Molecular Immunology 67 (2015) 46–57

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Cancer immunotherapy utilizing gene-modified T cells: From the bench to the clinic☆

Connie P.M. Duong[a,b,c], Carmen S.M. Yong[a,b], Michael H. Kershaw[a,b,c,d], Clare Y. Slaney[a,b,*,1], Phillip K. Darcy[a,b,c,d,*,1]

[a] Cancer Immunology Research Program, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia
[b] Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria 3010, Australia
[c] Department of Pathology, University of Melbourne, Parkville, Victoria 3010, Australia
[d] Department of Immunology, Monash University, Clayton, Australia

ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form
12 December 2014
Accepted 17 December 2014
Available online 13 January 2015

Keywords:
Adoptive immunotherapy
Chimeric antigen receptor
Tumor infiltrating lymphocytes
T cell receptor

ABSTRACT

The immune system plays a critical role in the elimination and suppression of pathogens. Although the endogenous immune system is capable of immune surveillance resulting in the elimination of cancer cells, tumor cells have developed a variety of mechanisms to escape immune recognition often resulting in tumor outgrowth. The presence of immune infiltrate in tumors has been correlated with a good prognosis following treatment (Sato et al., 2005; Loi et al., 2013; Clemente et al., 1996; Galon et al., 2006). As such, immune cells such as T cells, have been harnessed in order to target cancer. Tumor reactive lymphocytes, called tumor-infiltrating lymphocytes (TILs) have been isolated and expanded from the tumor and reinfused back into patients for the treatment of melanoma. The promise of adoptive immunotherapy utilizing TILs as a robust treatment for cancer has been highlighted in patients with advanced melanoma with greater than 50% of patients responding to treatment (Dudley et al., 2005). Although TIL therapy has shown promising results in melanoma patients, it has proved difficult to translate this approach to other cancers, given that the numbers of TILs that can be isolated are generally low. To broaden this therapy for other cancers, T cells have been genetically modified to endow them with tumor reactivity using either a T cell receptor (TCR) (Parkhurst et al., 2009, 2011; Chinnasamy et al., 2011) or a chimeric antigen receptor (CAR) (Grupp et al., 2013; Park et al., 2007). This review will outline the origins and development of adoptive immunotherapy utilizing TILs leading to genetic modification strategies to redirect T cells to cancer. Potential hurdles and novel strategies will be discussed for realizing the full potential of adoptive immunotherapy becoming a standard of care treatment for cancer.

1. Introduction

The maintenance of a healthy host is largely dependent on the ability for the immune system to identify, suppress and eliminate pathogens. Although the endogenous immune system is rarely capable of eliminating established cancer, the presence of immune infiltrate in tumors has been correlated with good prognosis following therapy (Sato et al., 2005; Loi et al., 2013; Clemente et al., 1996; Galon et al., 2006). Therefore, components of the immune system such as T cells have been utilized in order to target cancer. This has involved isolating and expanding tumor reactive lymphocytes from the tumor, called tumor-infiltrating lymphocytes (TILs). The promise of adoptive immunotherapy utilizing TILs as a robust treatment for cancer has been recently highlighted in patients with advanced melanoma, with greater than 50% of patients responding to treatment (Dudley et al., 2005). However, TILs are challenging to isolate from cancers other than melanoma. To overcome this problem, T cells have been genetically engineered endowing them with tumor reactivity. This has involved modifying T cells with αβ T cell receptor (TCR) transgenes (Parkhurst et al., 2009, 2011; Chinnasamy et al., 2011). TCR modified T cells have had some promising results in clinical trials in melanoma patients (Morgan et al., 2006). However, tumors are capable of escaping detection by the immune system by downregulating molecules

☆ This article belongs to Special Issue on Therapeutic Antibodies.

* Corresponding authors at: Cancer Immunology Program, Peter MacCallum Cancer Centre, Victoria, Australia. Tel.: +61 3 96563769; fax: +61 3 96561411.
E-mail addresses: clare.slaney@petermac.org (C.Y. Slaney), phil.darcy@petermac.org (P.K. Darcy).

[1] These authors contributed equally as senior authors.

http://dx.doi.org/10.1016/j.molimm.2014.12.009
0161-5890/

such as MHCI. Therefore, recombinant receptors called chimeric antigen receptor (CAR) (Grupp et al., 2013; Park et al., 2007) have been developed, arming the T cell with anti-tumor activity regardless of MHCI expression. The tremendous promises of the approach have been recently highlighted utilizing CAR T cells specific for CD19, which has resulted in remarkable responses in hematological malignancies (Maude et al., 2014). In this review, we outline the origins and development of adoptive immunotherapy utilizing TILs, TCR modified T cells and CAR modified T cells. Finally we discuss potential hurdles and novel strategies for the potential of adoptive immunotherapy becoming a standard of care treatment for cancer.

2. Adoptive immunotherapy

2.1. Early preclinical studies and clinical trials

It has long been proposed that the presence of a lymphocytic infiltration within tumors is associated with a good prognosis. In 1972, it was reported that a gastric cancer patient with hepatic metastases had a total regression of metastases in his liver in the absence of therapy (Rosenberg et al., 1972). The dense infiltration of lymphocytes in the resected gastric biopsy suggested the importance of these TILs against the growing cancer (Rosenberg et al., 1972). The presence of TILs have since been associated with a favorable prognosis in other cancers including ovarian (Sato et al., 2005), breast (Loi et al., 2013), melanoma (Clemente et al., 1996) and colon cancer (Galon et al., 2006)

Whilst TILs are capable of recognizing tumor associated antigens through their endogenous TCRs, the small number of TILs isolated from patient tumors without expansion are insufficient for clinical intervention. The discovery of interleukin-2 (IL-2) (Smith et al., 1980a, 1980b) allowed for the *ex vivo* expansion of TILs isolated from patients, with early clinical trials initiated by the pioneers of adoptive immunotherapy, Rosenberg et al. (Rosenberg et al., 1988a; Topalian et al., 1988; Lotze and Rosenberg, 1986) at the National Institutes of Health. The first clinical trial of adoptive immunotherapy involved the administration of IL-2 expanded TILs from 12 patients with advanced renal or breast cancer, colon carcinoma or melanoma. A single dose of cyclophosphamide was used to precondition the patients who were infused with 8×10^9–2.3×10^{11} cells and varying doses of IL-2. Partial tumor regression was reported in one patient with renal carcinoma, one patient with breast cancer and one patient with melanoma (Topalian et al., 1988). These encouraging results led to further trials to assess the combination of lymphodepleting regimens and the use of IL-2 for enhancing the efficacy of adoptive T cell therapy.

Early preclinical and clinical studies for metastatic melanoma were pioneered by Rosenberg et al. (Rosenberg et al., 1988a; Lotze and Rosenberg, 1986). Tumor reactive TILs were cultured with fresh melanoma samples or melanoma cell lines, and assessed for their ability to secrete cytokines and lyse target cells *in vitro* (Topalian et al., 1987). Following selection for antigen recognition, these TILs were then activated and expanded *ex vivo* in the absence of an immunosuppressive tumor microenvironment. Patients also received high doses of IL-2 to support the activation and proliferation of infused T cells (Forni et al., 1986; Testa et al., 1990). In a preliminary clinical trial with metastatic melanoma, Rosenberg et al. found that 11/20 of patients treated with this regimen had objective regression of their tumors in various sites of the body that lasted from 2 to greater than 13 months (Rosenberg et al., 1988a). The protocol used in this trial became the foundation of future clinical trials.

In these early trials, although some patients experienced long lasting regression, the majority of anti-tumor responses were short-lived. To determine the persistence and distribution of the infused TILs in patients, Rosenberg et al. retrovirally transduced TILs to express the neomycin resistance gene, which allowed the cells to be tracked following infusion (Rosenberg et al., 1990). Using polymerase chain reaction (PCR) analysis, they reported minimal numbers of TILs in the patients' peripheral blood and tumor biopsy and that these numbers decreased significantly after 3 weeks. The relatively short life span and poor infiltration of the infused TILs into the tumor bed warranted further investigation to enhance the tumoricidal capacity of the transferred T cells.

2.2. Lymphodepletion in adoptive immunotherapy

Murine models have demonstrated that lymphodepletion using nonmyeloablative sublethal total body irradiation (TBI) prior to TIL infusion greatly augmented the efficacy of adoptive immunotherapy (Wrzesinski et al., 2010; Gattinoni et al., 2005a). The mechanisms as to why lymphodepletion is beneficial prior to the infusion of T cells are still not completely understood. It has been shown that there is an increase in freely available homeostatic cytokines such as IL-2, IL-7 and IL-15 in the absence of cellular 'sinks', a phenomenon where endogenous lymphocytes such as T cells and natural killer cells (NKs) compete with transferred T cells for cytokine support (Wrzesinski et al., 2010; Gattinoni et al., 2005a; Klebanoff et al., 2005a). Elimination of immunosuppressive cells such as myeloid derived suppressor cells (MDSCs) and T regulatory cells (T_{regs}) and their secreted inhibitory cytokines such as IL-10 may also play a role in this enhanced anti-tumor effect (Gajewski et al., 2013; Gabrilovich et al., 2012). It is also believed that damage to the mucosal surfaces releases toll like receptor (TLR) agonists, inducing dendritic cells (DCs) to mature and stimulate expansion of the activated infused T cells (Kieper et al., 2005; Hill et al., 1997). Therefore, it appears that lymphodepletion using irradiation and chemotherapy are able to skew the tumor microenvironment, depleting pro-tumoral elements and subsequently allowing for a beneficial shift in the ratio of infused T cells to suppressive cells.

These preclinical findings prompted the investigation of the preconditioning regimen including TBI and lymphodepleting prior to adoptive transfer, which results in the temporary elimination of host immune cells from the patient. Several clinical trials treating patients with metastatic melanoma utilized increasing doses of lymphodepletion, achieving objective response rates of 49–72% (Dudley et al., 2005; Rosenberg et al., 2008, 2011) (the response evaluation criteria in solid tumors (RECIST) was used in these studies). In a report consisting of three sequential clinical trials of 93 patients with measurable metastatic melanoma, the combination of chemotherapy, TBI and adoptive immunotherapy led to the regression of melanoma metastasis in the lung, liver, brain, bone, lymph nodes and subcutaneous tissue, which resulted in objective response rates of 72%, with some complete responses reported for more than 3 years at the time of publication (Rosenberg et al., 2011). The results were more remarkable given that some tumors were rendered inoperable due to their size and location.

2.3. Optimal phenotype of infused T cells

While the *ex vivo* culture conditions for T cell expansion favor a more effector phenotype, several studies have focused on investigating which phenotype of infused T cells will thrive and induce the greatest anti-tumor effect following infusion. Early pre-clinical and clinical protocols for the generation of TILs involved the activation and expansion of T cells into large numbers *in vitro* with IL-2. T cells with attributes such as interferon-γ (IFN-γ) secretion (Barth et al., 1991) and cytotoxicity toward tumor cells (Aebersold et al., 1991; Schwartzentruber et al., 1994) were isolated and infused into patients. These T cells were highly activated and had an effector

phenotype, however more recent investigations indicate that additional parameters such as TIL differentiation status and memory phenotypes are important determinants in anti-tumor activity and persistence following transfer (Klebanoff et al., 2005b; Gattinoni et al., 2005b; Wolfl et al., 2011).

Data emerging from mouse models demonstrated that less differentiated TILs have superior *in vivo* antitumor properties. Gattinoni et al. (2005b) performed studies where B16 melanoma tumor-bearing mice were treated with active immunization, IL-2 and T cells in varying differentiation states. They reported that despite mature effector T cells (T_{EFF}) demonstrating a greater capacity for IFN-γ secretion and cytotoxicity *in vitro*, the differentiation stages of T_{EFF} cells were inversely related to their anti-tumor activity and proliferative capacity *in vivo*. In addition to the maturation status, emerging evidence also demonstrated that T cells with memory properties are superior candidates for adoptive immune therapy. Based on their phenotypic markers, function and migratory properties, memory T cells can be categorized into two populations, central memory (T_{CM}) and effector memory (T_{EM}) cells (Kaech et al., 2002). Gene expression analysis of these tumor-reactive $CD8^+$ T cell memory subsets have showed that T_{CM} cells have lower expression of pro-apoptotic signaling genes, Bid, Bnip3 and Bad and higher expression of genes associated with trafficking to secondary lymphoid organs, that include CD62L, CXCR3, CCR7 compared to T_{EM} cells (Klebanoff et al., 2005b). Tumor-reactive T_{CM} cells also mediated greater tumor regression and survival of B16 melanoma-bearing mice compared to T_{EM} cells, indicating that T cells with a T_{CM} phenotype are superior over T_{EM} cells for adoptive immunotherapy.

There has also been increasing interest in a population of $CD8^+$ T memory stem (T_{SCM}) cells. T_{SCM} cells were first identified in a model of graft *vs* host disease (GVHD) (Zhang et al., 2005), as self-renewing T cells capable of generating T_{CM}, T_{EM} and T_{EFF} cells, expressing CD27 and lymphoid homing molecules such as CCR7 and CD62L (Gattinoni et al., 2011). These properties make T_{SCM} cells suited for use in adoptive immunotherapy as they are able to mediate long-term immunity and generate various T cell phenotypes. A comparison of T_{EM}, T_{CM} and T_{SCM} cells demonstrated that T_{SCM} cells were superior to T_{EM} and T_{CM} cells in the ability to mediate tumor regression in a mouse model (Klebanoff et al., 2011).

In addition to the differentiation status and memory phenotype, it is also believed that shorter *ex vivo* culture processing is beneficial as prolonged cell culture conditions can result in cells reaching replicative senescence. Hinrichs et al. reported that T_{EFF} cells derived from naïve $CD8^+$ T cells exhibited greater anti-tumor immunity compared to the T cells derived from T_{CM} cells. The T_{EFF} cells derived from naïve T cells showed low levels of KLRG-1 expression, a marker for replicative senescence and cell terminal differentiation (Hinrichs et al., 2009). This study implies that naïve rather than T_{CM} cells may be the important source of T_{EFF} cells and prevention of terminal differentiation is beneficial in the generation of T_{EFF} cells for adoptive immunotherapy of cancer. A lack of telomerase activity and shortening of telomere lengths have also been correlated with poor persistence of infused TIL in patients with metastatic melanoma (Shen et al., 2007). In human $CD8^+$ T cells, telomeres are longer in naïve T cells than memory T cell subsets (Weng et al., 1995) and this may account for the improved persistence of TILs generated from naïve T cells. In clinical trials performed by Rosenberg et al., the number and percent of $CD8^+$ $CD27^+$ T cells in cultures as well as the length of telomere repeats in chromosomes corresponded closely with improved patient outcomes (Rosenberg et al., 2011).

It is possible that the suboptimal results of several early clinical trials were due to the extended culturing process (Topalian et al., 1987; Rosenberg et al., 1988b). Investigations into the ideal phenotype of infused T cells by several groups led to clinical trials utilizing 'young' or short-term cultured TILs (Besser et al., 2010; Dudley et al., 2010). The benefits of the protocol to generate younger TILs, included a much shorter culture period which is more cost effective and less labor intensive. Other advantages of the shorter protocol include the generation of T cells with higher levels of CD27 and longer telomeres (Tran et al., 2008) and increased survival of $CD4^+$ T cells, which may also contribute to tumor immunity (Bevan, 2004; Ossendorp et al., 1998), although this requires further investigation.

In a Phase II clinical trial using short-term cultured TILs in a cohort of 20 metastatic melanoma patients, Besser et al. (2010) reported that non-selected 'young' TILs that had been expanded for 14 days could mediate tumor regression in 50% of recipients. In another trial, Dudley et al. (2010) compared two different lymphodepletion protocols in combination with 'young' TIL therapy. Objective responses were observed in 58% and 48% of patients treated with young TILs and non-myeloablative conditioning or TBI respectively. As a result of these preclinical and clinical studies, more naïve and less differentiated T cells appear to be better suited to adoptive immunotherapy. Phase III clinical trials comparing 'young' TIL and existing TIL protocols need to be initiated in order to determine which populations are best for the treatment of cancer.

3. Genetic modification of T cells

Due to the success of adoptive immunotherapy for the treatment of metastatic melanoma, numerous attempts have been made to isolate and expand tumor reactive TILs in other malignancies, including renal cell carcinoma (RCC), ovarian, colorectal, and breast cancer (Baxevanis et al., 1994; Bouet-Toussaint et al., 2000; Freedman et al., 1994; Yannelli et al., 1996; Mulder et al., 1995). Figlin et al. (1997) reported that a clinical trial combining nephrectomy, IL-2 and $CD8^+$ TILs for the treatment of metastatic RCC resulted in moderate clinical benefit, where 9.1% and 25.5% of patients had a complete response and partial response respectively. However, a subsequent Phase III trial with the same protocol showed no clinical benefit (Figlin et al., 1999). The poor outcome of the adoptive immunotherapy trials in solid cancers other than melanoma may be due to several factors including the reduced immunogenicity of tumor-associated antigens (TAAs) in cancers other than melanoma. In addition, exomic sequencing data indicated that melanomas contain more mutations than other type of solid cancers (Walia et al., 2012). Therefore, it is likely that T cells recognizing these neo-antigens have not been deleted by the thymus.

Nevertheless, the poor clinical response in other solid cancer settings and some inherent limitations of TIL therapy, such as the need to perform invasive procedures to obtain TILs and the inability to grow TILs from some patients, has led to the development of alternative approaches to generate tumor reactive T cells. Development in genetic modification techniques has resulted in the ability to engineer T cells to endow them with anti-tumor activity, using TCRs or CARs specific for a range of TAAs. To date, the use of gene-modified T cells has been investigated for the treatment of several types of cancer including ovarian cancer, leukemia, lymphoma and RCC (Kershaw et al., 1996, 2006; Lamers et al., 2006; Till et al., 2008). The types of T cells used in adoptive immunotherapy are shown in Fig. 1.

3.1. Preclinical studies using TCR modified T cells

TCR gene therapy involves the transfer of tumor-specific TCRαβ genes into autologous T cells, which are then expanded *ex vivo* and re-infused back into the patient. The choice of TCR is dependent on the target antigen and human leukocyte antigen (HLA) alleles expressed by the patient. TCR gene therapy has been

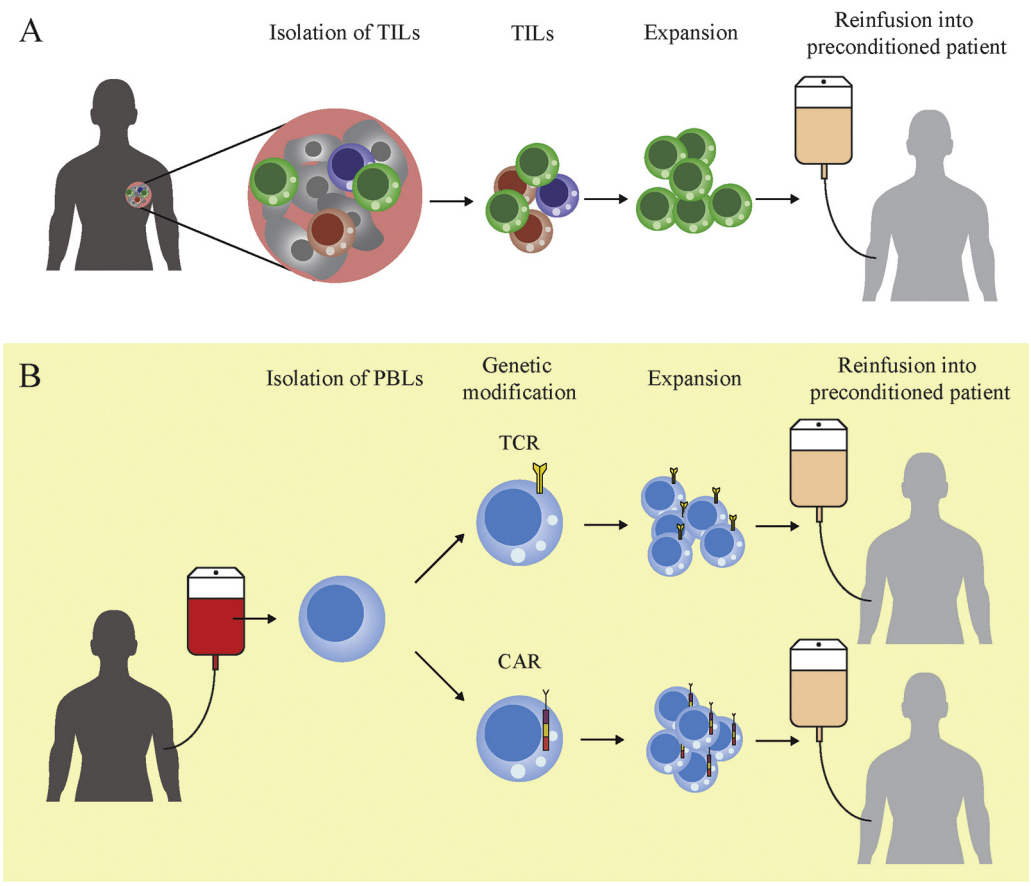

Figure 1. Treating patients with TILs or genetically modified tumor-reactive T cells.
In melanoma patients treated with TILs **(A)**, tumors are surgically resected and the TILs are isolated and expanded before reinfusion back into the patient, who may undergo pre-conditioning with chemotherapy and TBI. In order to generate tumor-specific T cells for targeting other malignancies. **(B)**, T cells from patient peripheral blood are isolated and expanded in culture and genetically modified to express either a TCR with specificity for tumor peptide presented on MHC or express a CAR containing a scFv extracellular domain with specificity for TAA linked to transmembrane and cytoplasmic T cell signaling domains. Depicted is a third generation CAR containing the costimulatory domains CD28 and 4-1BB linked to the CD3ζ chain. These *ex vivo* expanded T cells are reinfused back into pre-conditioned patients. PBL, peripheral blood lymphocyte; TBI, total body irradiation.

investigated for the treatment of cancer and viral-induced malignancies (Gehring et al., 2011; Scholten et al., 2011; Jurgens et al., 2006).

Early preclinical studies utilizing TCR modified T cells focused on the treatment of melanoma, using TCR specific for antigens including melanoma antigen recognized by T cells-1 (MART1), tyrosinase and gp100. Although efforts have been made to isolate TCRs for TAAs in other cancers, tumor specific TILs have proved difficult to isolate and expand, therefore TCRs specific for TAAs in other cancers have been generated using alternative techniques (Parkhurst et al., 2009; Stanislawski et al., 2001). One such technique involved immunizing transgenic mice such as HLA-A2.1 mice with human peptides, resulting in the expansion of murine $CD8^+$ T cell clones with peptide specific reactivity. Subsequently, the TCR αβ chains were cloned into viral vectors and used to transduce TILs. This technique was first used to isolate p53 reactive TCRs (Stanislawski et al., 2001). Another method to enhance the affinity of isolated TCRs is to use phage display techniques. This involves the display of millions of TCR sequences on bacteriophage coat proteins and the identification of tumor reactive TCRs which bind to tumor antigen peptide (Li et al., 2005). These approaches have been successfully used to isolate and transduce T cells with TCRs specific for multiple antigens including Wilms tumor 1 (WTI) (Xue et al., 2005), carcinoembryonic antigen (CEA) (Parkhurst et al., 2009) and murine double-minute 2 (MDM2) (Stanislawski et al., 2001). The use of TCRs isolated from patients or generated using other techniques has been evaluated in clinical trials for the treatment of cancers including melanoma and colorectal cancer (Robbins et al., 2011; Parkhurst et al., 2011).

3.2. *Clinical trials using TCR modified T cells*

TCR gene therapy was first investigated for the treatment of patients with metastatic melanoma. In an encouraging pre-clinical study, TCR αβ genes were isolated from a tumor reactive cytotoxic T lymphocyte (CTL) clone specific for the TAA MART-1 (Cole et al., 1995). Transduction of human peripheral blood lymphocytes (PBL) with the TCR α and β chains was shown to produce CTL with anti-tumor reactivity *in vitro* and could potentially treat patients with metastatic melanoma (Clay et al., 1999). Subsequently, the first clinical trial using genetically engineered MART-1 specific TCRs involved 15 metastatic melanoma patients (Morgan et al., 2006). In this trial, two patients experienced objective responses and modified cells were detected 1 year after treatment. This was the first demonstration of clinical efficacy due to TCR modified T cells in metastatic melanoma. Another clinical trial by the same group (Johnson et al., 2009) utilized higher affinity MART-1 specific T cells and reported objective responses in 30% and 19% of patients respectively. However, investigators also reported the targeting of normal melanocytes in the skin, eye and ear in some patients. This was the first report of "on-target/off-tumor" autoimmunity from TCR modified T cells. Other melanoma target differentiation antigens studied include tyrosinase and gp100, also derived from patients that had

been successfully treated with TIL therapy (Kawakami et al., 1995; Kawakami and Rosenberg, 1997).

Cancer-testis antigens (CTAs) are good candidates for TCR gene therapy as CTAs are expressed by some tumors and are restricted to male germ cells in testis but not in adult somatic tissues. Robbins et al. (2011) utilized TCR modified PBLs targeting the CTA NY-ESO-1 that is expressed on a range of epithelial cancers including breast, lung, prostate and ovarian (Barrow et al., 2006; Chen et al., 1997; Goydos et al., 2001; Kienstra et al., 2003). Response rates of 45% and 67% were reported in patients with melanoma and synovial cell sarcoma, respectively. No antigen specific toxicity was observed. This study highlighted the potential of using CTAs as targets in adoptive immunotherapy. Table 1 provides a summary of some of the clinical trials using TCR modified T cells for the treatment of cancer.

3.3. Limitations associated with TCR modified T cells

There are several limitations for using TCR modified T cells for adoptive immunotherapy. One of the main hurdles is that the effect is dependent on HLA/MHC presentation. However, a variety of cancers downregulate their HLA/MHCI (Bubenik, 2004) as a method of immune escape. In addition, TCR mispairing has also proven to be an issue. In preclinical mouse models, the combination of endogenous and transduced TCR has resulted in TCR mispairing, with the formation of potentially auto-reactive TCRs (Bendle et al., 2010). Strategies to reduce the incidence of mispairing have included the introduction of cysteine bonds, leucine zipper motifs and human-murine hybrid TCRs in order to stabilize TCR chains (Chang et al., 1994; Cohen et al., 2006, 2007; Kuball et al., 2007). Furthermore, the safety of retroviral transduction and its potential to insert and enhance dormant oncogenes, on-target/off-tumor toxicity due to infused T cells targeting antigen on normal tissues are also of concern (Parkhurst et al., 2011; Johnson et al., 2009; Linette et al., 2013; Morgan et al., 2013). Strategies to address these concerns are discussed in the latter part of the review, in Section 4.2.

3.4. The development of chimeric antigen receptors

In the past few decades, a novel form of adoptive immunotherapy involving the generation of modified CTL expressing CARs has been developed, which has broadened the application of adoptive immunotherapy to cancers other than melanoma. CARs are generally composed of the single-chain variable fragment (scFv) specific for a TAA (Wang et al., 2010; Kalos et al., 2011). The scFv is linked *via* hinge and transmembrane domains to intracellular cytoplasmic signaling domains, which are capable of triggering T cell activation. Thus, CARs do not require MHCI presentation and provide an alternative to TCR gene therapies. Eshhar et al. (1993) first reported the use of CAR in T cells which had been endowed with the extracellular scFv specific for the hapten 2,4,6-trinitrophenyl (TNP) linked to either the intracellular FcRγ or CD3ζ chain. T cells modified with this CAR were shown to mediate specific IL-2 secretion and induce target cell lysis. Since then, CAR design has progressed from the initial first generation CARs (with FcRγ or CD3ζ chains) to second generation CARs (incorporating additional cytoplasmic co-stimulatory domains such as CD28 and CD137 (Kalos et al., 2011; Moeller et al., 2005) and subsequently third generation CARs (comprising three signaling domains) (Zhong et al., 2010; Zhao et al., 2009; Kershaw et al., 2013). However, it remains unclear what number and combination of signaling domains are necessary for eliciting maximal anti-tumor responses.

The use of CARs confer several advantages over TCR transgenes. In addition to the recognition of antigen independent of the expression of MHC, CARs are able to bypass some of the mechanisms that tumors employ to evade immune detection. For example, CARs contain intracellular signaling domains that are designed to compensate the down-regulation of co-stimulatory molecules by cancer. Furthermore, CARs are not only able to target protein antigens but also carbohydrate (Westwood et al., 2005, 2009; Mezzanzanica et al., 1998; Craddock et al., 2010) and lipid antigens as well as any antigens that can be recognized by an antibody.

Nevertheless, CARs are only able to detect antigens on the cell surface and if tumors lose these antigens, they can escape CAR detection. Furthermore, current CARs incorporate only a fraction of the signaling molecules normally associated with TCR-mediated signaling, and as such CAR-mediated responses still fall short of achieving optimal activity from T cells, as illustrated by the robust TCR-mediated responses against pathogens (van Lier et al., 2003). Therefore studies are currently being performed to determine which combination of signaling molecules will provide optimal activation of a T cell.

3.5. Clinical trials utilizing chimeric antigen receptors

Due to promising preclinical results, clinical trials have been performed to evaluate the efficacy of different CARs against a range of cancers and these trials have generated remarkable responses in patients with hematological malignancies (Maude et al., 2014; Gill et al., 2014; Brentjens et al., 2013). A summary of these trials is shown in Table 2. First generation CARs containing the signaling domain CD3ζ or FcRγ used for the treatment of various cancers including ovarian, colorectal, renal cell carcinoma and neuroblastoma have generated mixed results (Park et al., 2007; Kershaw et al., 2006; Warren et al., 1998; Louis et al., 2011; Lamers et al., 2013). The first study to demonstrate complete responses in patients treated with CAR modified T cells in solid cancer involved the treatment of 3 neuroblastoma patients with the first generation GD2 specific CAR containing the CD3ζ signaling domain (Park et al., 2007; Louis et al., 2011). However, other trials utilizing first generation CARs have resulted in only modest anti-tumor activity (Kershaw et al., 2006; Lamers et al., 2011), therefore CARs with additional signaling domains were tested subsequently.

Clinical trials utilizing second generation CARs for the treatment of hematological malignancies have resulted in the most encouraging therapeutic responses (Grupp et al., 2013; Kalos et al., 2011; Brentjens et al., 2011, 2013; Porter et al., 2011; Kochenderfer et al., 2010). Kalos et al. (2011) utilized a CD19 specific CAR comprising the domains CD137 and CD3ζ to treat patients with chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL). Two out of the three CLL patients experienced complete responses, whilst the third patient experienced a partial response for 8 months. This landmark study was the first to demonstrate the curative potential of CAR T cells. This study also reported that infused T cells were able to expand >1000 fold, infiltrate the bone marrow and remained detectable 6 months post-infusion. Furthermore, each infused T cell was calculated to be responsible for the elimination of more than 1000 CLL cells, indicating the extensive cytotoxic capabilities of CAR modified T cells. In the ALL study, both patients experienced molecular remission, however one patient relapsed with CD19 negative disease (Grupp et al., 2013). Brentjens' et al. also utilized a second generation CD19 specific CAR, with the signaling domains CD28–CD3ζ for the treatment of ALL. They achieved 88% (14 patients) complete responses out of the 16 patients enrolled in the trial (Davila et al., 2014). However, clinical trials targeting the CD19 antigen have reported on-target/off-tumor toxicity where patients experienced B cell aplasia, due to the targeting of CD19 on B cells (Kochenderfer et al., 2010).

The use of third generation CAR T cells in clinical trials has been limited to trials for lymphoma (Till et al., 2012) and colon cancer (Morgan et al., 2010). Till et al. (2012) treated lymphoma patients with a CD20 CAR composed of CD3ζ, CD28 and CD137

Table 1
Summary of clinical trials utilizing TCR modified T cells for adoptive immunotherapy.

Target antigen	Cancer	Results (number of patients treated in trial)	Reference
gp100	Melanoma	One complete response and two partial responses (16)	Johnson et al. (2009)
MART-1/Melan-A	Melanoma	One partial response (15)	Duval et al. (2006)
		Four objective responses (31)	Morgan et al. (2006), Burns et al. (2009)
		Six partial responses (20)	Johnson et al. (2009)
p53	Melanoma	One partial response (14)	Davis et al. (2010)
NY-ESO-1	Melanoma and synovial sarcoma	Two complete responses and seven partial responses (17)	Robbins et al. (2011)
	Multiple myeloma	Three complete responses and seven partial responses (11)	Rapoport et al. (2010)
CEA	Colorectal	One partial response (3)	Parkhurst et al. (2011)
MAGE A-3	Melanoma, esophageal, synovial sarcoma	One complete response and four partial responses (9)	Morgan et al. (2013)
	Melanoma and multiple myeloma	Lethal cardiac toxicity (2)	Linette et al. (2013)
HBsAg	HBV-related hepatocellularcarcinoma (HCC)	No clinical response (1)	Qasim et al. (2014)

Abbreviations: CEA, carcinoembryonic antigen; HBsAg, Hepatitis B surface antigen; MART-1, melanoma antigen recognized by T cells; Melan-A, melanocyte antigen; MAGE A-3, melanoma associate antigen 3; NY-ESO-1, New York esophageal squamous cell carcinoma 1.

and reported two of the four patients treated experienced no disease progression for 24 months, with the third patient showing objective partial regression. These early results are promising although a larger cohort of patients will need to be assessed in order to determine whether this approach is better than other FDA approved therapies targeting CD20, such as Rituximab (McLaughlin et al., 1998; Coiffier et al., 2002). Morgan et al. utilized a similar CAR targeted to human epidermal growth factor receptor 2 (Her2/erbB2) in colon cancer, however the first patient treated experienced respiratory failure. This was likely due to the targeting of infused T cells to erbB2$^+$ lung epithelial cells and subsequently resulted in the cessation of the trial (Morgan et al., 2010). The results of these two trials highlight the need for further trials using third generation CARs.

Table 2
Summary of clinical trials utilizing CAR modified T cells for adoptive immunotherapy.

Target antigen	Cancer	Receptor	Results (number of patients treated in trial)	Reference/Clinical Trial ID
CD19	CLL	scFv-CD137-CD3ζ	Two complete responses and one partial response (3)	Kalos et al. (2011), Porter et al. (2011)
	ALL	scFv-CD137-CD3ζ	Molecular remission in both patients, one relapsed with antigen negative disease (2)	Grupp et al. (2013) Lee et al. (2014) Brentjens et al. (2011)
	ALL	scFv-CD28-CD3ζ	Overall complete response rate of 88% (16)	
	ALL and non-Hodgkin lymphoma	scFv-CD28-CD3ζ	Overall complete response rate of 66.7% (21)	
	CLL and B-ALL	scFv-CD28-CD3ζ	Stabilized disease (4)	
erbB2	Colorectal	scFv-CD28-CD137-CD3ζ	Trial ceased due to patient death (1)	Morgan et al. (2010)
anti-FR	Ovarian	scFv- FcεR1γ	No response (14)	Kershaw et al. (2006)
GD2	Neuroblastoma	scFv- CD3ζ	Three complete responses (19)	Louis et al. (2011)
Lewis Y	AML	scFv-CD28-CD3ζ	One patient experienced cytogenetic remission (4)	Ritchie et al. (2013)
CD20	NHL and mantle cell lymphoma	scFv-CD3ζ	Two complete response, one partial response, four with stable disease (7)	Till et al. (2008)
	NHL	scFv-CD137-CD28-CD3ζ	One partial response, two patients without evaluable disease remained progression free (3)	Till et al. (2012)
CAIX	Metastatic RCC	scFv-CD3ζ	No response (3)	Lamers et al. (2006)
	Metastatic RCC		No response (9) (Two out of nine experienced autoimmune liver toxicity)	Lamers et al. (2013)
CD171	Neuroblastoma	scFv- CD3ζ	One partial response (6)	Park et al. (2007)
GD2	Neuroblastoma	scFv-CD28-OX40-CD3ζ	Currently in progress	NCT01822652
	Non-neuroblastoma GD2$^+$ solid tumors			NCT02107963
FR	Ovarian	scFv-CD137- CD3ζ	Proposed	Kandalaft et al. (2012)
F19 protein (FAP)	FAP$^+$ malignant pleural mesothelioma (FAPME-1)	scFv-CD28- CD3ζ	Proposed	Petrausch et al. (2012)
erbB2	Head and neck squamous cell carcinoma	scFv-CD28-CD3ζ	Proposed	van Schalkwyk et al. (2013)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAIX, carbonic anhydrase IX; CLL, chronic lymphocytic leukemia; erbB2, erythroblastosis oncogene B2; FAP, fibroblast activation protein (associated with asbestos); FR, folate receptor; GD2, ganglioside; NHL, non-Hodgkin's lymphoma; RCC, renal cell carcinoma.

Table 3
Adverse events associated with adoptive immunotherapy clinical trials.

Adoptive immunotherapy	Target antigen	Tumor	Adverse events	References
TIL with lymphodepletion and IL-2	Various	Melanoma	Vitiligo (4/13), uveitis (1/1)	Dudley et al. (2002)
Young TIL with lymphodepletion and IL-2	Various	Metastatic melanoma	Vitiligo (1/20)	Besser et al. (2010)
CTL	MART-1	Melanoma	Melanocyte destruction (1/1)	Yee et al. (2000)
TIL and IL-2	Various	Melanoma	Autoimmune thyroiditis (1/25)	Ridolfi et al. (2003)
TIL and IL-2	Various	Melanoma	Systemic and ocular autoimmunity (1/1)	Yeh et al. (2009)
Gene-modified T cells expressing TCR	MART-1, gp100	Melanoma	Melanocyte destruction (29/36)	Johnson et al. (2009)
Gene-modified T cells expressing TCR	MAGE-A3	Melanoma	Neurological toxicity (3/9)	Morgan et al. (2013)
Gene-modified T cells expressing TCR	MAGE-A3	Melanoma and myeloma	Cardiac toxicity (2/2)	Linette et al. (2013)
Gene-modified T cells expressing TCR	CEA	Colorectal cancer	Colitis (3/3)	Parkhurst et al. (2011)
CTL with CAR for CD19	CD19	CLL and ALL	B cell aplasia (3/3)	Kalos et al. (2011)
CTL with CAR for CAIX	CAIX	RCC	Liver toxicity (grade III-IV) (3/3)	Lamers et al. (2006)
CTL with CAR for erbB2	erbB2	Colon cancer	Respiratory failure (1/1)	Morgan et al. (2010)

Abbreviations: ALL, acute lymphoblastic leukemia; CAIX, carbonic anhydrase IX; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukemia; CTL, cytotoxic T lymphocyte; TCR, erbB2, erythroblastosis oncogene B2; MART-1, melanoma antigen recognized by T cells; T cell receptor; TIL, tumor infiltrating lymphocyte.

Clinical trials in neuroblastoma and hematological malignancies highlight the potential of using CAR modified T cells for the treatment of cancer. However, in order to fully harness the capabilities of CAR T cells for the treatment of solid cancers, further studies to characterize and optimize CARs are required.

4. The future of adoptive immunotherapy

Whilst CAR modified T cells have shown great potential in the clinic, future studies are still required to potentially enhance the activity of these chimeric receptors whilst ensuring toxicity does not occur. This includes testing the combination of these CARs with therapies such as checkpoint inhibitors, addressing autoimmunity and the widespread implementation of adoptive immunotherapy as a standard of care therapy.

4.1. Combination of adoptive immunotherapy with existing therapies

Although adoptive immunotherapy using CAR modified T cells for some cancers such as hematological malignancies and neuroblastoma have shown early promise, clinical trials against other solid cancers have only shown moderate results to date. To potentially enhance the anti-tumor effect, CAR T cell therapy could be combined with immune checkpoint blockade antibodies, such as antibodies against cytotoxic T lymphocyte protein-4 (CTLA-4) (Hodi et al., 2010) and programmed death receptor-1 (PD-1) (Topalian et al., 2012) which have shown tremendous anti-tumor activity in patients as single agents or together in combination, particularly against metastatic melanoma with response rates of 40% (Wolchok et al., 2013). Studies have demonstrated that combining adoptive immunotherapy with an anti-PD-1 antibody (Peng et al., 2012; John et al., 2013) resulted in enhanced efficacy of infused T cells. This was found to be due to increased level of IFN-γ from CAR T cells at the tumor site (Peng et al., 2012). Other checkpoint pathways currently under investigation include lymphocyte-activation gene 3 (LAG-3) (Huang et al., 2004), which is being tested in a phase III breast cancer trial and T-cell immunoglobulin domain and mucin domain 2 (TIM-3) (Zhu et al., 2005), which is still in preclinical development. Therapeutic agents have also been developed to target the inhibitory ligands B7-H3 (Loo et al., 2012) (currently in clinical trials) and B7-H4 (He et al., 2011). Several studies have also shown that targeting multiple checkpoint pathways such as PD-1 and TIM-3 can result in the reversal of T cell exhaustion (Fourcade et al., 2010; Sakuishi et al., 2010), and therefore the combination of multiple checkpoint inhibitors with CAR T cells warrants further investigation. Another target currently under investigation is the ectonucleotidase, CD73, which is upregulated in some cancers and converts immunostimulatory ATP to immunosuppressive adenosine. The accumulation of adenosine, which binds to the A2A receptor impairs anti-tumor T cell responses and NK cell function resulting in the promotion of tumor growth (Spychala, 2000; Deaglio et al., 2007; Beavis et al., 2013). The use of A2A receptor antagonists to target the CD73 pathway has been used in combination with CTLA-4 blockade, which resulted in enhanced anti-tumor immune responses (Iannone et al., 2014). Furthermore, it has been proposed that through other rational combinations, that include epigenetic modulators, such as histone deacetylase inhibitors (Sigalotti et al., 2005), it may be possible to change the immune profile of tumor cells, and therefore increase their recognition and susceptibility to the immune system (Skov et al., 2005; Magner et al., 2000). In future studies, it will be interesting to determine which immune therapies when combined with CAR T cells mediate the most effective therapeutic responses.

4.2. Adverse events

The targeting of normal healthy tissue by genetically modified infused T cells has been reported in several clinical trials (Lamers et al., 2006; Morgan et al., 2010, 2013; Kochenderfer et al., 2010; Cameron et al., 2013) in some cases resulting in the death of patients (summarized in Table 3). Several strategies have been developed (summarized in Table 4) in order to increase the safety of adoptive immunotherapy, including the incorporation of suicide genes (Bonini et al., 1997; Budde et al., 2013; Marin et al., 2012; Sato et al., 2007; Straathof et al., 2005; Tiberghien et al., 1994) and the administration of antibodies which are capable of depleting auto-reactive infused cells (Kieback et al., 2008; Vogler et al., 2010; Wang et al., 2011). The incorporation of suicide genes such as herpes simplex virus-thymidine kinase (Bonini et al., 1997; Berger et al., 2006) or inducible caspase 9 (Budde et al., 2013; Straathof et al., 2005; Di Stasi et al., 2011; Quintarelli et al., 2007; Hoyos et al., 2010) allows cells to be eliminated with the administration of ganciclovir or a chemical inducer of dimerization respectively. Both are currently being evaluated in clinical trials. The use of FDA approved antibodies Rituximab (Vogler et al., 2010; Serafini et al., 2004; Introna et al., 2000) and Cetuximab (Wang et al., 2011) are currently being investigated in preclinical models to deplete infused genetically

Table 4
Approaches to increase the safety of CAR T cells in adoptive immunotherapy.

Approach	Advantage	Disadvantage	Status	References
HSV-TK suicide gene	Eliminate T cells with the anti-viral drug ganciclovir	Ganciclovir treatment for viral infections will eliminate infused T cells HSV-TK specific T cells detected Loss of therapy	Clinical trial in GVHD	Park et al. (2007), Bonini et al. (1997), Berger et al. (2006)
Inducible caspase 9 (iCasp9)	Eliminate T cells with chemical inducer of dimerization such as AP1903	Loss of therapy	Clinical trial in GVHD Clinical trial in CAR T cells commenced	Budde et al. (2013), Straathof et al. (2005), Di Stasi et al. (2011), Quintarelli et al. (2007), Hoyos et al. (2010)
CD20	Eliminate T cells with Rituximab, a FDA-approved antibody used in non-Hodgkin's lymphoma	Elimination of endogenous B cells Loss of therapy	Preclinical studies in GVHD mouse models	Vogler et al. (2010), Serafini et al. (2004), Introna et al. (2000)
mTMPK	Eliminate T cells with AZT, a FDA-approved drug used in HIV	Loss of therapy	*In vitro* data using T cell line No data using CARs	Sato et al. (2007)
Truncated EGFR	Eliminate T cells with Cetuximab, a FDA-approved antibody used in colon cancer	Hypomagnesaemia in patients treated with Cetuximab Loss of therapy	Preclinical studies in mouse model using CARs	Wang et al. (2011)
Myc TAG in CAR	Eliminate T cells with Myc-specific antibody	Loss of therapy	Preclinical studies in mouse models using TCR	Kieback et al. (2008)
Targeting multiple TAAs	Complete activation of T cells occurs when two CARs for different TAAs bind to the same tumor cell	Suboptimal activation of T cells in tumors that lose expression of an antigen	Preclinical studies in mouse models using CARs	Duong et al. (2011), Wilkie et al. (2012), Anurathapan et al. (2014)
Combination of activating signals	Activation is dependent on presence of multiple antigens	Suboptimal activation of T cells in tumors that lose expression of an antigen	Preclinical studies in mouse models using CARs	Kloss et al. (2013)
Targeting TAA and normal tissue antigen	Preservation of normal tissue as activation occurs only in presence of TAA	If inhibition is not complete, damage to normal tissue can still occur	Preclinical studies in mouse models using CARs	Fedorov et al. (2013)

Abbreviations: AZT, 3′-azido-3′-deoxythymidine; CAR, chimeric antigen receptor; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; GVHD, graft *versus* host disease; HIV, human immunodeficiency virus; HSV-TK, herpes simplex virus thymidine kinase; TAA, tumor associated antigen; TCR, T cell receptor.

modified cells. However, as the administration of antibodies or other agents to eradicate infused cells can also ablate the cancer therapy, efforts to address autoimmunity by using a novel approach of multiple chimeric receptors have been investigated (Fedorov et al., 2013; Kloss et al., 2013; Duong et al., 2011). One approach is to genetically modify a T cell so that it expresses two different CARs specific for two TAAs, called dual-specific T cells (Duong et al., 2011). Dual-specific T cells were generated to express CARs specific for the TAAs, erythroblastosis oncogene B2 (erbB2) and folate binding protein (FBP). The dual specific T cells secreted more cytokine and lysed tumor target cells expressing both FBP and Her2, compared to cells expressing FBP or Her2 alone. Although this approach resulted in greater tumor specific responses, cells expressing either antigen alone were not spared completely, therefore autoimmunity could still occur, although possibly to a lesser extent. A recent study utilized an approach where activation of a T cell was dependent on combinatorial recognition of two prostate cancer antigens (Kloss et al., 2013). This approach utilized a combination of anti-prostate specific membrane antigen (PSMA) CAR, which provided suboptimal activation as well as a chimeric costimulatory receptor weakly specific for prostate stem cell antigen (PSCA) in the same T cell. This resulted in the elimination of $PSCA^+PSMA^+$ but not $PSCA^+PSMA^-$ tumor cells in mice. The same group also published a study where they used PD-1 or CTLA-4 incorporated into CARs in an effort to alter the activity of CD19 specific CARs in the same T cell (Fedorov et al., 2013). They generated a PSMA specific chimeric inhibitory receptor (CIR) containing a PD-1 intracellular signaling domain and expressed it in T cells, which had been modified to express a CD19 specific CAR. They demonstrated that T cells, which expressed both CARs exhibited a decrease in effector function when exposed to target cells expressing both CD19 and PSMA, compared to CD19 alone. In the clinical setting, it will be crucial to find the optimal balance between tumor targeting and normal tissue preservation. This could be achieved by altering the ratio of CIRs to CARs, the level of receptor expression (based on the strength of the promoter) and careful selection of antigen based on its expression level and distribution in addition to chimeric receptor affinity. Although most studies have focused on identifying TAAs, some investigations have sought to characterize antigens on normal tissue (Uhlen et al., 2010). In order to translate this approach to the clinic, further investigations are required to determine which normal tissue antigens are expressed on the majority of normal tissue but down-regulated or absent on tumor tissue. One possible target that CIR could be specific for is MHCI (Connor and Stern, 1990) as it is often down-regulated by tumors in order to escape immune detection. Cell surface tumor suppression antigens such as OPMCL, which is frequently inactivated by methylation in cancers including lung, colon and breast (Cui et al., 2008), could also be targeted.

4.3. *Transition of adoptive immunotherapy in clinical trials to a standard of care treatment option*

In order for adoptive immunotherapy to become standard of care, several issues need to be resolved. There is a need to develop consistent clinical protocols, which can be used as a baseline in multiple institutions. This includes the choice of gene modification technique and which chimeric receptor is used for a particular antigen target. In the past 5 years, several papers have been published pertaining to the use of CD19 CAR modified T cells for hematological malignancies (Grupp et al., 2013; Brentjens et al., 2011, 2013; Kochenderfer et al., 2010, 2012; Savoldo et al., 2011). Although some patients have experienced remarkable remissions, other

patients have only experienced partial responses. This is likely due to several factors, including differences in signaling domains present in the CAR, preconditioning regimens and tumor burden prior to treatment. Furthermore, once the most effective signaling domains in CARs for a particular cancer have been established, a cost effective and consistent genetic modification approach needs to be investigated. Some clinical protocols expand and activate T cells using beads (Grupp et al., 2013), however other groups use high dose IL-2 and the anti-CD3 antibody, OKT3 to stimulate cells prior to infusion (Ritchie et al., 2013). Currently, Carl June's group utilizes a lentiviral vector to modify T cells, however the majority of clinical trials have used retroviral transduction (Ritchie et al., 2013). Laurence Cooper's team is investigating a less complex and cheaper technique involving the electroporation of T cells and using the Sleeping Beauty transposon system (Singh et al., 2013). At present, there have been few studies comparing the efficacy of CAR T cells using these different genetic modification approaches, however in order for adoptive immunotherapy to progress, the optimal genetic modification and culturing technique needs to be determined. Although, adoptive immunotherapy currently costs approximately $50,000 per patient, it is anticipated that due to the development of closed systems (Klapper et al., 2009) and following the initial investment into the construction of good manufacturing practice grade facilities, the overall cost of adoptive immunotherapy may significantly decrease. Furthermore, it is hoped that once CAR T cells are an established treatment for cancer, the use of cell banks, similar to those currently being used for Epstein–Barr virus specific T cells (Leen et al., 2013) can be used to store cryopreserved allogeneic CAR T cells. Resolution of these various issues relating to adoptive immunotherapy will enable better comparison of clinical trial results, which would allow for the true value of immunotherapy to be evaluated and possibly its widespread application for the treatment of various types of cancer.

5. Concluding remarks

Adoptive immunotherapy has come of age with success in recent clinical trials, in particular using TCR modified cells in melanoma and CAR T cells in hematological malignancies. However devising new strategies for improving the activity but limiting toxicity of these gene modified T cells in patients may broaden the approach to other cancers and increase the feasibility of adoptive immunotherapy as a standard of care treatment for cancer.

Funding

This work was funded by Program and Project grants from the National Health and Medical Research Council (NHMRC) (#1013667, #1030436). C. Y. Slaney was supported by a National Breast Cancer Fellowship (#PF-12-14). P. K. Darcy and M. H. Kershaw were supported by NHMRC Senior Research Fellowships (#1041828 and 1058388 respectively).

References

Aebersold, P., et al., 1991. Lysis of autologous melanoma cells by tumor-infiltrating lymphocytes: association with clinical response. J. Natl. Cancer Inst. 83 (13), 932–937.
Anurathapan, U., et al., 2014. Kinetics of tumor destruction by chimeric antigen receptor-modified T cells. Mol. Ther. 22 (3), 623–633.
Barrow, C., et al., 2006. Tumor antigen expression in melanoma varies according to antigen and stage. Clin. Cancer Res. 12 (3 Pt 1), 764–771.
Barth Jr., R.J., et al., 1991. Interferon gamma and tumor necrosis factor have a role in tumor regressions mediated by murine CD8+ tumor-infiltrating lymphocytes. J. Exp. Med. 173 (3), 647–658.
Baxevanis, C.N., et al., 1994. Tumor specific cytolysis by tumor infiltrating lymphocytes in breast cancer. Cancer 74 (4), 1275–1282.
Beavis, P.A., et al., 2013. Blockade of A2A receptors potently suppresses the metastasis of CD73+ tumors. Proc. Natl. Acad. Sci. U. S. A. 110 (36), 14711–14716.
Bendle, G.M., et al., 2010. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat. Med. 16 (5), 565–570, 1p following 570.
Berger, C., et al., 2006. Analysis of transgene-specific immune responses that limit the in vivo persistence of adoptively transferred HSV-TK–modified donor T cells after allogeneic hematopoietic cell transplantation. Blood 107 (6), 2294–2302.
Besser, M.J., et al., 2010. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin. Cancer Res. 16 (9), 2646–2655.
Bevan, M.J., 2004. Helping the CD8(+) T-cell response. Nat. Rev. Immunol. 4 (8), 595–602.
Bonini, C., et al., 1997. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science 276 (5319), 1719–1724.
Bouet-Toussaint, F., et al., 2000. Interleukin-2 expanded lymphocytes from lymph node and tumor biopsies of human renal cell carcinoma, breast and ovarian cancer. Eur. Cytokine Netw. 11 (2), 217–224.
Brentjens, R.J., et al., 2011. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118 (18), 4817–4828.
Brentjens, R.J., et al., 2013. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci. Transl. Med. 5 (177), 177ra38.
Bubenik, J., 2004. MHC class I down-regulation: tumour escape from immune surveillance? (review). Int. J. Oncol. 25 (2), 487–491.
Budde, L.E., et al., 2013. Combining a CD20 chimeric antigen receptor and an inducible caspase 9 suicide switch to improve the efficacy and safety of T cell adoptive immunotherapy for lymphoma. PLOS ONE 8 (12), e82742.
Burns, W.R., et al., 2009. Lack of specific gamma-retroviral vector long terminal repeat promoter silencing in patients receiving genetically engineered lymphocytes and activation upon lymphocyte restimulation. Blood 114 (14), 2888–2899.
Cameron, B.J., et al., 2013. Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells. Sci. Transl. Med. 5 (197), 197ra103.
Chang, H.C., et al., 1994. A general method for facilitating heterodimeric pairing between two proteins: application to expression of alpha and beta T-cell receptor extracellular segments. Proc. Natl. Acad. Sci. U. S. A. 91 (24), 11408–11412.
Chen, Y.T., et al., 1997. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. U. S. A. 94 (5), 1914–1918.
Chinnasamy, N., et al., 2011. A TCR targeting the HLA-A*0201-restricted epitope of MAGE-A3 recognizes multiple epitopes of the MAGE-A antigen superfamily in several types of cancer. J. Immunol. 186 (2), 685–696.
Clay, T.M., et al., 1999. Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. J. Immunol. 163 (1), 507–513.
Clemente, C.G., et al., 1996. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77 (7), 1303–1310.
Cohen, C.J., et al., 2006. Enhanced antitumor activity of murine-human hybrid T-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability. Cancer Res. 66 (17), 8878–8886.
Cohen, C.J., et al., 2007. Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond. Cancer Res. 67 (8), 3898–3903.
Coiffier, B., et al., 2002. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N. Engl. J. Med. 346 (4), 235–242.
Cole, D.J., et al., 1995. Characterization of the functional specificity of a cloned T-cell receptor heterodimer recognizing the MART-1 melanoma antigen. Cancer Res. 55 (4), 748–752.
Connor, M.E., Stern, P.L., 1990. Loss of MHC class-I expression in cervical carcinomas. Int. J. Cancer 46 (6), 1029–1034.
Craddock, J.A., et al., 2010. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J. Immunother. 33 (8), 780–788.
Cui, Y., et al., 2008. OPCML is a broad tumor suppressor for multiple carcinomas and lymphomas with frequently epigenetic inactivation. PLoS ONE 3 (8), e2990.
Davila, M.L., et al., 2014. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci. Transl. Med. 6 (224), 224ra25.
Davis, J.L., et al., 2010. Development of human anti-murine T-cell receptor antibodies in both responding and nonresponding patients enrolled in TCR gene therapy trials. Clin. Cancer Res. 16 (23), 5852–5861.
Deaglio, S., et al., 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J. Exp. Med. 204 (6), 1257–1265.
Di Stasi, A., et al., 2011. Inducible apoptosis as a safety switch for adoptive cell therapy. N. Engl. J. Med. 365 (18), 1673–1683.
Dudley, M.E., et al., 2002. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298 (5594), 850–854.
Dudley, M.E., et al., 2005. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J. Clin. Oncol. 23 (10), 2346–2357.
Dudley, M.E., et al., 2010. CD8+ enriched "young" tumor infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin. Cancer Res. 16 (24), 6122–6131.

Duong, C.P., et al., 2011. Enhancing the specificity of T-cell cultures for adoptive immunotherapy of cancer. Immunotherapy 3 (1), 33–48.

Duval, L., et al., 2006. Adoptive transfer of allogeneic cytotoxic T lymphocytes equipped with a HLA-A2 restricted MART-1 T-cell receptor: a phase I trial in metastatic melanoma. Clin. Cancer Res. 12 (4), 1229–1236.

Eshhar, Z., et al., 1993. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc. Natl. Acad. Sci. 90 (2), 720–724.

Fedorov, V.D., Themeli, M., Sadelain, M., 2013. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Sci. Transl. Med. 5 (215), 215ra172.

Figlin, R.A., et al., 1997. Treatment of metastatic renal cell carcinoma with nephrectomy, interleukin-2 and cytokine-primed or CD8(+) selected tumor infiltrating lymphocytes from primary tumor. J. Urol. 158 (3 Pt. 1), 740–745.

Figlin, R.A., et al., 1999. Multicenter, randomized, phase III trial of CD8(+) tumor-infiltrating lymphocytes in combination with recombinant interleukin-2 in metastatic renal cell carcinoma. J. Clin. Oncol. 17 (8), 2521–2529.

Forni, G., et al., 1986. Tumour inhibition by interleukin-2 at the tumour/host interface. Biochim. Biophys. Acta 865 (3), 307–327.

Fourcade, J., et al., 2010. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J. Exp. Med. 207 (10), 2175–2186.

Freedman, R.S., et al., 1994. Large-scale expansion in interleukin-2 of tumor-infiltrating lymphocytes from patients with ovarian carcinoma for adoptive immunotherapy. J. Immunol. Methods 167 (1–2), 145–160.

Gabrilovich, D.I., Ostrand-Rosenberg, S., Bronte, V., 2012. Coordinated regulation of myeloid cells by tumours. Nat. Rev. Immunol. 12 (4), 253–268.

Gajewski, T.F., Schreiber, H., Fu, Y.X., 2013. Innate and adaptive immune cells in the tumor microenvironment. Nat. Immunol. 14 (10), 1014–1022.

Galon, J., et al., 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313 (5795), 1960–1964.

Gattinoni, L., et al., 2005a. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J. Exp. Med. 202 (7), 907–912.

Gattinoni, L., et al., 2005b. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. J. Clin. Invest. 115 (6), 1616–1626.

Gattinoni, L., et al., 2011. A human memory T cell subset with stem cell-like properties. Nat. Med. 17 (10), 1290–1297.

Gehring, A.J., et al., 2011. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. J. Hepatol. 55 (1), 103–110.

Gill, S., et al., 2014. Efficacy against human acute myeloid leukemia and myeloablation of normal hematopoiesis in a mouse model using chimeric antigen receptor-modified T cells. Blood 123 (15), 2343–2354.

Goydos, J.S., Patel, M., Shih, W., 2001. NY-ESO-1 and CTp11 expression may correlate with stage of progression in melanoma. J. Surg. Res. 98 (2), 76–80.

Grupp, S.A., et al., 2013. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N. Engl. J. Med. 368 (16), 1509–1518.

He, C., et al., 2011. The inhibitory role of b7-h4 in antitumor immunity: association with cancer progression and survival. Clin. Dev. Immunol. 2011, p695834.

Hill, G.R., et al., 1997. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood 90 (8), 3204–3213.

Hinrichs, C.S., et al., 2009. Adoptively transferred effector cells derived from naive rather than central memory CD8+ T cells mediate superior antitumor immunity. Proc. Natl. Acad. Sci. U. S. A. 106 (41), 17469–17474.

Hodi, F.S., et al., 2010. Improved survival with ipilimumab in patients with metastatic melanoma. N. Engl. J. Med. 363 (8), 711–723.

Hoyos, V., et al., 2010. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 24 (6), 1160–1170.

Huang, C.T., et al., 2004. Role of LAG-3 in regulatory T cells. Immunity 21 (4), 503–513.

Iannone, R., et al., 2014. Adenosine limits the therapeutic effectiveness of anti-CTLA4 mAb in a mouse melanoma model. Am. J. Cancer Res. 4 (2), 172–181.

Introna, M., et al., 2000. Genetic modification of human T cells with CD20: a strategy to purify and lyse transduced cells with anti-CD20 antibodies. Hum. Gene Ther. 11 (4), 611–620.

John, L.B., et al., 2013. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin. Cancer Res. 19 (20), 5636–5646.

Johnson, L.A., et al., 2009. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114 (3), 535–546.

Jurgens, L.A., et al., 2006. Transduction of primary lymphocytes with Epstein–Barr virus (EBV) latent membrane protein-specific T-cell receptor induces lysis of virus-infected cells: a novel strategy for the treatment of Hodgkin's disease and nasopharyngeal carcinoma. J. Clin. Immunol. 26 (1), 22–32.

Kaech, S.M., Wherry, E.J., Ahmed, R., 2002. Effector and memory T-cell differentiation: implications for vaccine development. Nat. Rev. Immunol. 2 (4), 251–262.

Kalos, M., et al., 2011. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci. Transl. Med. 3 (95), 95ra73.

Kandalaft, L.E., Powell Jr., D.J., Coukos, G., 2012. A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer. J. Transl. Med. 10, 157.

Kawakami, Y., Rosenberg, S., 1997. Immunobiology of human melanoma antigens MART-1 and gp100 and their use for immuno-gene therapy. Int. Rev. Immunol. 14.

Kawakami, Y., et al., 1995. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J. Immunol. 154 (8), 3961–3968.

Kershaw, M., et al., 1996. The use of chimeric human Fc(epsilon) receptor I to redirect cytotoxic T lymphocytes to tumors. J. Leukoc. Biol. 60 (6), 721–728.

Kershaw, M.H., et al., 2006. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin. Cancer Res. 12 (20 Pt. 1), 6106–6115.

Kershaw, M.H., Westwood, J.A., Darcy, P.K., 2013. Gene-engineered T cells for cancer therapy. Nat. Rev. Cancer 13 (8), 525–541.

Kieback, E., et al., 2008. A safeguard eliminates T cell receptor gene-modified autoreactive T cells after adoptive transfer. Proc. Natl. Acad. Sci. U. S. A. 105 (2), 623–628.

Kienstra, M.A., et al., 2003. Identification of NY-ESO-1, MAGE-1, and MAGE-3 in head and neck squamous cell carcinoma. Head Neck 25 (6), 457–463.

Kieper, W.C., et al., 2005. Recent immune status determines the source of antigens that drive homeostatic T cell expansion. J. Immunol. 174 (6), 3158–3163.

Klapper, J.A., et al., 2009. Single-pass, closed-system rapid expansion of lymphocyte cultures for adoptive cell therapy. J. Immunol. Methods 345 (1–2), 90–99.

Klebanoff, C.A., et al., 2005a. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol. 26 (2), 111–117.

Klebanoff, C.A., et al., 2005b. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc. Natl. Acad. Sci. U. S. A. 102 (27), 9571–9576.

Klebanoff, C.A., et al., 2011. Determinants of successful CD8+ T-cell adoptive immunotherapy for large established tumors in mice. Clin. Cancer Res. 17 (16), 5343–5352.

Kloss, C.C., et al., 2013. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat. Biotechnol. 31 (1), 71–75.

Kochenderfer, J.N., et al., 2010. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood 116 (20), 4099–4102.

Kochenderfer, J.N., et al., 2012. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119 (12), 2709–2720.

Kuball, J., et al., 2007. Facilitating matched pairing and expression of TCR chains introduced into human T cells. Blood 109 (6), 2331–2338.

Lamers, C.H., et al., 2006. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J. Clin. Oncol. 24 (13), e20–e22.

Lamers, C.H., et al., 2011. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. Blood 117 (1), 72–82.

Lamers, C.H., et al., 2013. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol. Ther. 21 (4), 904–912.

Lee, D.W., et al., 2014. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. http://dx.doi.org/10.1016/S0140-6736(14)61403-3. Epub ahead of print.

Leen, A.M., et al., 2013. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood 121 (26), 5113–5123.

Li, Y., et al., 2005. Directed evolution of human T-cell receptors with picomolar affinities by phage display. Nat. Biotechnol. 23 (3), 349–354.

Linette, G.P., et al., 2013. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 122 (6), 863–871.

Loi, S., et al., 2013. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J. Clin. Oncol. 31 (7), 860–867.

Loo, D., et al., 2012. Development of an Fc-enhanced anti-B7-H3 monoclonal antibody with potent antitumor activity. Clin. Cancer Res. 18 (14), 3834–3845.

Lotze, M.T., Rosenberg, S.A., 1986. Results of clinical trials with the administration of interleukin 2 and adoptive immunotherapy with activated cells in patients with cancer. Immunobiology 172 (3–5), 420–437.

Louis, C.U., et al., 2011. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 118 (23), 6050–6056.

Magner, W.J., et al., 2000. Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. J. Immunol. 165 (12), 7017–7024.

Marin, V., et al., 2012. Comparison of different suicide-gene strategies for the safety improvement of genetically manipulated T cells. Hum. Gene Ther. Methods 23 (6), 376–386.

Maude, S.L., et al., 2014. Chimeric antigen receptor T cells for sustained remissions in leukemia. N. Engl. J. Med. 371 (16), 1507–1517.

McLaughlin, P., et al., 1998. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J. Clin. Oncol. 16 (8), 2825–2833.
Mezzanzanica, D., et al., 1998. Transfer of chimeric receptor gene made of variable regions of tumor-specific antibody confers anticarbohydrate specificity on T cells. Cancer Gene Ther. 5 (6), 401–407.
Moeller, M., et al., 2005. Adoptive transfer of gene-engineered CD4+ helper T cells induces potent primary and secondary tumor rejection. Blood 106 (9), 2995–3003.
Morgan, R.A., et al., 2006. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 314 (5796), 126–129.
Morgan, R.A., et al., 2010. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol. Ther. 18 (4), 843–851.
Morgan, R.A., et al., 2013. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J. Immunother. 36 (2), 133–151.
Mulder, W.M., et al., 1995. Culture of tumour-infiltrating lymphocytes from melanoma and colon carcinoma: removal of tumour cells does not affect tumour-specificity. Cancer Immunol. Immunother. 41 (5), 293–301.
Ossendorp, F., et al., 1998. Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J. Exp. Med. 187 (5), 693–702.
Park, J.R., et al., 2007. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol. Ther. 15 (4), 825–833.
Parkhurst, M.R., et al., 2009. Characterization of genetically modified T-cell receptors that recognize the CEA:691-699 peptide in the context of HLA-A2.1 on human colorectal cancer cells. Clin. Cancer Res. 15 (1), 169–180.
Parkhurst, M.R., et al., 2011. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol. Ther. 19 (3), 620–626.
Peng, W., et al., 2012. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res. 72 (20), 5209–5218.
Petrausch, U., et al., 2012. Re-directed T cells for the treatment of fibroblast activation protein (FAP)-positive malignant pleural mesothelioma (FAPME-1). BMC Cancer 12, 615.
Porter, D.L., et al., 2011. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. N. Engl. J. Med. 365 (8), 725–733.
Qasim, W., et al., 2014. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells targeting HBsAg in a liver transplant patient. J. Hepatol. http://dx.doi.org/10.1016/j.jhep.2014.10.001. Epub ahead of print.
Quintarelli, C., et al., 2007. Co-expression of cytokine and suicide genes to enhance the activity and safety of tumor-specific cytotoxic T lymphocytes. Blood 110 (8), 2793–2802.
Rapoport, A.P., et al., 2010. Adoptive transfer of gene-modified T-cells engineered to express high-affinity TCRs for cancer-testis antigens (CTAs) NY-ESO-1 or Lage-1, in MM patients post auto-SCT. ASH Annu. Meet. Abstr. 120 (21), 472.
Ridolfi, L., Ridolfi, R., Riccobon, A., De Paola, F., Petrini, M., Stefanelli, M., Flamini, E., Ravaioli, A., Verdecchia, G.M., Trevisan, G., Amadori, D., 2003. Adjuvant adoptive immunotherapy in patients with stage III and resected stage IV melanoma: a pilot study. J. Immunother. 26 (2), 156–162.
Ritchie, D.S., et al., 2013. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol. Ther. 21 (11), 2122–2129.
Robbins, P.F., et al., 2011. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J. Clin. Oncol. 29 (7), 917–924.
Rosenberg, S.A., Fox, E., Churchill, W.H., 1972. Spontaneous regression of hepatic metastases from gastric carcinoma. Cancer 29 (2), 472–474.
Rosenberg, S., et al., 1988a. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N. Engl. J. Med. 319 (25), 1676–1680.
Rosenberg, S.A., et al., 1988b. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N. Engl. J. Med. 319 (25), 1676–1680.
Rosenberg, S.A., et al., 1990. Gene transfer into humans – immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N. Engl. J. Med. 323 (9), 570–578.
Rosenberg, S.A., et al., 2008. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat. Rev. Cancer 8 (4), 299–308.
Rosenberg, S.A., et al., 2011. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin. Cancer Res. 17 (13), 4550–4557.
Sakuishi, K., et al., 2010. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J. Exp. Med. 207 (10), 2187–2194.
Sato, E., et al., 2005. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc. Natl. Acad. Sci. U. S. A. 102 (51), 18538–18543.
Sato, T., et al., 2007. Engineered human tmpk/AZT as a novel enzyme/prodrug axis for suicide gene therapy. Mol. Ther. 15 (5), 962–970.
Savoldo, B., et al., 2011. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J. Clin. Invest. 121 (5), 1822–1826.
Scholten, K.B., et al., 2011. Generating HPV specific T helper cells for the treatment of HPV induced malignancies using TCR gene transfer. J. Transl. Med. 9, 147.
Schwartzentruber, D.J., et al., 1994. In vitro predictors of therapeutic response in melanoma patients receiving tumor-infiltrating lymphocytes and interleukin-2. J. Clin. Oncol. 12 (7), 1475–1483.
Serafini, M., et al., 2004. Characterization of CD20-transduced T lymphocytes as an alternative suicide gene therapy approach for the treatment of graft-versus-host disease. Hum. Gene Ther. 15 (1), 63–76.
Shen, X., et al., 2007. Persistence of tumor infiltrating lymphocytes in adoptive immunotherapy correlates with telomere length. J. Immunother. 30 (1), 123–129.
Sigalotti, L., et al., 2005. Epigenetic modulation of solid tumors as a novel approach for cancer immunotherapy. Semin. Oncol. 32 (5), 473–478.
Singh, H., et al., 2013. Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLOS ONE 8 (5), e64138.
Skov, S., et al., 2005. Cancer cells become susceptible to natural killer cell killing after exposure to histone deacetylase inhibitors due to glycogen synthase kinase-3-dependent expression of MHC class I-related chain A and B. Cancer Res. 65 (23), 11136–11145.
Smith, K.A., et al., 1980a. The functional relationship of the interleukins. J. Exp. Med. 151 (6), 1551–1556.
Smith, K.A., Gilbride, K.J., Favata, M.F., 1980b. Lymphocyte activating factor promotes T-cell growth factor production by cloned murine lymphoma cells. Nature 287 (5785), 853–855.
Spychala, J., 2000. Tumor-promoting functions of adenosine. Pharmacol. Ther. 87 (2–3), 161–173.
Stanislawski, T., et al., 2001. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. Nat. Immunol. 2 (10), 962–970.
Straathof, K.C., et al., 2005. An inducible caspase 9 safety switch for T-cell therapy. Blood 105 (11), 4247–4254.
Testa, U., et al., 1990. Interleukin 2 in cancer therapy. Ann. Ist. Super. Sanita 26 (3–4), 283–334.
Tiberghien, P., et al., 1994. Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: an approach for specific in vivo donor T-cell depletion after bone marrow transplantation? Blood 84 (4), 1333–1341.
Till, B.G., et al., 2008. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood 112 (6), 2261–2271.
Till, B.G., et al., 2012. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. Blood 119 (17), 3940–3950.
Topalian, S.L., et al., 1987. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. J. Immunol. Methods 102 (1), 127–141.
Topalian, S.L., et al., 1988. Immunotherapy of patients with advanced cancer using tumor-infiltrating lymphocytes and recombinant interleukin-2: a pilot study. J. Clin. Oncol. 6 (5), 839–853.
Topalian, S.L., et al., 2012. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N. Engl. J. Med. 366 (26), 2443–2454.
Tran, K.Q., et al., 2008. Minimally cultured tumor-infiltrating lymphocytes display optimal characteristics for adoptive cell therapy. J. Immunother. 31 (8), 742–751.
Uhlen, M., et al., 2010. Towards a knowledge-based human protein atlas. Nat. Biotechnol. 28 (12), 1248–1250.
van Lier, R.A., ten Berge, I.J., Gamadia, L.E., 2003. Human CD8(+) T-cell differentiation in response to viruses. Nat. Rev. Immunol. 3 (12), 931–939.
van Schalkwyk, M.C., et al., 2013. Design of a phase I clinical trial to evaluate intratumoral delivery of ErbB-targeted chimeric antigen receptor T-cells in locally advanced or recurrent head and neck cancer. Hum. Gene Ther. Clin. Dev. 24 (3), 134–142.
Vogler, I., et al., 2010. An improved bicistronic CD20/tCD34 vector for efficient purification and in vivo depletion of gene-modified T cells for adoptive immunotherapy. Mol. Ther. 18 (7), 1330–1338.
Walia, V., et al., 2012. Delving into somatic variation in sporadic melanoma. Pigment Cell Melanoma Res. 25 (2), 155–170.
Wang, L.X., et al., 2010. Tumor ablation by gene-modified T cells in the absence of autoimmunity. Cancer Res. 70 (23), 9591–9598.
Wang, X., et al., 2011. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood 118 (5), 1255–1263.
Warren, R.S., et al., 1998. Clinical studies of regional and systemic gene therapy with autologous CC49-z modified T cells in colorectal cancer metastatic to the liver. (Abstract, 7th International Conference on Gene Therapy of Cancer). Cancer Gene Ther. 5 (6 Suppl.), S1–S2.
Weng, N.P., et al., 1995. Human naive and memory T lymphocytes differ in telomeric length and replicative potential. Proc. Natl. Acad. Sci. U. S. A. 92 (24), 11091–11094.
Westwood, J.A., et al., 2005. Adoptive transfer of T cells modified with a humanized chimeric receptor gene inhibits growth of Lewis-Y-expressing tumors in mice. Proc. Natl. Acad. Sci. U. S. A. 102 (52), 19051–19056.
Westwood, J.A., et al., 2009. The Lewis-Y carbohydrate antigen is expressed by many human tumors and can serve as a target for genetically redirected T cells despite the presence of soluble antigen in serum. J. Immunother. 32 (3), 292–301.
Wilkie, S., et al., 2012. Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. J. Clin. Immunol. 32 (5), 1059–1070.
Wolchok, J.D., et al., 2013. Nivolumab plus ipilimumab in advanced melanoma. N. Engl. J. Med. 369 (2), 122–133.

Wolfl, M., et al., 2011. Primed tumor-reactive multifunctional CD62L+ human CD8+ T cells for immunotherapy. Cancer Immunol. Immunother. 60 (2), 173–186.
Wrzesinski, C., et al., 2010. Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. J. Immunother. 33 (1), 1–7.
Xue, S.A., et al., 2005. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. Blood 106 (9), 3062–3067.
Yannelli, J.R., et al., 1996. Growth of tumor-infiltrating lymphocytes from human solid cancers: summary of a 5-year experience. Int. J. Cancer 65 (4), 413–421.
Yee, C., et al., 2000. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of T cell-mediated vitiligo. J. Exp. Med. 192 (11), 1637–1644.
Yeh, S., et al., 2009. Ocular and systemic autoimmunity after successful tumor-infiltrating lymphocyte immunotherapy for recurrent, metastatic melanoma. Ophthalmology 116 (5), 981-989 e1.
Zhang, Y., et al., 2005. Host-reactive CD8+ memory stem cells in graft-versus-host disease. Nat. Med. 11 (12), 1299–1305.
Zhao, Y., et al., 2009. A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity. J. Immunol. 183 (9), 5563–5574.
Zhong, X.S., et al., 2010. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI3kinase/AKT/Bcl-XL activation and CD8+ T cell-mediated tumor eradication. Mol. Ther. 18 (2), 413–420.
Zhu, C., et al., 2005. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat. Immunol. 6 (12), 1245–1252.