

Chapter 3

Adoptive T Cell Transfer

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Abstract The clinical use of adoptively-transferred T or NK lymphocytes with anti-tumor activity is gaining in popularity as reports of success accumulate. High specific activity with minimal off target effects contribute to a class of therapy with minimal toxicity that is transformative for cancer patients, who with their physicians have come to accept severe short and long term toxicities as the cost of a frequently small hope of cure or extension of life. However, the successes of cell therapies are as yet in the minority and scientists are developing and testing strategies to improve the function and persistence of adoptively transferred lymphocytes in the face of multiple, potent immune evasion strategies used by tumors and their accessory cells to coexist with an intact immune response. These strategies include combination with chemotherapies and/or radiation and genetic modifications that improve T cell function and tumor targeting. However many of these strategies come with a price and re-introduce toxicities including death in rare cases. This chapter will outline the development of tumor-specific lymphocyte therapies, highlighting successes and difficulties and discussing potential ways forward in this exciting field.

3.1 Introduction

Potent cytotoxicity with exquisite specificity is the *sine qua non* of novel cancer therapeutics. Nowhere in nature are these two qualities better exemplified than in the cytotoxic T lymphocyte (CTL).

Humans possess a diverse T cell repertoire with approximately 2.5×10^7 distinct T cell receptors (TCRs) recognizing unique peptide sequences or antigens¹. Each

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T cell is capable of massive clonal expansion and potent cytotoxic activity after pathogen recognition, yet the T cell response to infection is coordinated and controlled. Infected cells are efficiently destroyed with minimal damage to surrounding healthy tissues, and T cell numbers rapidly return to homeostatic levels once the infection is resolved. Tumor immunotherapy attempts to harness the power and specificity of the cellular immune response and direct or amplify it against malignant cells. Indeed, the stimulation of tumor-specific T cells is the end goal of cancer vaccines, while the ability to recruit natural killer (NK) cells is an important component of anticancer antibodies. Perhaps the most direct way to confer antitumor cellular immunity to patients is by adoptive T cell transfer, which refers to the *ex vivo* culture and subsequent infusion of autologous or donor-derived T lymphocytes. Not far behind, the use of NK cells as mediators of antitumor responses in an adoptive immunotherapy setting is gaining increased attention over the last few years owing to improvements in the understanding of their function, activation and *ex vivo* expansion.

In this chapter, we will discuss the three major adoptive T cell transfer platforms that have been used clinically, (1) Epstein–Barr virus (EBV)-specific T cells for EBV-associated malignancies, (2) tumor-infiltrating lymphocytes (TIL) for metastatic melanoma and ovarian cancer, and (3) genetically modified T cells directed against various solid and hematological malignancies, and conclude with a brief discussion focused on NK cells as an immunotherapy platform.

3.2 EBV-Associated Malignancies

One of the first challenges to adoptive T cell transfer for tumor immunotherapy is the identification of antigens that are uniquely expressed by malignant cells and can serve as suitable targets for T cells. Antigens for T cell targeting should meet several requirements. They should (1) be presented on the cell surface by human leukocyte antigen (HLA) major histocompatibility complex (MHC) antigens, (2) be expressed on malignant cells but absent or expressed at low levels on normal cells, (3) have no expression on vital organs, and (4) ideally have some essential role in supporting tumor growth or maintenance. Malignancies associated with viruses are optimal targets for T cell therapy as many viral antigens fulfill all of these requirements. Several viruses are known to be associated with cancer, but in this section, we will focus on Epstein-Barr virus (EBV)-associated malignancies as there has been extensive experience using adoptive T cell transfer as immunotherapy for EBV-expressing tumors.

3.2.1 EBV-Associated Posttransplant Lymphoproliferative Disorder

Hematopoietic stem cell transplantation and solid organ transplantation recipients receive intensive and/or prolonged immunosuppression putting them at increased risk for developing lymphoma². At least 90% of these “lymphomas” express EBV

antigens, implicating the virus as a causative agent³. Today this condition is referred to as posttransplant lymphoproliferative disease, reflecting the fact that this is a heterogeneous malignancy that could present as a polyclonal hyperplasia or a monoclonal, aggressive non-Hodgkin's lymphoma⁴. EBV-associated lymphoproliferative disease has also been documented in patients with acquired immunodeficiency syndrome and congenital immunodeficiency, further supporting the hypothesis that a deficient cellular immune response to EBV is a major contributor in its development⁵.

One strategy to combat this complication in hematopoietic stem cell transplant recipients is the infusion of unmanipulated donor-derived leukocytes. If the stem cell donor is EBV seropositive then donor lymphocyte infusions should have protective cellular immunity against the virus, which can be transferred to the recipient by T cell infusion. This strategy proved effective, resulting in complete responses in 17 of 30 patients⁶. Unfortunately, a significant fraction of unmanipulated donor T cells are alloreactive, putting patients at significant risk for developing graft versus host disease (GVHD). In the previous study, it was reported that 17% of patients receiving donor lymphocyte infusions developed GVHD.

3.2.2 EBV-Specific CTL for Prophylaxis and Treatment of EBV-Associated Posttransplant Lymphoproliferative Disease

To reduce the incidence of GVHD associated with donor lymphocyte infusions, ex vivo expanded, EBV-specific T cells for the prevention and treatment of posttransplant lymphoproliferative disease have been evaluated. Posttransplant lymphoproliferative disease provides an excellent model in which to evaluate the efficacy of adoptively transferred EBV-specific CTL because the tumor cells express all latent-cycle virus-encoded antigens (EBNAs 1, 2, 3A, 3B, 3C and LP, BHRF1, BARF1 and LMP1, 2A and 2B), most of which are targets for virus-specific immune responses⁷⁻¹⁰. Furthermore, immortalized lymphoblastoid cell lines, that express the same viral antigens, can readily be generated from essentially any donor by infecting B cells with a laboratory strain of EBV. Lymphoblastoid cell lines function as superb antigen-presenting cells, expressing lytic and latent-cycle EBV antigens as well as costimulatory molecules that facilitate CTL generation. The ex vivo stimulation of peripheral blood mononuclear cells for several weeks with lymphoblastoid cell lines and interleukin (IL)-2 produces a highly enriched population of polyclonal EBV-specific CTL (Fig. 3.1).

Our group has treated 101 hematopoietic stem cell transplant recipients prophylactically with EBV-specific CTL. None developed EBV-related posttransplant lymphoproliferative disorder with up to 17 years of follow-up, compared with 5 of 42 (11%) patients enrolled on the same transplantation protocol who did not receive EBV-specific CTL¹⁴. Of 13 patients who received CTLs as treatment for biopsy proven or probable EBV-related posttransplant lymphoproliferative

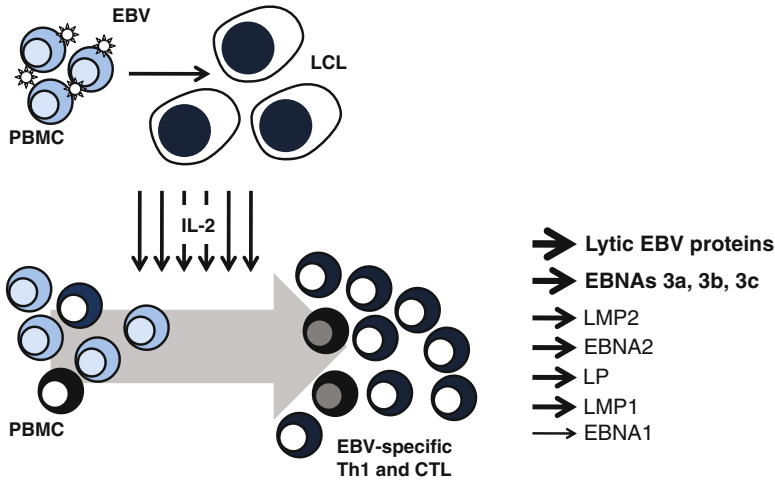


Fig. 3.1 Generation of EBV-specific cytotoxic T lymphocytes (CTLs). In the first step, EBV-transformed B lymphoblastoid cell lines (EBV-LCLs) are generated from the patient or stem cell donor for use as antigen-presenting cells (APCs). Peripheral blood mononuclear cells (PBMCs) are infected with the B95-8 strain of EBV in the presence of cyclosporin A to inhibit EBV-specific T cells. A permanently growing cell line can be established from most healthy donors within 4 to 6 weeks, but is frequently slower from patient blood. In the second step, the EBV-LCL is irradiated and used to stimulate PBMCs from the same donor to activate EBV-specific T cells. The responder T cells are restimulated weekly with the irradiated EBV-LCL from day 9 and IL-2 is added twice weekly from day 14 until sufficient T cells have been expanded. These T cell lines comprise CD4+ and CD8+ T cells specific for a range of EBV lytic cycle and latency-associated antigens. In clinical productions, the virus is drawn from a clinical grade, working virus bank and the EBV-LCLs are cultured for at least 2 weeks in ganciclovir to prevent the release of infectious virus.

disorder, 11 (85%) achieved complete remission with no recurrence. Importantly, CTL infusions were safe and not associated with *de novo* GVHD. Gene-marking studies also showed that the infused CTL could expand by several logs₁₀ *in vivo*, contribute to the memory pool (persisting for up to 9 years), and traffic to tumor sites^{11–14}. The safety and efficacy of donor-derived EBV-specific CTL for the prophylaxis and treatment of EBV-related posttransplant lymphoproliferative disorder has been subsequently confirmed by other investigators^{15,16}.

While EBV-specific CTLs reconstitute immunity to EBV and prevent EBV-related posttransplant lymphoproliferative disorder after hematopoietic stem cell transplant, their use as therapy is limited by the time required (~3 months) for production, in addition to logistical and cost issues. Two different avenues of research are being pursued to overcome the time limitation. The first involves the use of banked, allogeneic EBV-specific CTLs, which are readily available as an “off-the-shelf” therapy. In one multicenter clinical trial, 31 solid organ transplant and 2 hematopoietic stem cell transplant recipients with EBV-related posttransplant lymphoproliferative disorder who had failed conventional therapies received allogeneic EBV-specific CTL. These infusions were well tolerated and the overall

response rate was 52% at 6 months including 14 patients with a complete response¹⁷. Other strategies aimed at shortening the production time of EBV-specific CTLs include the use of (1) EBV-specific peptides for overnight stimulation of donor peripheral blood mononuclear cells that can then be selected based on their secretion of interferon (IFN)- γ ^{18,19}, (2) HLA-peptide multimers that can directly select viral peptide-specific T cells from peripheral blood mononuclear cells²⁰, or (3) dendritic cells nucleofected with DNA plasmids encoding immunodominant EBV antigens to expand EBV-specific CTL rapidly over 7–10 days^{19,21}. Only the first strategy has been evaluated clinically for EBV. For example, Moosmann et al. treated 6 patients with EBV-specific CTL activated with peptides derived from 11 EBV antigens. They observed three complete responses in patients with early EBV-related posttransplant lymphoproliferative disease, whereas three patients with late-stage disease progressed after therapy¹⁹. While these results are encouraging, all responding patients in this study received additional therapies making it difficult to ascribe the anti-EBV-related posttransplant lymphoproliferative disease effects to CTL alone. The clinical safety and efficacy both of tetramers and of selection based on IFN- γ secretion have been evaluated for cytomegalovirus^{22,23}. However, further studies are necessary to evaluate the efficacy and safety of rapidly generated T cells specific for EBV.

3.2.3 *EBV-Associated Lymphoma and Nasopharyngeal Carcinoma*

The success of EBV-specific CTL adoptive transfer to treat EBV-related posttransplant lymphoproliferative disease led to the extension of this therapy to other EBV-associated malignancies. Nearly 100% of undifferentiated nasopharyngeal carcinoma as well as 40% of Hodgkin's and about 20% of non-Hodgkin's lymphomas tumors express EBV antigens^{24,25}. However, in contrast to EBV-related posttransplant lymphoproliferative disease, EBV-associated nasopharyngeal carcinoma and lymphomas develop in seemingly immunocompetent hosts and display a restricted expression pattern of EBV antigens. Whereas EBV-related posttransplant lymphoproliferative disease expresses all ten EBV proteins that are associated with the virus latent cycle (type III latency), the malignant cells of Hodgkin's lymphoma and nasopharyngeal carcinoma are typically characterized by a type II latency pattern, expressing only LMP1, LMP2, EBNA1, and BARF1^{26,27}. Of these viral antigens, only LMP1, LMP2, and BARF1 are efficiently processed and presented by HLA class I molecules and thus targets for CTL therapy²⁸. Although EBNA1 is rarely presented on HLA class I molecules²⁹, it is frequently presented on class II molecules and may also be of value^{29,30}.

Our group used EBV-LCL-activated EBV-specific CTL to treat 14 patients with relapsed Hodgkin's disease. Of 11 patients with clearly measurable disease at the time of CTL infusion, 2 experienced complete remissions, 1 had a partial response,

5 had stable disease, and 3 had no response to CTL therapy³⁰. Chua et al.³¹ used EBV-specific CTL to treat four patients with advanced nasopharyngeal carcinoma. These investigators found the treatment to be well tolerated, and they observed an increase in EBV-specific immunity for 2–3 weeks after CTL infusion. Unfortunately, the patients treated had very advanced stage disease, and the authors could not clearly evaluate an antitumor effect. Our group has also administered EBV-specific CTL to 23 nasopharyngeal carcinoma patients with relapsed or refractory disease. At the time of CTL infusion, 8 patients with relapsed nasopharyngeal carcinoma were in remission and 15 had active disease. Of those treated in remission, 62% (5/8) remain disease-free (17–75 months), while 48.7% (7/15) patients with active disease achieved a complete (33.3%) or partial response (15.4%) to therapy^{31,32}. Collectively, these results were encouraging and showed that in some cases EBV-specific CTLs were therapeutically beneficial for patients with Hodgkin's lymphoma or nasopharyngeal carcinoma. Still, the clinical responses were relatively limited when compared with the responses observed in EBV-related posttransplant lymphoproliferative disorder patients.

3.2.4 Improving EBV-Specific CTL Therapy for EBV-Related Lymphoma and Nasopharyngeal Carcinoma

Work being conducted in our laboratory and others led to the hypothesis that at least two important differences between EBV-related posttransplant lymphoproliferative disorder and lymphoma/nasopharyngeal carcinoma patients might contribute to the lower clinical responses seen in the latter group. First, only a minor component of our EBV-specific CTL lines recognizes the EBV antigens expressed on lymphoma and nasopharyngeal carcinoma tumors (EBNA1, LMP1, LMP2 and BARF1). Second, in the stem cell transplant setting of EBV-related posttransplant lymphoproliferative disorder, EBV-specific CTLs are generated from healthy donors and infused into a lymphopenic environment created by the pre-transplant conditioning regimen. This provides the transferred cells immunologic space and access to antigen, homeostatic cytokines, and growth factors. In nasopharyngeal carcinoma and most lymphoma patients, the T cells are derived from the patient and T cells specific for the viral tumor antigens could be anergized within the tumor sites. Thus, EBV-specific CTL therapy for lymphoma and nasopharyngeal carcinoma patients might be improved by increasing the frequency of T cells specific for the EBV-specific LMP1 or LMP2 antigens, increasing the potency of antigen-presenting cells for T cell activation and infusing the cells after lymphodepletion of the host.

We evaluated a combination strategy of lymphodepletion prior to transfer of EBV-specific CTL in patients with refractory or relapsed nasopharyngeal carcinoma³³. Administration of an anti-CD45 monoclonal antibody (mAb) resulted in a transient lymphopenia in all patients and an increase in serum concentrations of IL-15, an important T cell survival cytokine, in six out of eight patients. At the time

of lymphopenia, patients were infused with EBV-specific CTL, and all showed an increase in the frequency of these EBV-specific CTLs in their peripheral blood that was not seen in patients who received EBV-specific CTLs without lymphodepletion. Encouragingly, the three patients with greatest and longest lasting rise in their EBV-specific immunity had clinical benefit (one complete response and two stable disease), suggesting that continued investigation into the strategy of using lymphodepletion before CTL transfer is warranted.

We also tested the hypothesis that EBV-specific CTL enriched for LMP2 and/or LMP1 could mediate superior antitumor activity in lymphoma patients. Protocols were developed to generate LMP2 or LMP1 and LMP2-enriched EBV-specific CTL and used to treat patients with EBV-positive Hodgkin's or non-Hodgkin's lymphoma³⁴. Sixteen patients received LMP2-specific CTLs and 33 received LMP1/2-specific CTLs without toxicity. The number of LMP-specific T cells in peripheral blood rose 2–70-fold and persisted for up to 3 months. Lymph node biopsies from three patients taken 3–6 months post CTL infusion showed selective accumulation of LMP-specific T cells in lymph nodes compared to peripheral blood. Preliminary results in patients who received LMP2 or LMP1 plus LMP2-specific T cells show tumor responses in about 70% of patients and complete responses in over 60%³⁵.

These studies suggest that the in vivo antitumor activity of EBV-specific CTL can be improved by increasing the frequency of cells with specificity for the appropriate latency antigen(s). Other strategies to improve EBV-specific CTL for patients with EBV-associated lymphoma and nasopharyngeal carcinoma involve genetic modifications aimed at making CTL resistant to the immunosuppressive mechanisms of the tumor and grafting CTL with chimeric receptors to allow recognition of nonviral antigens expressed on tumor cells. These strategies will be discussed in more detail later in this chapter.

3.2.5 Summary of EBV-Associated Malignancies

EBV-associated malignancies provide an excellent platform for evaluating the feasibility and safety of adoptive T cell transfer. As prophylaxis and treatment of EBV-related posttransplant lymphoproliferative disorder, EBV-specific CTLs have proven safe and highly effective. The extension of EBV-specific CTL therapy to EBV-associated malignancies developing in immune competent hosts has been more challenging. EBV-specific CTL therapy has produced complete tumor regressions in some patients with EBV-associated lymphoma and in nasopharyngeal carcinoma, but in other cases certain limitations must be overcome to increase the overall effectiveness of adoptive T cell transfer in this patient population. Strategies to increase the antitumor activity of EBV-specific CTL therapy include lymphodepletion of the host, enriching for CTL with specificity toward particular EBV latency antigens, and genetic modifications of the CTL to improve their survival in the tumor microenvironment and to enhance tumor recognition.

3.3 Tumor-Infiltrating Lymphocytes

While EBV-specific CTLs have shown promise for the treatment of several EBV-associated malignancies, most tumors are not associated with known viruses and thus not targets for antiviral CTL therapy. Therefore, alternative strategies must be employed to generate tumor-specific T cells. In patients with melanoma, colorectal, and ovarian cancer, the presence of TIL is associated with better clinical outcomes³⁶⁻³⁸. Thus, investigators have attempted to use ex vivo expanded TIL as a source of tumor-specific T cells for adoptive T cell transfer. This strategy has been pioneered by Rosenberg et al., at the National Cancer Institute, who have been using TILs to treat patients with metastatic melanoma. Thus, we will focus much of our attention in this section on their results.

3.3.1 *Generating TILs for Adoptive T Cell Transfer of Metastatic Melanoma Patients*

The first major hurdle in developing TIL-based adoptive T cell transfer was cleared in 1987 when Muul et al. reported that TIL extracted from surgically resected metastases from patients with malignant melanoma could be expanded ex vivo in medium containing IL-2³⁹. Responsive lymphocytes were cytotoxic to autologous melanoma cells and could be expanded >90,000-fold in culture while retaining tumor specificity. Shortly thereafter, a clinical trial was initiated using large doses of ex vivo expanded TIL (>10¹¹ cells) plus high-dose IL-2 to treat patients with metastatic melanoma. A cohort of patients was also given low-dose cyclophosphamide 36 hours prior to infusion for immunomodulation. Overall, an objective clinical response rate of 34% was reported with no significant difference in response between patients treated with TIL plus IL-2 (31%) and those given cyclophosphamide (35%) prior to infusion of TIL⁴⁰. Unfortunately, most of the clinical responses were transient and few complete responses were observed; however, several critical findings were made that would improve TIL therapy in future studies. The investigators found that patients who responded to treatment were significantly more likely to have received TIL which (1) were from younger cultures, (2) had shorter doubling times, and (3) exhibited higher lysis against autologous tumor targets. Furthermore, patients receiving TIL expanded from subcutaneous tumor deposits had higher response rates (49%) compared with those receiving TIL from lymph nodes (17%).

3.3.2 *Improving TIL Therapy: Modified Culture and Increased Lymphodepletion*

In the previous study, TILs were isolated by digestion of melanoma tumors, to form a single-cell suspension, which was expanded in a single culture. A modified

protocol for growing TIL was adopted that involved mincing tumors into tiny fragments and establishing multiple cultures. Interestingly, this method generally succeeded in expanding several different TIL cultures from the same tumor specimen, often with qualitative and quantitative differences in antigen-specific reactivity. Those cultures with the highest reactivity against autologous tumor cells underwent rapid expansion using the T cell stimulating antibody OKT3 plus IL-2. Using this method, a total of 10^{10} – 10^{11} T cells could be obtained in as little as 5 weeks⁴¹. Three subsequent clinical protocols were initiated utilizing this method of TIL preparation and focused on increasing amounts of lymphodepletion prior to cell infusion. In the first trial, 43 patients received a non-myeloablative chemotherapy regimen of cyclophosphamide (60 mg/kg) for two consecutive days followed by fludarabine (25 mg/m^2) for an additional 5 days. In the second trial, 25 patients were given the same chemotherapy regimen followed by 200 cGy whole-body irradiation the day before cell infusion. In a third trial of 25 patients, the total body irradiation was intensified by giving 200 cGy twice a day for 3 consecutive days for a total of 1,200 cGy. Hematopoietic stem cell rescue was performed by administration of autologous CD34⁺ cells one day after TIL infusion in both trials where total body irradiation was used⁴².

Overall, objective clinical responses were 49%, 52%, and 72% for the 3 trials, respectively. Of the responding patients, 12 experienced complete responses (3 in trial 1, 2 in trial 2, and 7 in trial 3) that are ongoing from 18 to 75 months⁴². Importantly, cancer regressions were observed at distant metastatic sites including the lung, liver, lymph nodes, subcutaneous tissues, and brain, suggesting that T cells migrate across the blood–brain barrier. Interestingly, the data also suggests that more aggressive lymphodepletion prior to TIL infusion could lead to an improvement in overall survival, though that conclusion can only be definitively drawn after a randomized trial. Still, preclinical data supports the assertion that, at least in melanoma, increasing amounts of total body irradiation are directly correlated with increased treatment efficacy. Moreover, the ratio of tumor-specific CD8⁺ T cells to endogenous host cells with inhibitory potential was increased in animals receiving the highest doses of total body irradiation, suggesting that a severely lymphodepleted host provides the optimal environment for transferred T cells⁴³ as previously observed for EBV-specific T cells in the stem cell transplant setting. However, lymphodepleting regimens come with a significant risk of toxicity, and therefore, the potential benefits must be appropriately weighed against the risks.

3.3.3 TIL Therapy for Ovarian Cancer

While much of the pioneering work with TIL therapy has been performed in patients with metastatic melanoma, TILs can also be found in ovarian tumors and have been expanded ex vivo for adoptive T cell transfer of ovarian cancer patients. An early study conducted in 1994 used ex vivo expanded TILs, isolated from solid metastases or malignant effusions, to treat eight patients with advanced epithelial

ovarian carcinoma⁴⁴. The generated lines were primarily CD4⁺ T cells and these were infused into patients who also received recombinant IL-2. Unfortunately, no objective antitumor responses were observed in this trial, though the investigators reported some signs of clinical activity including ascites regression in two patients. In a study by Fujita et al., 13 ovarian cancer patients treated with surgical resection and cisplatin-containing chemotherapy who showed no detectable disease after treatment were given TIL to prevent relapse⁴⁵. A similar control group was established who did not receive TIL. With an average of 3 years of follow-up (36 months in TIL group and 33 months in the control group), the estimated 3-year disease-free survival rate was significantly ($p < 0.05$) higher in the TIL group (82.1%) versus the control group (54.5%). Thus, this study concluded that in ovarian cancer patients with minimal residual disease after surgery and chemotherapy, TIL could significantly extend disease-free survival.

Current work suggests a critical factor affecting patient outcome is the ratio of CD8⁺ effector T cells to CD4⁺ regulatory T cells⁴⁶. However, the factors that affect these ratios from one patient to the next are largely unknown. A better understanding of the immune response in patients with a high effector to regulatory T cell ratio might help to improve future adoptive T cell transfer strategies for ovarian cancer.

3.3.4 Summary of TIL Studies

Where available, ex vivo expanded TILs provide an excellent source of tumor-specific T cells for use in adoptive T cell transfer. TILs have proven particularly successful in the treatment of patients with metastatic melanoma, and preliminary evidence suggests that an intensive lymphodepleting regimen of chemotherapy and total body irradiation with stem cell rescue could enhance the antitumor activity of the transferred cells. Ovarian and colon carcinomas have also been treated with TILs (TILs), though the clinical experience with adoptive T cell transfer for these cancers is limited. While TILs were found to be successful in extending disease-free survival in ovarian cancer patients with minimal residual disease, they did not produce objective clinical responses in patients with advanced stage disease. Still, patients with ovarian cancer who have a high effector to regulatory T cell ratio have significantly better outcomes, suggesting further investigation into T cell-based immunotherapy is warranted.

3.4 Genetically Modified T Cells

While TILs have produced antitumor responses in melanoma patients, the broader application of this strategy is limited by the fact that TILs are not available or difficult to isolate from most tumors, and even when TILs are available, it is not always possible to expand a large number of tumor-specific CTLs⁴⁷. However,

advances in immunology and vector biology have allowed the development of tools, including the genetic modification of T cells to redirect their specificity toward tumor antigens, or increase their resistance to inhibitory ligands produced by tumors and their stroma, which might overcome some of the limitations of TIL and EBV-specific CTL therapy. In this section, we will discuss two strategies being used to redirect T cell specificity, TCR transfer, and chimeric antigen receptors (CARs), with a particular emphasis on those studies that have entered phase I clinical trials.

3.4.1 T Cell Receptor Transfer

Over 25 years ago, it was discovered that T cells derive antigen specificity from a heterodimeric complex of two immunoglobulin-like proteins that form part of the TCR complex^{48,49}. From early on, investigators recognized that cloning and transferring these TCR genes into T cells offered the potential to redirect T cell specificity toward any antigen of interest. However, it was not until several years later that advances in vector technology have made redirecting T cell specificity through TCR gene transfer possible.

Retroviruses, in particular the Moloney murine leukemia virus, have revolutionized gene therapy approaches by allowing for high transduction efficiency of primary cells and a relatively high and stable expression of the transgene⁵⁰. However, even with this technology, the transfer of TCR genes has proved difficult. Since a functional TCR requires both the α and β TCR chains, these genes must be transferred into T cells either by two different retroviral vectors, requiring two separate transductions, or on a single vector containing an internal ribosomal entry site or a viral 2A sequence capable of producing high-level expression of both chains. Further, mispairing between transgenic and endogenous TCRs can create unwanted specificities and reduce expression of the transgenic pair resulting in T cells with low avidity for tumors.

3.4.2 Adoptive T Cell Transfer for Metastatic Melanoma Using TCR Transfer

The first clinical trial to use TCR transfer was conducted in patients with metastatic melanoma⁵¹. The genes for a MART-1-specific TCR were cloned from TIL with proven antitumor activity and transferred into peripheral blood T cells of the study patients. While this study was the first to demonstrate the feasibility of this strategy in man, only 4 of 31 patients (13%) experienced any clinical response and none achieved a complete response, despite the fact that these MART-1-specific T cells engrafted and persisted for several months after infusion^{51,52}. The investigators

noted that none of their patients receiving genetically modified T cells experienced side effects such as skin rash or melanocyte toxicity in the eye or ear, which had previously been associated with robust antitumor responses in TIL studies. After extensive *in vitro* study, they concluded that a TCR with a higher avidity for the target antigen might be necessary to achieve clinical response rates similar to those achieved with naturally occurring TIL. In a follow-up study, Johnson et al. cloned a high-affinity TCR from a human T cell that recognized an HLA-A2-restricted MART-1 epitope⁵². Using HLA-A2 transgenic mice, they also cloned a high-affinity murine TCR recognizing the gp100 154–162 epitope, which is the most highly presented peptide from the gp100 protein in the context of HLA-A2. Six of 20 (30%) patients receiving the high-affinity MART-1 TCR achieved clinical regression of melanoma. While the numbers were low, it appeared that the high-affinity TCR was associated with better clinical response rates than the low-affinity TCR. When the high-affinity murine TCR to gp100 was transferred into patient T cells, they observed clinical responses in 3 of 16 patients (19%). While encouraging, these response rates are still well below the >50% response rate observed when using naturally occurring TIL to treat metastatic melanoma; further, high-affinity TCRs destroyed normal melanocytes in the skin, eye, and ear requiring local steroid treatment to treat uveitis and hearing loss⁵². More recently, severe off-target effects associated with the use of enhanced, high affinity TCRs specific for MAGE-A3 have been reported to the Recombinant DNA Advisory committee (RAC).

Another problem brought to light in the wake of these clinical studies is the frequent mispairing of introduced α and β TCR chains with the endogenous α and β chains^{53,54}. This mispairing can result in two major problems: the cloned TCR failing to achieve wild-type expression levels, thus lowering the overall avidity of transgenic T cells and inhibiting their effector functions, and the generation of a novel TCR with autoreactive potential. Strategies currently being investigated to decrease mispairing of introduced α and β chains include modifying the constant regions with disulfide bonds, using hybrid TCRs that consist of murine constant regions fused to human variable regions, and silencing of the endogenous TCR^{55–57}.

While the problem of TCR mispairing may be solvable, the use of cloned TCR genes means that tumor killing is HLA restricted, thus limiting this strategy to patients with common HLA types for which a high-affinity TCR has been cloned. Additionally, tumors have been found to downregulate class I MHC molecules as means of escaping TCR recognition⁵⁸. Many of these limitations may be overcome with another technology that utilizes a hybrid TCR known as the CAR (chimeric antigen receptor).

3.4.3 Chimeric Antigen Receptors

First-generation CARs consist of an extracellular antigen recognition domain, a short hinge, a transmembrane domain, and an intracellular signaling domain derived from the TCR CD3- ζ chain⁵⁹ (Fig. 3.2). The extracellular antigen recognition domain is

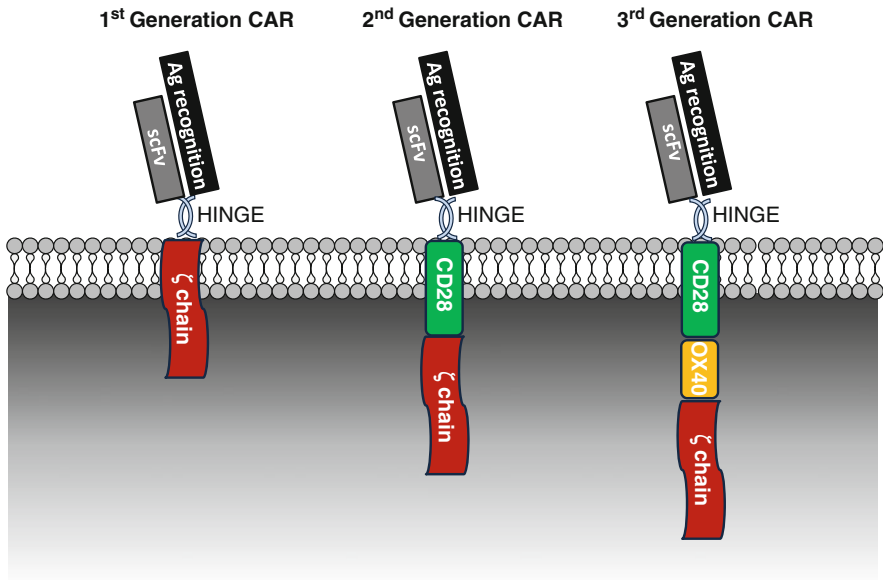


Fig. 3.2 Enhancing the activity of chimeric antigen receptor (CARs). First generation CARs linked the single chain variable fragment of an antibody to the intracellular signaling domain of the TCR CD3- ζ chain, via a hinge region and a transmembrane region of various origins. When expressed on T cells, this molecule mediates killing of tumor cells recognized by the antibody domain, but unless the tumor cell expresses costimulatory molecules, it does not mediate proliferation. Since most tumors do not express costimulatory domains, second generation CARs introduce the intracellular signaling domain of CD28 to provide second tier costimulation and added to that in third generation CARs are costimulatory domains from third tier costimulatory molecules such as OX40 or 4-1BB.

typically composed of a single-chain variable fragment from a mAb. When the single-chain variable fragment binds its cognate ligand, a signal is transmitted through the CD3- ζ chain resulting in T cell activation⁶⁰. Since antigen recognition is through an antibody–ligand interaction, CARs can be used for any patient regardless of HLA type. Additionally, CARs can recognize tumors which have downregulated class I MHC, and they can be generated against virtually any tumor-associated antigen that is expressed on the cell surface, including carbohydrates and glycolipids⁵⁷, and that is minimally expressed on essential normal tissues.

Owing to the many advantages that CARs offer over traditional TCRs, substantial interest has surrounded their use in adoptive immunotherapy with several phase I trials currently underway to assess the safety and efficacy of this approach. To date, five phase I clinical trials have been completed using CARs to treat cancer, and the results have been published (Table 3.1). Unfortunately, in most of the initial studies, clinical responses have been modest, and limited persistence of the CAR-expressing T cells is hypothesized to be one of the major obstacles to antitumor efficacy.

Table 3.1 Completed phase I clinical trials using first generations CARs for cancer

Tumor-associated antigen	Targeted malignancy	Clinical response (# of pts.)	Ref.
α -Folate receptor	Ovarian cancer	NR (14)	Kershaw et al. ¹⁰¹
Carbonic anhydrase (CAIX)	Renal cell carcinoma	NR (3)	Lamers et al. ¹⁰²
CD20	Lymphoma	PR (1); SD (4); NED (2)	Till et al. ¹⁰³
CD171	Neuroblastoma	PR (1); NR (5)	Park et al. ¹⁰⁴
G _{D2}	Neuroblastoma	CR (3); PR (1); SD (1); PD (4); TN (2)	Pule et al. ⁶¹

CR, complete response; PR, partial response; NR, no response; SD, stable disease; PD, progressive disease; TN, tumor necrosis by biopsy

3.4.4 Costimulatory Domains to Improve CAR Function

When T cells are activated by professional antigen-presenting cells, they not only receive stimulation through the TCR but also receive essential costimulatory signals through CD28 and tumor necrosis factor receptors like CD27, 4-1BB, and OX40^{62–64}. See chapter 8 for details of T cell co-signaling. Since T cells expressing a first-generation CAR receive only CD3- ζ stimulation in the absence of a costimulatory signal, their activation is incomplete, and this is thought to be one of the major reasons for their limited persistence in vivo since most tumors do not express costimulatory molecules and inhibit the activation of local professional antigen-presenting cells that do. Therefore, several investigators have made improvements to the original CAR by including a CD28 signaling domain in addition to the CD3- ζ chain, now known as the second-generation CAR^{65–68}. T cells expressing second-generation CARs show increased proliferation and important cytokine secretion (e.g., IFN- γ , IL-2, and TNF- α), after stimulation with target cells expressing the cognate antigen, compared to T cells expressing a first-generation CAR. Furthermore, T cells with second-generation CARs persist longer in vivo when used in immunodeficient (SCID) mouse xenograft tumor experiments and displayed superior in vivo antitumor activity when compared with first-generation CAR-expressing T cells^{67,68}. A clinical study describing an inpatient comparison of activated T cells expressing first- and second-generation CD19-specific CARs also demonstrated increased persistence of the CD28-containing CAR⁶⁹, but clinical responses were not produced.

Researchers have therefore added even more costimulatory domains into CARs. Thus, the third-generation CAR was developed, which contained an additional signaling domain (OX40 or 4-1BB) sandwiched between the CD28 and CD3- ζ domains. Several preclinical studies have now shown that third-generation CARs mediate superior in vivo tumor regression because of their enhanced cytokine secretion, expansion, and persistence^{70–72}. Recently three complete tumor remissions were obtained in response to T cells expressing a CD19-specific CAR expressing the 4-1BB endodomain when infused after lymphodepletion into

patients with chronic lymphocytic leukemia. However, severe toxicity associated with cytokine storm was also reported^{73,74}. Thus, increasing the number of costimulatory domains is not without risk, and two deaths have recently been reported in patients receiving T cells genetically modified with second- or third-generation CAR^{61,75}. While it is unclear exactly what caused the deaths of these two patients, there is concern that second- and third-generation CARs could be easily triggered, such that even low avidity off-target binding could cause potent activation, again leading to cytokine storm⁷⁶. Thus, while adoptive T cell transfer has an excellent safety record overall, these two cases highlight the need to tread cautiously when testing new-generation CARs in humans.

3.4.5 Expressing CARs on Virus-Specific CTL

Another strategy to increase the persistence of CAR-expressing T cells is to modify virus-specific CTL with CARs, rather than using T cells nonspecifically activated with anti-CD28 and/or anti-CD3 antibodies (that activate the TCR)^{75,77,78}. As discussed earlier, EBV-specific T cells persist for up to 9 years in some patients, and we hypothesized that EBV-specific CTL expressing CARs could be stimulated through their native EBV-specific TCRs in vivo after transfer into EBV-seropositive hosts, thus allowing CAR-expressing T cell to persist longer and expand to greater numbers thereby increasing their potential for antitumor activity.

We recently infused neuroblastoma patients with both EBV-specific CTL and anti-CD3-stimulated T cells, each expressing a G_{D2}-specific CAR (distinguished only by a unique DNA barcode in each vector that allowed for PCR detection of transgenic EBV-specific CTL or transgenic CD3⁺ T cells). Indeed, tenfold more G_{D2}-CAR EBV-specific CTLs than CD3⁺ T cells were detected in the peripheral blood of infused patients. Furthermore, G_{D2}-CAR EBV-specific CTL could be detected for more than 6 weeks after infusion, whereas G_{D2}-CAR CD3⁺ T cells could only be detected for 2–3 weeks post infusion⁶¹. The latest clinical data from this study show that of 11 patients with detectable disease at the time of CTL infusion, 3 achieved complete responses, 1 had a partial response, 1 had stable disease, 4 had progressive disease, and 2 had detectable tumor necrosis. This was the first study to report complete clinical regression of solid tumors after treatment with CAR-expressing T cells and suggests that the increased expansion and persistence of G_{D2}-CAR EBV-specific CTL could contribute to more robust antitumor activity in patients.

3.4.6 Overcoming Tumor Immune Evasion Strategies

T cells expressing CARs are an effective way to redirect T cell specificity to tumors as well as overcome the downregulation of class I MHC molecules. Still,

tumors have a plethora of other immune evasion strategies to avoid recognition and destruction by T cells, including secretion of inhibitory cytokines (e.g., TGF- β and IL-10), upregulation of the inhibitory ligand PD-L1, and the recruitment of regulatory T cells⁷⁹. Additional genetic modifications of T cells to overcome immune evasion are in preclinical and clinical development. These strategies include modifying T cells with chemokine receptors for increased tumor homing^{80,81}, transgenic expression of cytokines/receptors that support T cell proliferation and effector function^{82,83}, constitutive activation of Akt in T cells to resist regulatory T cells⁸⁴, as well as the expression of mutant proteins allowing T cell function in the presence of TGF- β and the therapeutic drug rapamycin⁸⁵. Finally, combining T cell transfer with DNA demethylating agents or histone deacetylase inhibitors to modify the tumor microenvironment and increase antigen presentation on tumor cells might improve clinical outcomes in patients⁸⁶.

3.4.7 Summary of TCR Transfer and CARs

The ability to redirect T cell specificity using TCR transfer or CARs is a powerful tool that makes it possible to target virtually any tumor with T cell immunotherapy. Thus far, the only clinical trial to use TCR transfer has been in patients with metastatic melanoma. Clinical tumor regression was observed, though the response rate was lower than in patients receiving unmodified, expanded TILs. While a potentially useful strategy, the use of TCRs means that tumor killing is HLA restricted and thus only available to patients with common HLA types. This limitation can be overcome by redirecting T cell specificity with a CAR. CARs use monoclonal antibodies to recognize surface molecules in an HLA-unrestricted fashion and can therefore be used to treat any patient whose tumor expresses the appropriate antigen. Although first-generation CARs were largely unsuccessful in phase I studies, strategies to improve the persistence of CAR-expressing T cells, including the addition of costimulatory domains and expressing CARs on virus-specific T cells, should improve the clinical efficacy of CAR-expressing T cells. Further genetic modifications to overcome elements of the immunosuppressive tumor microenvironment should also lead to more effective T cell therapies.

3.5 Adoptive Therapy Using Natural Killer Cells

Our discussion throughout this chapter has focused on T cells because they are widely used in clinical adoptive transfer studies. However, NK cells represent another important population of cells with potent antitumor potential. Indeed, interest in NK cells to treat cancer grew substantially after the discovery that IL-2 activation of NK cells could result in cytotoxic activity against previously NK-resistant tumors⁸⁷. Various protocols have been developed for isolating NK

cells from peripheral blood, and more recently new methods have been described for expanding NK cells *ex vivo*⁸⁸. While a detailed review of clinical adoptive NK cell transfers is beyond the scope of this chapter, we will conclude with a brief discussion of the current progress and challenges in the field of NK cell transfer⁸⁹.

3.5.1 Adoptive Transfer of Allogeneic NK Cells

In retrospect, the use of NK cells in adoptive immunotherapy should have been evident from the outset. After all, the existence of NK cells in immunodeficient SCID mice all but resurrected the theory of tumor immune surveillance, after apparently receiving a significant blow following the findings that immunocompromised mice are no more at risk of getting spontaneous tumors⁹⁰ (although that view has been updated now to demonstrate the importance of T cells and IFN- γ in cancer immune surveillance; please see chapter 1 for details). As their name implies, NK cells have an innate ability to mediate target cell destruction without any previous priming event (as required by T cells)⁹¹. While most of the conceivable applications of tumor immunotherapy with these cells center on this particular effector function, their ability to mediate immunoregulatory effects between the adaptive and innate immune systems also lends them increased applicability in the clinical setting⁹².

NK cells recognize self from nonself through a system of activating and inhibitory ligands, as well as receptors that mediate antibody-dependent cytotoxicity. The balance of the corresponding ligands on the target cells determine whether the NK cells choose to exert their cytolytic function or to retreat into immune tolerance⁹³.

Results from different groups all seem to suggest that NK cell transfer is more effectively accomplished in the allogeneic setting, where the presence of KIR mismatches (among other things) allows for unimpeded NK cell activity against the tumor. A retrospective study by Ruggeri et al.⁹⁴ has shown the positive effects of allogeneic NK cell transfer in a haplotype-mismatched hematopoietic stem cell transplant setting. Miller et al. reported complete remissions in cancer patients given haploidentical NK cells⁹⁵, an observation they did not find when they previously infused autologous NK cells⁹⁶.

To date, several studies demonstrate the feasibility of using allogeneic NK cells in such a therapeutic approach. Shi et al.⁹⁷ infused KIR–ligand mismatched NK cells depleted of T cells in patients with advanced multiple myeloma. Besides demonstrating that the therapy is safe and well tolerated, the authors reported an encouraging (near) complete remission rate of 50%.

On the other hand, Dillman et al.⁹⁸ utilized autologous NK cells as part of a population of cells termed lymphokine-activated killer cells that are comprised of approximately 77% T cells and 23% NK cells. These lymphokine-activated killer cells were a leukapheresis product and injected intralesionally during surgery. A median survival of 20.5 months from diagnosis was observed following the use of this therapy as an adjunct in glioblastoma multiforme, higher than the 15-month

median survival seen in standard temozolomide therapy⁹⁸ and the 12-month survival associated with the disease.

3.5.2 Hurdles in the Use of NK Cells for Immunotherapy

Despite these and many other promising results, several significant hurdles are actively being addressed to improve the chances further of using NK cells as cancer immune therapy.

Their poor number has been a constant concern, since NK cells represent only 3–20% of circulating lymphocytes. To expand NK cells, several new approaches have been proposed, among them the use of feeder K562 cells genetically modified to express 41BBL and IL-15⁹⁹.

Related to their limited numbers is the need for IL-2 coadministration in vivo. Because IL-2 administration is associated with a host of adverse events, several investigators are looking at the possibility of delivering IL-2 locally—along with the NK cells—using genetic modification⁹³. NK cells were engineered to express IL-2 via retroviral transduction. In vitro studies using the NK cell line NK-92 showed that expression of IL-2 increased cytotoxicity against tumor lines and IL-2 independence¹⁰⁰.

Not all the functions of NK cells are currently understood, most of their activating ligands are unknown, and it is not clear how they might be induced to persist in vivo. A more comprehensive review of their functions is required to harness better the potential of this therapy in combating malignancy.

3.5.3 Summary on NK Cell Transfers

Adoptive transfer of NK cells is an emerging immunotherapy for patients with malignant disease. While it has long been known that NK cells possess an innate ability to recognize and kill tumor cells, their use has been limited by difficulties in expanding large number of cells needed for adoptive transfer and the ability of these cells to persist in vivo. Recently discovered methods for expanding NK cells in culture and genetic modifications that improve NK cell persistence and function in vivo will undoubtedly increase the use of these cells in future clinical adoptive transfer studies.

3.6 Conclusions

Adoptive transfer of antigen-specific T cells presents a highly specific means to eliminate tumors with minimal toxicity. The efficacy of adoptively transferred T cells has been linked to their ability to proliferate massively after infusion to

numbers able to eliminate large tumors. Unfortunately, most tumors have low immunogenicity, poorly present weak tumor antigens, and have multiple ways to inhibit every stage of T cell activation, proliferation, and effector functions. They also inhibit local antigen-presenting cells that might otherwise cross present tumor antigens, so that infused T cells rarely proliferate sufficiently, except under conditions of extreme lymphopenia. These characteristics of tumors likely explain the disappointing clinical effects of tumor vaccines. Fortunately, T cells are readily modified with immunomodulatory transgenes that can redirect their specificity and alter their migration and response to tumor-derived inhibitory ligands. These modifications will likely be required to ensure the optimal antitumor activity of T cells that can be further improved by combination with small molecules that change the tumor microenvironment in favor of T cells and increase tumor antigen presentation. The problems of NK cells may also be solved by genetic modulation and combination with small molecules.

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