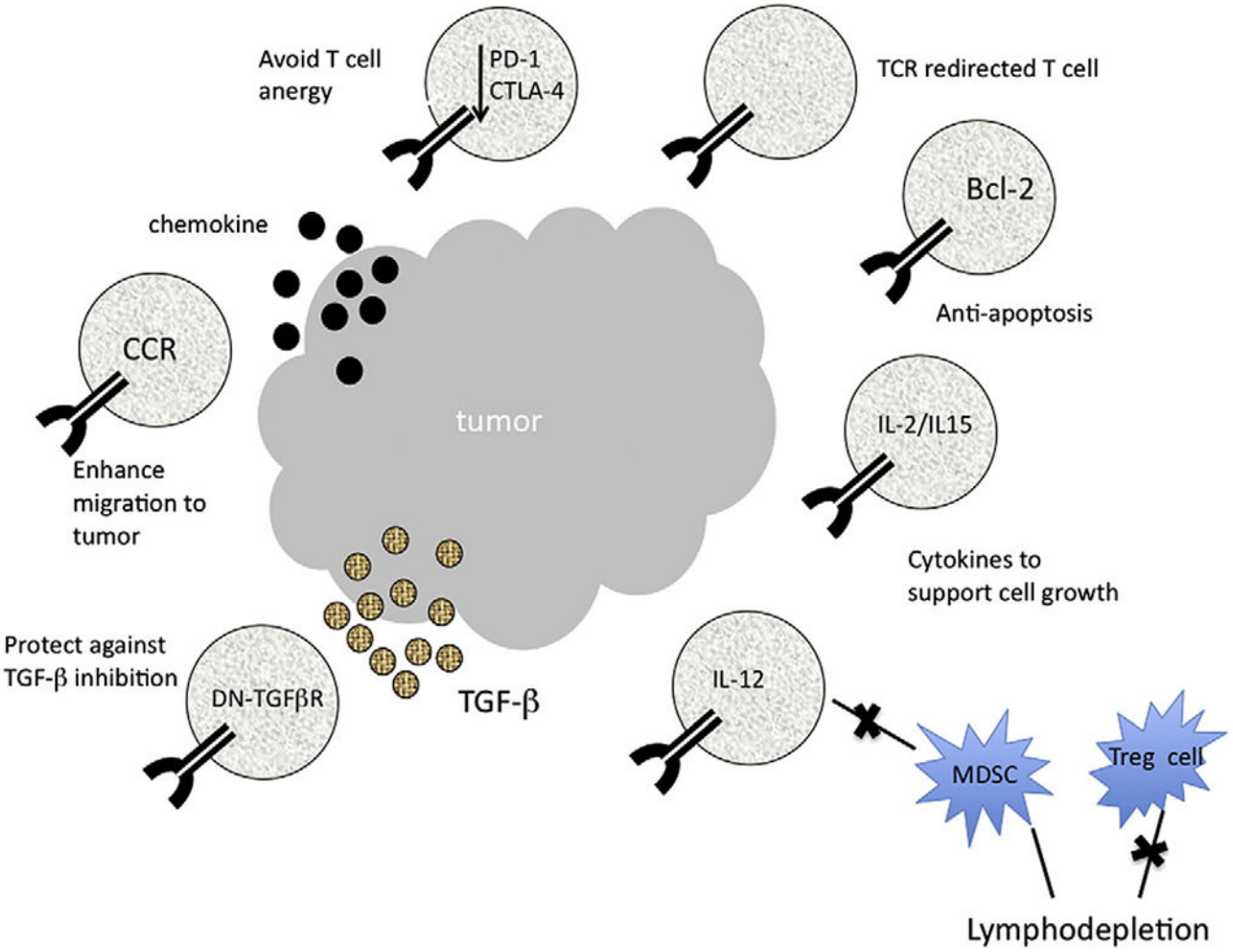

Fig. 2.

Strategies for improving ACT to treat human cancer. Shown are the potential ways to facilitate engraftment, persistence, and activity of adoptively transferred TCR gene-modified cells (see text for details). To potentially lessen T cell anergy, antibodies to CTLA-4 or PD-1 could be co-administered with TCR gene-engineered T cells. In addition TCR gene engineering, T cells could be further modified to resist apoptosis, to preferentially migrate to tumors, or to be resistant to inhibitory factors at the tumor environment and to deliver inflammatory cytokines to tumor site. DN-TGFβR indicates dominant-negative TGFβ receptor; CCR, chemokine receptor; MDSC, myeloid-derived suppressor cells; Treg, T regulatory cell.

Table 1

TCR gene therapy trials registered in the United States.
Data from: ClinicalTrial.gov 08/04/2011.

Disease	TCR-target	Lymphodepletion	Gene transfer	ClinicalTrial.gov identifier
Melanoma	MART-1	Y	RTV	NCT00509288 NCT00910650
Melanoma	gp100	Y	RTV	NCT00509496
Renal cancer	DR4-TRAIL	Y	RTV	NCT00923390
p53 expressing metastatic cancer	p53	Y	RTV	NCT00393029
HIV infection	HIV-Gag	N	LTV	NCT00991224
CEA expressing metastatic cancer	CEA	Y	RTV	NCT00923806
NY-ESO-1 expressing metastatic cancer	NYE-ESO-1	Y	RTV	NCT00670748
MAGE-A3.12 expressing metastatic cancer	MAGE-A3/12	Y	RTV	NCT01273181
Melanoma	NY-ESO-1/MAGE-A3	Y	LTV	NCT01350401
Myeloma	MAGE-A3/NY-ESO	N	LTV	NCT01352286

Abbreviation: RTV, retrovirus; LTV, lentivirus.