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Treating Cancer with Genetically Engineered T Cells

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Abstract

Administration of *ex-vivo* cultured, naturally occurring tumor-infiltrating lymphocytes (TILs) have been shown to mediate durable regression of melanoma tumors. However, the generation of TIL is not possible in all patients and there has been limited success in generating TIL in other cancers. Advances in genetic engineering have overcome these limitations by introducing tumor-antigen-targeting receptors into human T lymphocytes. Physicians can now genetically engineer lymphocytes to express highly active T-cell receptors (TCRs) or chimeric antigen receptors (CARs) targeting a variety of tumor antigens expressed in cancer patients. In this review we discuss the development of TCR and CAR gene transfer technology and the expansion of these therapies into different cancers with the recent demonstration of the clinical efficacy of these treatments.

Introduction

The ability of lymphocytes to eradicate tumor cells in cancer patients has been demonstrated in metastatic melanoma where the T cell cytokine interleukin-2 (IL-2, aldesleukin), now an FDA-approved therapy, can mediate measurable responses in 15% of patients treated^{1, 2}. The immunogenic nature of melanoma tumors has served as the foundation for the development of other immune-based therapies for the treatment of this and other cancers. Non-specific immune stimulation with interleukin-2 and anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA4, Ipilimumab) antibody leads to the activation of anti-tumor lymphocytes *in vivo*, and has been shown to mediate tumor regression in metastatic melanoma and renal cell cancer³. Currently the most effective immune-based therapy for melanoma is adoptive cell therapy involving the generation of T lymphocytes with anti-tumor activity. When these TILs are infused into patients along with IL-2 and reduced-intensity chemotherapy to temporarily knock-down the patient's circulating immune cells, TIL can mediate tumor responses in up to 70% of patients, with a significant portion of these being durable complete responses (defined as the disappearance of all target lesions)⁴.

The protein that the T cell utilizes to identify foreign epitopes (or in the case of TIL, tumor antigens) is the T-cell receptor. The TCR is a member of the immunoglobulin gene super family and is a heterodimer composed of an alpha and beta chain. TCR genes can be isolated from tumor reactive T cell clones, (clones which mediated clinical responses), inserted into gene transfer vectors, and used to genetically engineer normal T lymphocytes to re-direct them with antitumor specificity. These genetically engineered T cells were shown to result in objective responses in a small number of metastatic melanoma patients in 2006⁵. Progress in the ability to mediate responses with the above immune based therapies in metastatic

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melanoma had prompted the translation of these therapies to treat cancers of other tissues and organs. Recently, a series of new clinical trials have shown measurable responses can be achieved using gene modified T cells in cancers other than melanoma including; colorectal cancer, lymphoma, neuroblastoma, and synovial sarcoma^{6–10}. In this review we will discuss the development of T cell genetic engineering, then discuss two specific gene modifications, and conclude with the clinical applications of these biotechnologies.

Initial studies using natural anti-tumor T cell therapy

Adoptive immunotherapy using the transfer of viral antigen-specific T cells is a now a well-established procedure resulting in effective treatment of transplant-associated viral infections and rare viral-related malignancies. In these approaches, allogeneic peripheral blood lymphocytes (PBL) are first isolated from the bone marrow donor. PBL with reactivity to human cytomegalovirus (CMV) or Epstein-Barr virus (EBV) are isolated and expanded and then intravenously infused into patients receiving allogeneic hematopoietic stem cell transplantation¹¹ in order to treat post-transplant viral infections. The direct targeting of human tumors using autologous tumor infiltrating lymphocytes was first demonstrated to mediate tumor regression in 1988, though these results were modest and often not durable¹². A significant improvement in the response rate and durability of response occurred with the addition of a preconditioning regimen with lymphocyte-depleting chemotherapy, increasing the measurable response rate to up to 50% with durable responses in patients rendered disease free.⁴ The addition of whole body irradiation to further condition the patient, improved these results with measurable responses as high as 70 percent with a 32 percent complete response rate, the majority of these being durable to >3 years.

Limitations of TIL therapy include the requirement for surgery to isolate the tumor, as well as, the ability to consistently generate T cells with antitumor activity. This latter point may be overcome with recent trials utilizing “young TIL” where the lymphocytes are grown briefly and introduced into patients without testing for reactivity¹³. In these trials, the response rate was comparable to conventional TIL.

Development of engineered T cells: T cell receptor gene transfer

As an alternative to TIL therapy, highly avid TCRs can be cloned from naturally occurring T cells and, by using gene transfer vectors, introduced into patient's lymphocytes, creating the opportunity to generate large quantities of antigen specific T cells for treatment (Figure 1)^{14, 15}. The first step in TCR gene therapy is to isolate a high affinity T cell clone for a defined target antigen. These TCRs can be isolated from patients with rare, highly reactive T cell clones that recognize and lyse target tumor cells¹⁶. The isolation of these rare tumor reactive T cell clones is often the rate-limiting step in this procedure and these clones often have low affinity for the target antigen.

One of the most important applications of biotechnology to human immunology has been the development transgenic mice, which are engineered with human immune system genes. Transgenic mice containing the human leukocyte antigen system (HLA) can be used to generate TCRs against human antigens. This is done by immunizing HLA transgenic mice with human-specific antigenic peptides, and isolating the resultant mouse T cells, which will contain a TCR that recognizes a human peptide. Using this approach, investigators have been able to generate multiple murine TCRs against a variety of human tumor antigens from different histologies^{17, 18}. Another method that does not require patient material to obtain a tumor antigen reactive TCR, is the use of phage display technology for TCR isolation. Phage display technology has the advantage that it does not depend on the ability to generate T cell clones yet allows for the selection of high-affinity TCRs reactive against a variety of

antigens^{19, 20}. One potential drawback to TCRs isolated by phage display is that caution must be exercised in the selection of very high-affinity TCRs, which have been shown to lose specificity²¹. In theory, these nonhuman TCR isolation technologies create the possibility to provide the patient with a tailored therapy based on their unique antigen expression pattern, potentially ushering in a new era of personalized cancer immunotherapy.

With either method, after the high-avidity T-cell clone is obtained, the TCR alpha and beta chains are isolated and cloned into a gene expression vector (Figure 2). To assure co-expression of both chains, the TCR alpha and beta genes are most commonly linked via a picornavirus 2A ribosomal skip peptide²². For human applications, gene transfer platforms that can mediate stable gene transfer are the systems of choice (e.g., gamma-retroviral, lentiviral vectors, or transposons)^{23–25}. The two viral based systems are complex biologic reagents that require extensive safety testing for human applications but they mediate very high gene transfer efficiencies and have been used for over two decades in human studies. Transposons are a relative newcomer in the human gene therapy field and have the advantage that they are plasmid DNA-based, are much simpler to produce, and require less upfront safety testing. Ex vivo gene transfer is accomplished by first stimulating T cell growth and the activated cells are then transduced and expanded in culture to numbers sufficient for clinical applications (generally $> 1 \times 10^8$ cells).

The genetic transfer of an antigen-specific TCR can generate antigen-specific T cells from any naturally occurring T cell. It has been shown that the transduced lymphocytes exhibit the specificity of the parental clone^{26, 27}. These TCR gene engineered T cells can secrete cytokines upon encountering tumor antigen positive targets, exhibit tumor cell specific lysis, and expand upon antigenic stimulation.

Unlike antibodies, the affinity of many naturally occurring TCRs for their target peptide is low (in the μ molar range), and therefore, steps to improve the performance of TCRs through protein engineering have been made. These include strategies to improve TCR affinity, increase cell surface expression, and prevent mixed dimer formation between the introduced and endogenous TCR chains (such mixed dimers would not target the tumor antigen)²⁸. Single or dual amino acid substitutions in the complementary determining region (CDR) of the alpha or beta chain has been shown to improve antigen specific reactivity in T cells²⁹. Development of hybrid TCRs where the human constant region is replaced by a murine constant region has been shown to improve specific chain pairing as well as facilitate stronger association with T cell signaling proteins of the CD3 complex. T cells engineered with these hybrid TCRs exhibit superior surface expression, cytokine release and cytolytic activity^{30–32}. Introduction of an additional cysteine bridge in the constant region of the TCR alpha and beta chains was also shown to improve pairing^{32, 33}. Inverse exchange of an amino acid pair at the interface the TCR alpha or beta constant regions that normally forms a “knob-into-hole” configuration into a “hole-into-knob”, has been shown to favor selective assembly of the introduced TCR with preserved function of the receptors³⁴. In addition, it is possible to produce a chimeric molecule by fusing the CD3zeta gene to the TCR alpha and beta chains, and in cell lines engineered with these chimeric molecules, specific alpha-beta chain pairing was reported³⁵.

An alternative non-genetic approach is to use $\gamma\delta$ T cells for adoptive therapy, in which $\alpha\beta$ heterodimers can be introduced without the concern for heterogenous pairing. However, whether $\gamma\delta$ T cells will function and persist as well as in $\alpha\beta$ T cells in the setting of adoptive T cell therapy is still under investigation^{36, 37}. All of these modifications have the potential to increase the anti-tumor activity of the engineered T cells. The main advantage of using TCRs to target tumors is that they function through well understood T cell signaling pathways and are the natural means by which the body clears foreign elements. The main

disadvantage of TCR-based anti-cancer therapies is that the biology of the TCR restricts it to one HLA type and alpha/beta TCRs cannot target non- protein tumor antigens (i.e., carbohydrate or lipid antigens).

Development of Engineered T cells: Chimeric Antigen Receptors

Redirection of T cell specificity by TCRs is constrained by HLA restriction, which limits the applicability of TCR therapy to patients who express the particular HLA type (similar to organ or bone marrow transplantation). In addition, tumors can lose their antigen expression by down regulation of HLA³⁸. Chimeric antigen receptors (CAR) can avoid these limitations as they can confer non-HLA restricted specificity to a T cell based on antibody recognition. CARs consist of a tumor antigen-binding domain of a single-chain antibody (scFv) fused to intracellular signaling domains capable of activating T cells upon antigen stimulation, a concept first reported by Eshhar and colleagues in 1989 (Figure 2)³⁹.

CARs generally incorporate the scFv from a murine monoclonal antibody as the antigen-targeting domain. This is fused to a protein spacer element followed by a transmembrane spanning domain and intracellular signaling elements^{40, 41}. Thus the CAR protein contains both tumor antigen recognition domains and T cell signal domains in the same hybrid molecule. The design of CARs has evolved over the decades since their first description with the goal of enhancing T cell signaling functions. In the first generation CARs intracellular signaling domains were based on the CD3zeta, and conferred the engineered T cells the ability to secrete cytokine and mediate lysis of target cells. The second generation of CARs incorporated another intracellular domain, usually from T cell costimulatory molecules such as CD28, resulting in enhanced cell proliferation upon contact with target antigen in addition to cytokine release and lysis. Third generation CARs incorporate additional signaling domains (i.e., 41BB or OX40) to further improve effector function and survival.

Antigen selection for CAR therapy includes the requirement of the antigen to be expressed on the cell surface (a disadvantage in comparison to TCRs, which can recognize both intracellular and extracellular processed peptides). In addition to proteins, CARs can recognize nonprotein surface molecules such as carbohydrates and glycolipids, which can also be uniquely associated with tumors. As many of the antibodies used for CAR design are murine monoclonal antibodies, it is not surprising that human anti-mouse antibody immune responses have been reported, and this may potentially limit their long-term clinical use^{42, 43}. In general, CARs have been shown to be extremely robust anti-tumor reagents and because the number of anti-tumor antigen antibodies far exceeds the number of known anti-tumor TCRs, CARs will likely be the main platform for anti-cancer T cell engineering.

Clinical trials using engineered T Cells

As first documented in melanoma, genetically engineered T cells can recognize and destroy large established tumors in cancer patients, an example of this is shown in figure 3 (this particular patient had a complete elimination of melanoma tumors and remains disease free >four years post-treatment). Recently, several clinical trials have been reported documenting the clinical efficacy of gene modified T cell for the treatment of other cancers (Table 1). These trials used both TCR and CAR engineered T cells and have shown clinical benefit in several different cancer histologies including melanoma, colorectal cancer, synovial cell cancer, neuroblastoma, and lymphoma.

Carcinoembryonic antigen (CEA) TCR trial

Carcinoembryonic antigen (CEA) is a 180-kDa tumor-associated glycoprotein over expressed in many epithelial cancers, most notably in colorectal adenocarcinoma. The first clinical trial utilizing lymphocytes transduced with a TCR specific for CEA was recently reported⁹. The anti-CEA TCR was raised in HLA transgenic mice against a CEA peptide, and TCR reactivity was enhanced by introducing a single amino acid substitution in the CDR3 region of the α chain¹⁷. 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 response⁹. Severe transient colitis was also observed in the patients presumptively due to targeting CEA, which is also expressed in normal intestinal epithelial cells. The development of on-target/off-tumor toxicity was previously reported in targeting melanocyte differentiation antigens and in a CAR-based kidney cancer trial^{44, 45}. The severe intermittent inflammatory colitis observed in this trial represented a dose limiting toxicity, though the colitis resolved in all three patients. This is the first report of cancer regression in a solid organ tumor other than melanoma using adoptive cell therapy with TCR-gene modified lymphocytes. Additionally, this is another example of how targeting self-antigens with highly active T cell therapy can mediate cancer regression but the ability of these lymphocytes to recognize normal tissue(s) can be a limitation to treatment.

NY-ESO-1 TCR trial

In light of these on-target/off-tumor toxicities, many investigators have been focusing on cancer testis (CT) antigens as a target for adoptive cell therapy. More than 110 CT antigens have been identified⁴⁶. These antigens are expressed in the germ line but also in various tumor types, including melanoma, and carcinomas of bladder, liver, and lung. While CT antigens are expressed in a wide variety of epithelial cancers, their expression is restricted in normal adult tissues to the testes, whose cells do not express HLA molecules, and are thus not susceptible to damage by a TCR. In vitro examples of TCR gene therapy approaches targeting CT antigens include studies directed against the NY-ESO-1 and MAGE-A proteins^{47, 48}. The first clinical studies targeting NY-ESO-1 using TCR gene therapy have now been reported¹⁰.

The NY-ESO-1 antigen is expressed in 10 to 50% of metastatic melanomas, breast, prostate, thyroid, and ovarian cancers^{49–51}. Of note, NY-ESO-1 is expressed in 80% of synovial cell sarcoma patients⁵². 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 reported. The TCR used in this report was also an affinity modified TCR in that it contained two amino acid substitutions in CDR3 that conferred to T cells, enhanced ability to recognize target cells expressing the NY-ESO-1 antigen²⁹. In this trial reported by Robbins *et al*, there was a measurable response rate in synovial cell cancer patients of 66% (4/6) and in melanoma patients of 45% (5/11) with two melanoma patients being ongoing complete responders¹⁰. In contrast to the vigorous on-target/off-tumor toxicity seen in the melanoma antigen TCR and the CEA TCR trials, none of the patients who received NY-ESO-1 specific T cells experienced toxicity. These objective regressions with the concomitant lack of toxicity exemplify the use of CT antigens as targets in adoptive cell therapy to mediate the regression of established tumors without damage to normal tissues. In addition this trial, along with the CEA TCR trial, is amongst the first reports of cancer regression in a solid organ tumor other than melanoma using adoptive cell therapy with TCR gene-modified lymphocytes.

Potential for Graft vs. Host Disease in TCR Gene Therapy Trials

There had been a report of a high incidence of lethal graft vs. host disease (GVHD) in mice receiving a lymphodepleting regimen followed by syngeneic cells transduced with genes encoding TCRs. The GVHD was manifested as cachexia, anemia, loss of hematopoietic reconstitution, pancreatitis, colitis, and death. The authors demonstrated that this was due to the formation of self-antigen-reactive mixed TCR dimers between the endogenous and introduced TCRs⁵³. Subsequently, an *in vitro* study by van Loenen *et al.* suggested that introduction of new TCRs into human lymphocytes could lead to the generation of mixed-TCR dimers with alloreactivity⁵⁴.

In contrast, in the human TCR gene trials at the National Cancer Institute, there was no evidence of GVHD in 106 patients using seven different antitumor TCRs. Each of these patients received lymphodepleting chemotherapy prior to administration of gene-transduced lymphocytes. The TCRs were of human origin in 77 patients and of mouse origin in 29 patients. Additionally, 6 more patients were treated with the lymphodepleting chemotherapy and 600cGy of whole body irradiation along with TCR-transduced cells and none of these patients exhibited any signs of GVHD. Furthermore, 44 additional patients received gene-modified lymphocytes without lymphodepletion and none of these patients exhibited signs of GVHD. The clinical course of the patients who received TCR-transduced cells was compared to 115 patients who received the adoptive transfer of autologous nontransduced TIL, and no meaningful differences were found. It thus appears that in contrast to the report of mouse models by Bendle *et al.*, humans receiving autologous T cells transduced with human or mouse TCRs did not develop evidence of GVHD⁵⁵. Because of differences in T cell growth and engraftment regimens in the mouse experiments, the possibility remains that GVHD may manifest in future human trials, stressing the importance of the ongoing evaluation and implementation of technologies that can prevent mixed dimers.

Neuroblastoma CAR trial

The first evidence of clinical response using a CAR was seen in a clinical trial utilizing a CAR against disialoganglioside GD2 for the treatment of neuroblastoma⁶. In this trial by Pule *et al.*, tumor necrosis was observed in half of the patients and there was 1 complete response out of 11 patients. Each patient was treated with two groups of cells, one group of EBV-specific CAR transduced cytotoxic T lymphocytes (CTLs), and another group of non-virus specific CAR transduced CTLs. The EBV-specific CTLs expressing the GD2 CAR demonstrated longer persistence than the CTLs expressing GD2 CAR in these neuroblastoma patients, suggesting that virus-specific memory T cells may be able to promote long-term engraftment.

Lymphoma CAR trials

The second CAR trial to report evidence of a clinical response was seen in a phase I trial using a first generation CAR against CD20⁷, which is frequently over expressed on lymphoma cells. This trial by Till *et al.*, was unique, in that it used T cells engineered by electroporation of plasmid DNA not viral vector-based gene transfer. While electroporation is technically less demanding to perform than viral vector-mediated gene transfer, the reported gene transfer efficiencies were very low and required extended cell culture to expand cells for treatment. The transferred cells were found in the circulation at very low levels post-infusion and did not persist. There was one measurable response reported, lasting 3 months, which was seen in one of seven patients.

Better success was reported in a CAR vector trial targeting CD19 in lymphoma. CD19 is expressed on malignant B cells, with normal tissue expression limited to mature B cells, B cell precursors and plasma cells^{56–58}. The attractiveness of CD19 as a target has led several

groups to pursue development of anti-CD19 CARs⁵⁹. A second generation CAR that consists of the variable regions of a mouse-anti-human CD19 antibody coupled to the signaling domains of CD28 and CD3zeta was used in a clinical trial to treat 6 patients at the NCI, and a summary of the first patient treated was recently published by Kochenderfer et al⁸. These patients were treated with lymphocyte-depleting chemotherapy followed by infusions of anti-CD19 CAR-transduced autologous T cells and high dose IL-2. There were 2 partial responses and one complete response⁶⁰. Main toxicities noted were hypotension and fever, and in two responding patients there was eradication of B-lineage cells from the bone marrow and blood. This elimination of normal B lineage cells is not attributable to the chemotherapy and indicates the CD19 CAR-specific eradication of B-lineage cells in addition to the lymphoma cells (this can be medically managed by giving patients intravenous immunoglobulin).

Adverse events in CAR trials

In addition to on-target/off-tumor toxicities reported in both TCR and CAR gene therapy clinical trials, two patient deaths have been reported in trials using CAR engineered lymphocytes. In one trial using a second generation CAR targeting CD19, one patient death was reported proximal to the infusion of the engineered T cells and was associated with elevated serum cytokine levels⁶¹. The precise cause of death in this case may have been complicated by the patients underlying disease and potential other co-morbid factors. We have also reported a patient death. In this clinical trial, CAR engineered T-cells were used in an attempt to treat cancer patients with ERBB2 over-expressing tumors using a CAR based on the humanized monoclonal antibody Trastuzumab (Herceptin)⁶². A third generation optimized CAR vector containing CD28, 4-1BB, and CD3zeta signaling domains was assembled in a gamma-retroviral vector and used to transduce autologous peripheral blood lymphocytes from a patient with colon cancer metastatic to the lungs and liver, refractory to multiple standard treatments. Soon after administration of these cells, the patient rapidly fell into respiratory distress and expired five days later. Localization of CAR transduced T cells to the lung immediately following cell infusion was thought to have triggered a cytokine storm by the recognition of low levels of ERBB2 on lung epithelial cells.

Future Directions

Active efforts are being made in the basic research arena to improve TCR and CAR function. For TCRs, site directed mutagenesis in the CDR region to improve antigen affinity and manipulations to improve TCR chain pairing are active areas of research. For CARs, second and third generation CARs with additional co-stimulatory signaling domains are being investigated for their ability to improve cell signaling, survival, and proliferation. Lessons learned from the clinical responses in patients treated with TCRs and CARs also demonstrate the importance of the choice of antigen. Active investigation is underway for the discovery of more candidate antigens, not only to increase the repertoire of different cancer histologies that can be targeted, but also to identify those antigens with selective expression to tumor and not to normal or vital tissues, which may avoid on-target/off-tumor toxicity. The CT antigens and other antigens that have limited to no expression in normal tissues (EGRFvIII) or expression limited to non-vital organs (CD19, FSH Receptor) seems to be the most reasonable candidates.

With the first report of the use TCR engineered lymphocytes in humans in 2006, significant progress has been made resulting in the demonstration of clinical efficacy in multiple tumor histologies using genetically engineered lymphocytes. With the active pursuit of TCRs and CARs targeting multiple cancer histologies, it is likely that soon the treatment of a cancer will evolve into a personalized immunotherapy targeting the antigen expression pattern unique to any cancer patient.

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Figure 1. Clinical application of gene-modified T cells. Shown is a diagram of the use of both natural (top) and gene modified T cells (bottom) for treatment of cancer.

Figure 2.

Producing anti-tumor T cells. Shown is the general schema for the construction of gene transfer reagents for the engineering of T cells with anti-tumor receptors. Step 1. Anti-tumor antigen receptor can be isolated as natural TCRs (left) or an antibody can be turned into a chimeric antigen receptor (right). Step 2. Both TCR and CARs are produced as fusion proteins to facilitate insertion into gene transfer vectors. Step 3. Gene transfer vector that afford the possibility for stable gene transfer include transposons, gamma-retroviral vectors, and lentiviral vectors. Abbreviations: V_H and V_L, immunoglobulin variable regions; 2A and G4S, linker peptides; Exo, extracellular domain; TM, transmembrane domain; IR/DR, inverted/direct repeat; pA, polyadenylation signal; LTR, long terminal repeat; SD, splice donor; SA, splice acceptor; Ψ⁺, packaging signal; sinLTR, self-inactivating LTR; RRE, rev responsive element; cPPT, central polypurine tract; WPRE, woodchuck hepatitis virus post-translation regulator element.

Figure 3.

Cancer regression using TCR gene-modified T cells. Shown is an X-ray computed tomography (CT) scan of the abdomen of a patient with metastatic melanoma before and >2 years after administration of anti-gp100 TCR gene transduced autologous T cells¹⁶. The dashed circle indicates the position of one of the patients' metastatic tumors in a pelvic lymph node. The long line-like element in the pre-treatment image is a biopsy needle. The patient continues to be disease free four years post-treatment.

Table 1

Recent Clinical Success using Gene Modified T Cells.

Cancer	Target Antigen	Gene-Vector	Comments	Reference
Neuroblastoma	GD2	CAR-RTV	Cell persistence better in viral-specific CTL	Pule et al., 2008
Indolent B-NHL and mantle cell lymphoma	CD20	CAR-EP	Successful demonstration of non-viral gene transfer	Till et al., 2008
Melanoma	MART-1	TCR-RTV	30% response rate with on-target/off – tumor toxicity	Johnson et al., 2009
Melanoma	gp100	TCR-RTV	19% response rate with on-target/off – tumor toxicity	Johnson et al., 2009
Lymphoma	CD19	CAR-RTV	Near complete response with concomitant elimination of B cells.	Kochenderfer et al., 2010
Colorectal cancer	CEA	TCR-RTV	Responses associated with on-target/off-tumor toxicity	Parkhurst et al., 2010
Synovial sarcoma and melanoma	NY-ESO-1	TCR-RTV	50% response rate with on toxicity.	Robbins et al., 2011

Abbreviations; CAR, Chimeric Antigen Receptor; TCR, T Cell Receptor; RTV, gamma-retroviral vector; EP, electroporation.