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Immunotherapy for solid tumors—a review for surgeons

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Abstract

Immunotherapy has evolved considerably in the last decade and is becoming an integral component of the armamentarium for the treatment of patients with advanced solid tumors. It is important for clinicians, especially surgeons, to understand the basic principles of novel immunotherapies and the immune system. This review summarizes the evolution of the most relevant immunotherapies, their mechanisms of action, the data supporting their clinical use, and integration of immunotherapy into multidisciplinary management of solid tumors. This review should serve as a primer for clinicians and surgeons to understand the rapidly evolving field of immunotherapy.

Keywords

Immunotherapy; Solid tumors; Adoptive cell transfer therapy

1. Introduction

Our understanding of the dialog between cancer and our immune systems has evolved considerably over the past decade. With recent scientific and conceptual advances, novel immunotherapeutic approaches have emerged as effective and promising treatments against cancer. Immunotherapy is rapidly being incorporated into everyday clinical care and is no longer relegated to the realm of the esoteric. Therefore, clinicians must understand the basic principles of immunotherapy and how it will be integrated into multidisciplinary cancer care.

2. Historical perspective

The clinical relevance of the bidirectional crosstalk between immune cells and cancer cells has been the subject of considerable controversy. In 1891, Dr William B. Coley published a

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remarkable account of an unresectable neck sarcoma that regressed completely after a severe episode of erysipelas [1]. Based on this observation, Coley speculated that the patient's response to the infection mediated tumor regression. In one of the first ventures into the realm of immunotherapy, Coley prepared a mixture of bacterial “toxins” that he used to treat a number of sarcoma and carcinoma patients. Like so many immunotherapy trials in the modern era, Coley's initially promising results could not be consistently reproduced. Although it would be decades before the link between immunity and regression of solid tumors would become widely accepted, Coley was instrumental in shaping our thinking about harnessing the immune system to destroy solid tumors.

Paul Ehrlich furthered the notion that the immune system has a protective effect against cancer, proposing the concept of immunosurveillance [2]. The immunosurveillance concept was expanded on by Burnet and Thomas, who formally proposed that the immune system was responsible for preventing cancer development in immunocompetent organisms [3,4]. However, others challenged the clinical relevance of cancer immunosurveillance through studies showing that immunocompetent mice had similar cancer susceptibility as immunodeficient mice [5]. However, nude mice used in these experiments were not completely immunodeficient because of the presence of functional macrophages and natural killer (NK) cells. It was not until the development of completely immunodeficient mouse models that the absence of a competent immune system was confirmed to increase susceptibility to neoplasia.

Advances in cytokine biology led to a deeper appreciation of the mechanisms through which immune cells combat cancer. Interferon-gamma (IFN γ), a cytokine that drives development of effector T cell subsets, was demonstrated to be integral to a successful antitumor response [6]. Research from the National Cancer Institute led by Dr Steven Rosenberg, demonstrated the therapeutic potential of interleukin (IL) 2, a crucial T cell growth factor, when used alone or in combination with adoptive cellular therapy [7]. Investigators at the National Cancer Institute subsequently reported on tumor specific T cell infusions, which we will expand upon below. Enthusiasm for solid tumor immunotherapy has grown with the development of agents designed to shut down immunoinhibitory pathways, enabling reversal of tumor-induced immunosuppression. Several promising monoclonal antibody products that block immunoinhibitory pathways are now under clinical development. The importance of addressing the immunosuppressive tumor microenvironment in addition to providing specific antitumor therapy is now well accepted.

This review summarizes clinically relevant basic immunology and immunotherapy principles with particular emphasis on recent clinical advances. The goal of this review is to provide an understanding of how immunotherapy will be integrated into the management of advanced solid tumors refractory to conventional therapy. We will focus on the importance of combining effective antitumor agents with products designed to reverse tumor-induced immunosuppression.

3. Tumor cell recognition, evasion, and escape

3.1. Immune system overview

A basic understanding of how the immune system functions and interacts with tumor cells is necessary to appreciate the clinical application of immunotherapy. The immune system can be broadly divided into innate and adaptive components. Innate immunity refers to the nonspecific first line of defense against danger signals from pathogens or tumor cells. Triggers for innate immune cells include bacterial cell wall products, endotoxin, and pathogen nucleic acids. The innate response is mediated by NK cells, macrophages, and dendritic cells. Adaptive immunity, orchestrated by T and B cells, functions with exquisite specificity and memory. Immunologic memory allows for enhanced T and B cell responses on reexposure to antigens or pathogens, providing the basis for immunosurveillance. It is important to note that there is significant overlap and communication between the innate and adaptive immune systems. For example, activation of innate immune cells can lead to the stimulation of T and B cells via cytokine secretion and antigen presentation (Fig.).

3.2. Tumor recognition by the immune system

Initially, it was thought that spontaneous tumors were not susceptible to immune responses because they originated from normal cells. The view held that immune cells could not recognize cancer antigens as foreign or nonself. However, naturally occurring tumor antigens that elicit adaptive immune responses have been identified [8,9]. Mutations may lead to altered proteins that are presented to CD8 or CD4 T lymphocytes [10]. Examples include mutated β -catenin, cyclin-dependent kinase 4, and caspase 8, found in melanoma, renal, and head and neck cancers, respectively [10]. Differentiation antigens expressed by tumors may also be immunogenic and examples include gp100, melan-A, and tyrosinase in melanoma [10]. Overexpressed proteins, such as carcinoembryonic antigen or HER2, can also be recognized by the immune system [11]. Finally, mutated tumor suppressor genes, such as p53, and oncogene products such as RAS, contain epitopes capable of inducing immune responses [12]. Therefore, proteins altered in structure or expression context may be recognized by the immune system. The concept of immune recognition of solid tumor antigens is further supported by tumor infiltrating lymphocyte (TIL) studies.

TIL studies provide strong evidence that immune responses to solid tumors are clinically relevant [13,14]. In melanoma, transcriptional profiling and immunohistochemical analyses demonstrate infiltration of tumors by CD8+ T cells [15]. We have shown that the T cell responses to colorectal cancer and neuroendocrine tumor liver metastases are independent predictors of outcome after resection [13,14,16]. Similar findings have been seen in primary colorectal cancer [17], ovarian cancer [18], and lung cancer [19]. Mlecnik [20] demonstrated a stronger association between T cell infiltration and colorectal cancer prognosis than the TNM staging system. Although patients with favorable endogenous immune responses to tumors have better prognoses, most patients with solid tumors are unable to generate effective antitumor immunity [14,16]. The immunosuppressive tumor microenvironment limits endogenous immunity and creates barriers to the effectiveness of immunotherapy in most patients.

4. Mechanisms of tumor immunosuppression

4.1. Tumor evasion and immune escape

The presence of immunogenic tumor antigens is not sufficient to induce a clinically meaningful immune response in most patients. Tumor cells actively influence their microenvironment, promoting an immunosuppressive milieu (Table 1, Fig.). Tumors not only evade cytotoxic responses but also actively produce immunosuppressive factors with local and systemic effects. These factors, through a variety of mechanisms, promote tumor growth and prevent eradication. As such, effective immunotherapy will require not only effective tumor targeting, but also reversal of immunosuppressive factors mediated by the tumor and host. Specific mechanisms of immune evasion are described below.

4.2. Soluble suppressive factors

Cancer cells can modulate their microenvironment by producing suppressive cytokines, promoting expansion of suppressor cells or directly inducing apoptosis of the cytotoxic T cells. Immunosuppressive cytokines include transforming growth factor- β (TGF β) and IL-10. TGF β inhibits T and NK cell function by limiting proliferation and cytokine production [21]. TGF β also promotes the expansion of regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC), both of which inhibit antitumor immunity [22,23]. IL-10 has also been demonstrated to mediate the suppression of antitumor immunity, and elevated IL-10 levels predict shorter survival time in patients with solid tumors [38].

Prostaglandins (PGs) have potent effects on the immune system, including inhibition of NK cell mediated toxicity, inhibition of tumor necrosis factor production (TNF), and suppression of B and T cell proliferation [31]. PGE₂ has been associated with immunosuppression in colorectal tumors [44]. Moreover, overexpression of cyclooxygenase-2 has been found in association with increased production of PGs and upregulation of anti-apoptotic proteins, such as B cell lymphoma gene 2[30]. As such, dialog between the host and tumor via secreted factors can result in a profoundly immunosuppressive milieu that prevents immune mediated tumor clearance and promotes aggressive tumor biology.

4.3. Induction of lymphocyte death

Apoptosis of activated T cells plays an important role in the regulation of the immune response to tumors. Cancer cells may also evade the immune system through the induction of T cell apoptosis by way of activation-induced cell death [45]. This process is mediated in part by Fas (CD95)-to-Fas ligand (FasL; CD95 L) interactions [39]. Tumors may express FasL that can engage Fas molecules on the surface of TIL, inducing apoptosis and suppressing the cytotoxic immune response. The tumor microenvironment may induce the expression of Fas on T cells as demonstrated in melanoma models [46].

4.4. Immunoinhibitory molecules

Suppressive or co-inhibitory signaling pathways also play an important role in tumor-induced suppression. The immunoinhibitory receptors receiving the most attention in laboratories and clinical trials are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1) [40,42]. Other immunoinhibitory pathways have been

characterized, but will not be addressed in this review. The PD-1 and CTLA-4 axes are pivotal regulators of T cell activity, that can be usurped by tumors to induce T cell suppression (Fig.). CTLA-4 is expressed on activated T cells and increases the stimulation threshold required for T cell cytokine production and proliferation. Blocking the activity of CTLA-4 enables reactivation and expansion of T cells [40]. CTLA-4 blockade also directly limits the suppressive activity of Treg, a cell type discussed in more detail below [47]. Ipilimumab, a humanized anti-CTLA-4 monoclonal antibody, was approved by the FDA for the treatment of advanced melanoma. Despite low response rates, ipilimumab significantly prolonged overall survival in advanced melanoma patients [41].

PD-1 is a co-inhibitory receptor with a similar structure to CTLA-4, but with distinct biologic activity [42]. PD-1 has two known ligands, programmed death ligand-1 (PD-L1), and programmed death ligand-2. PD-L1 is expressed by many tumors and suppressive immune cells, thereby promoting tumor immune evasion [43]. PD-1 engagement by PD-L1 results in T cell functional exhaustion. Exhausted T cells have a markedly diminished capacity for cytokine production, proliferation, and tumor lysis. Blockade of PD-1 can reinvigorate exhausted T cells and restore their antitumor function. Antibody-mediated blockade of both PD-1 and PD-L1 has shown promising clinical activity in patients with advanced non-small-cell lung cancer (NSCLC), melanoma, and renal cell cancer (Table 1; [42,48]). Recent clinical trials have shown durable objective response rates (ORRs) of 38% with PD-1/PD-L1 (programmed cell death 1 antigen/ligand) blockade [49]. The combination of anti-PD-1 with anti-CTLA-4 has also been studied, with a response rate of up to 53% and acceptable toxicity [50].

Although blockade of CTLA-4 or PD-1 may result in auto-immune sequelae and inflammatory tissue damage, targeting PD-1 may be safer. In an animal model, we have recently demonstrated that PD-1 blockade can resuscitate T cells within the liver while actually minimizing inflammatory injury to normal parenchyma [51]. Other investigators have also found that PD-1/PD-L1 inhibition can restore immunity while minimizing inflammation [52]. These animal model data may in part explain why the rates and severity of immune related adverse events with anti-PD-1 or anti-PD-L1 infusions have been less than what has been observed with other forms of immunotherapy [42,53]. As such, activation of antitumor T cells via PD-1/PD-L1 inhibition is particularly attractive given the favorable tradeoff between antitumor responses and toxicity.

4.5. Immunosuppressive cells

Treg-mediated suppression of antitumor immunity is an important obstacle to successful tumor immunotherapy (Fig.; [54]). Treg mediate tolerance by suppressing antigen-specific T cells [55]. Treg have been demonstrated to suppress effector T cells and the immune response against tumor cells [56]. Increased numbers of Treg have been documented in several types of cancers, and a high density of intratumoral Treg has been associated with poor outcomes [14,54]. Multiple mechanisms have been proposed to explain immunosuppression by Treg cells in the tumor microenvironment. Treg suppression is mediated through the activation of CTLA-4 [54], direct killing of T cells [32], induction of

indoleamine 2,3-dioxygenase [33], IL-10 production, and TGF β secretion [34,35]. Of note, Treg may also express PD-L1, which activates immunoinhibitory PD-1 on T cells [47].

MDSC work in concert with Treg in promoting an immunosuppressive environment within solid tumors (Fig.; [57]). MDSC are a heterogeneous group of cells derived from a myeloid lineage pathway [58]. Phenotypically, MDSC have features of immature neutrophils, monocytes, or NK cells. MDSC express PD-L1 and also promote T cell suppression through production of suppressive cytokines and enzymes [59]. Neoplastic cells can promote MDSC expansion within tumors, which may profoundly hinder antitumor immunity [60].

4.6. Summary of tumor-induced immunosuppression

Exciting advances in cellular and molecular immunology have provided insight into the nature of the dialog between the tumor microenvironment and the immune system. A new phase of investigation focused on restoring the host immune response against tumors has emerged with multiple potential targets to promote and activate effective antitumor immunity. Targeting suppressive cells and immunoinhibitory molecules will complement the more direct efforts designed to kill tumor cells. It is clear that effective immunotherapy must not only deliver an effective antitumor component, but also combat tumor induced immune dysfunction.

5. Immunotherapy approaches

Numerous immunotherapy strategies have undergone extensive development and clinical testing. Approaches range from nonspecific immunostimulation to the production of exquisitely specific genetically modified T cells. The first FDA approved immunotherapies include IL-2 and interferon- α 2b used for melanoma [61,62]. These approaches have yielded durable results in a select group of patients, with tumor regression in 10%e15% of melanoma and renal cell carcinoma (RCC) patients [62]. Cytokine-based approaches were found to induce high levels of antitumor T cells in some patients [63]. As noted earlier, TIL have been associated with improved outcome in several solid tumors [13,14]. These findings provide compelling rationale for the development of adoptive cellular therapeutics for cancer, which we review in the following section (Table 2).

5.1. Non–antigen-specific immunotherapy approaches

Following Coley's observations in 1891 that postsurgical erysipelas resulted in complete remission of an unresectable neck sarcoma, he and others reported limited success with bone and soft-tissue sarcomas [64]. Coley's toxins activated the innate arm of the immune system *via* stimulation of pattern recognition receptors that interact with, among other things, pathogen nucleic acids. For example, toll-like receptor 4 recognizes lipopolysaccharide from gram-negative bacteria. Unfortunately, with the exception of Bacillus Calmette–Guérin for bladder cancer, nonspecific immune stimulants alone have not been widely successful for the treatment of solid tumors. The clinical use of non–antigen-specific approaches has been limited by toxicity as well as unpredictable and heterogeneous responses [65].

5.2. Vaccination

The goal of vaccination for cancer treatment is to induce a specific antitumor immune response. This can be achieved by delivering tumor-associated antigens alone or loaded onto the surface of antigen presenting cell. Examples that have been tested clinically include vaccines against breast cancer (HER2) [66,67], lung cancer (MUC1) [68], pancreatic cancer (telomerase peptides) [69], and prostate cancer (prostatic acid phosphatase) [70]. Several vaccine strategies have resulted in demonstrable immune responses, but most of the phase 3 trials have failed to show a significant benefit. We and others speculate that a critical barrier to antitumor vaccine efficacy is the suppressive effects of the tumor microenvironment [40]. The use of vaccines plus other immunotherapy approaches has therefore been attempted.

The FDA recently approved sipuleucel-T for patients with metastatic castration-resistant prostate cancer based on a 4.1-mo prolongation of overall survival, reported in the Immunotherapy Prostate AdenoCarcinoma Treatment trial [71]. The vaccine consists of autologous peripheral-blood mononuclear cells activated *ex vivo* with a recombinant fusion protein (PA2024). PA2024 is a fusion protein containing prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor (GM-CSF), the latter being capable of antigen presenting cell activation. Available data strongly suggest that successful cancer vaccines will need to be combined with nonspecific immune stimulants, such as GM-CSF, or agents that block immunoinhibitory pathways. Establishing the optimal combinations and sequences to maximize efficacy and minimize toxicity will be crucial in the development of multifaceted approaches. While vaccines attempt to induce highly specific immune cell responses, more direct approaches involve the *ex vivo* production of antitumor T cells that can be infused into the patient.

5.3. Adoptive cell transfer immunotherapy

Adoptive cell transfer immunotherapy (ACT) involves the delivery of immune cells with antitumor activity into cancer patients. This requires isolation or production of autologous lymphocytes with antitumor activity [72]. Initially, one of the major obstacles to ACT was the inability to obtain a sufficient number of autologous lymphocytes from the cancer patient. Different methods to generate cells capable of lysing tumors have been described. Initially, tumor lysis with lymphokine-activated killer cells (LAK) was reported [63]. LAK are activated with IL-2 and capable of tumor lysis with an ORR of 44% and with a limited number of complete responses [73]. However, the large number of cells required along with high doses of IL-2 led to practical and toxicity limitations [63].

TIL were found to be 50–100 times more potent than LAK with smaller doses of IL-2 required to enhance their therapeutic efficacy [73]. TILs are isolated from patient tumor samples and then cultured and expanded to therapeutic levels. IL-2 and host lymphodepletion have been used to promote *in vivo* TIL expansion and improve therapeutic efficacy. Lymphodepletion or preconditioning has been achieved with combinations of cyclophosphamide, fludarabine, and total body irradiation before TIL infusion. In murine models, lymphodepletion enhances the antitumor effects of transferred T cells by several mechanisms. Elimination of Treg [74] and increased levels of homeostatic cytokines, such as IL-7 and IL-15, support TIL activity after preconditioning [75]. The combination of TIL,

IL-2, and preconditioning in patients with metastatic melanoma has resulted in response rates of 50%–70% [76].

Although ACT with autologous TIL showed impressive response rates in patients with advanced melanoma, the difficulty in isolating and expanding TIL from most solid tumors limits the application of this approach. Melanoma typically has higher numbers of TIL compared with other solid tumors, making the TIL approach well suited for melanoma. Even among melanoma patients, TIL isolation and expansion is successful in only 60% [76,77]. Given that the TIL approach is not applicable to the vast majority of solid tumors, a more direct approach to the production of highly specific ACT products has been developed.

Autologous T cells can now be engineered to express immune receptors specific for tumor antigens. T cells are genetically engineered with genes encoding T cell receptor (TCR) or antibody-based fusion proteins capable of recognizing specific tumor antigens. After production, genetically modified or designer T cells (dTc) are expanded in culture for the production of patient doses. Given that TIL isolation is not required, dTc can potentially be applied to a much larger group of patients and wider variety of malignancies. Recent reports provide encouraging results that support the use of dTc for cancer treatment.

The first successful treatments with dTc were demonstrated in melanoma patients. TCR-based dTc specific for melanoma antigens demonstrated objective regression in patients refractory to other treatments [78]. One limitation of TCR-based dTc products is that they are restricted to patients with specific HLA haplotypes. DTc with TCR-based receptors must recognize tumor antigen peptides within the context of HLA molecules on the surface of tumor cells. Immunoglobulin-based dTc specificity is not hindered by HLA restrictions.

Immunoglobulin chimeric antigen receptors (CARs) were developed by Eshhar and others [79] in an effort to increase the reactivity of dTc. The antigen reactive portions of immunoglobulin light and heavy chains were fused with T-cell intracellular signaling molecules. DTc transduced with immunoglobulin-based CARs are activated on recognition of tumor antigen. This technique has been used to target GD2 in neuroblastoma [80] and CD19+ B cells for non-Hodgkin lymphomas and chronic lymphocytic leukemia [81]. In chronic and acute lymphoid leukemia, the use of dTc with specificity for CD19 have resulted in complete remissions with acceptable toxicity [81,82]. We have recently developed a CAR that targets KIT+ gastrointestinal stromal tumors based on incorporation of the natural ligand for KIT, stem cell factor [83].

Optimization of dTc delivery to tumor sites is another strategy that may enhance efficacy and limit toxicity. We are investigating the regional hepatic artery infusion of dTc specific for carcinoembryonic antigen in our phase I Hepatic Immunotherapy for Metastases (HITM™, NCT01373047) trial. We speculate that regional delivery will improve the safety of highly potent dTc for liver metastases and increase intrahepatic tumor killing. Ultimately, although dTc are highly specific and potent, it may be necessary to combine dTc with immunoinhibitory blockade agents to address the tumor microenvironment. Successful immunotherapy for solid tumors will likely require both a highly specific antitumor product and immunomodulatory agents capable of blocking suppressive pathways.

6. Combining immunotherapy with conventional treatment

The notion that chemotherapy is strictly immunosuppressive is no longer valid, and evidence exists to support the notion that the effect of cytotoxic agents on antitumor immunity is complex. Suppression of Treg has been seen with low doses of cyclophosphamide [84]. Gemcitabine has been shown to reduce the number of MDSC [85]. Oxaliplatin has been linked to favorable cytokine release within the tumor micro-environment with the activation of dendritic cells and cytotoxic T cells [86]. Therefore, conventional chemotherapeutic agents may play a role as immune-modulating agents, which can be used in combination with adoptive cell therapy approaches.

Important interactions between the immune system and ionizing radiation therapy have also been reported. Ionizing radiation therapy may have systemic immunologic effects. The abscopal effect refers to a rare phenomenon of tumor regression at a site distant to the radiotherapy target [87]. The exact mechanism of the abscopal effect is not known, however, it is likely related to the promotion of systemic antitumor immunity [88]. Multiple studies have shown that radiation therapy can promote recruitment of effector T cells at tumor sites and induce expression of molecules that enhance tumor antigen recognition [89]. The use of radiotherapy as an adjuvant to immunotherapies is an emerging concept that merits exploration. A phase III randomized controlled trial is underway to evaluate the use of ipilimumab and radiation therapy for metastatic melanoma [88].

7. Immunotherapy trials

7.1. Trials evaluating immune checkpoint blocking agents

As reviewed earlier, CTLA-4 and PD-1 represent critical immune checkpoint molecules and their inhibition can activate antitumor T cells. Initial studies evaluating the activity of ipilimumab as a single dose in patients with advanced tumors, showed increased T cell reactivity suggesting increased anti-tumor immunity with the drug [90]. Later reports from the National Cancer Institute demonstrated a 12.5% ORR in stage IV melanoma patients when used with gp100 vaccine [91]. A phase III trial for stage IV melanoma patients demonstrated that ipilimumab was associated with an increase in median overall survival to 10.0 mo compared with 6.4 mo in the control group ($P < 0.001$) [41]. Grade 3 or 4 toxicity was seen in 10%–15% of the patients who received ipilimumab. Robert *et al.* [92] evaluated ipilimumab in combination with dacarbazine (DTIC) in a randomized phase III clinical trial. Overall survival at 3 y was 20.8% after treatment with DTIC and ipilimumab compared with 12.2% in those who received only DTIC. The median duration of response in the ipilimumab + DTIC group was 19.3 mo whereas only 8.1 mo in the DTIC alone group, at the expense of increased toxicity in those receiving the combination.

Currently, several trials are evaluating the use of CTLA-4 blockade in various solid tumors. Most of these trials are phases I and II in patients with chemotherapy resistant tumors. Anti-CTLA-4 monoclonal antibodies are being evaluated in the following settings: children and adolescents with sarcoma, neuroblastoma and Wilm's tumor resistant to conventional therapy [93], and malignant mesothelioma patients [94], advanced hepatocellular carcinoma [95], prostate cancer in combination with Sipuleucel-T vaccine [96], stage IV pancreatic

cancer [97] and in metastatic melanoma in multiple different combinations. We anticipate an increasing number of trials will test combinations of immunotherapeutic agents that work by distinct mechanisms.

Blockade of the immunoinhibitory PD-1/PD-L1 axis enhances antitumor activity [43]. Brahmer *et al.* [42] evaluated safety of PD-1/PD-L1 blockade in a phase I study. In patients with advanced NSCLC, melanoma, colorectal cancer, ovarian cancer, and pancreatic cancer that received the drug, 9% developed grade 3–4 adverse events. ORRs ranged from 6%–17% for the various tumors. The findings of this trial validated PD-1/PD-L1 blockade as an important immunotherapy target for different solid tumors.

In the phase I study by Hamid *et al.* [49], evaluating anti-PD-1 antibody lambroizilumab in patients with advanced melanoma, the response rate was 38% with a median progression free survival of 7 mo. Most of the adverse events seen were low grade and included fatigue, rash, pruritus, and diarrhea. Topalian *et al.* [48] also evaluated the safety of PD-1/PD-L1 blockade in patients with NSCLC, RCC, and melanoma. Grade 3–4 toxicity associated with nivolumab was 14%, with three reported deaths from pulmonary toxicity. ORRs seen ranged from 18%–28%, and as in the previous trial, the responses were durable with most of them lasting also at least 1 y. This trial also evaluated association between the expression of PD-L1 in tumor cells and the response rate. Interestingly, no response was seen in tumors lacking PD-L1 in contrast to a 36% response rate in the tumors expressing the ligand. The ability to predict response to immunotherapy based on patient, and tumor characteristics will play an increasingly prominent role.

Usage of multiple immune checkpoint blocking agents is a compelling strategy. Wolchok *et al.* [50] in a phase I study evaluated ipilimumab in conjunction with nivolumab as concurrent and sequenced therapy in patients with advanced melanoma. Response rates of 40% were seen with concurrent treatment. The maximum dose was well tolerated with reversible grade 3–4 adverse events in 53% of patients. Although the adverse events rate of 18% was lower in the sequenced treatment group, the response rate was 20%. Ongoing trials are evaluating the role of PD-1/PD-L1 blockade in patients with prostate cancer [98], hepatocellular carcinoma [99], RCC and NSCLC [100], and melanoma [101]. Targeting immune checkpoint molecules is attractive given that these immunosuppressive pathways are likely important drivers of progression in a large number of tumor types, potentially rendering these agents broadly applicable in the clinical setting.

7.2. Trials evaluating adoptive cell therapy

As noted previously, early experience with ACT was with TIL obtained from resected tumor and grown *in vitro*. The development of genetically modified T cells may broaden the applications of ACT. Initial reports using genetically modified T cells against MART-1 and gp100 in melanoma showed objective responses in two of 15 patients [78]. DTc specific for NY-ESO-1 cancer testes antigen mediated objective responses in five of seven patients with synovial cell sarcoma [102]. DTc cells specific for GD2 led to objective responses in patients three of 11 patients with evaluable neuroblastoma [103]. We recently completed a phase I trial evaluating safety of regional hepatic artery ACT with anti-carcinoembryonic antigen dTc for unresectable colorectal metastases [104]. Promising ongoing trials involve

dTc targeting NY-ESO-1 for esophageal cancer [105], epidermal growth factor receptor in refractory advanced NSCLC, ovarian and colorectal cancer with liver metastasis [106], prostate specific membrane antigen in patients with metastatic prostate cancer [107], and HER2 in patients with glioblastoma multiforme [108]. It is likely that dTc will be tested for a wide variety of solid tumors in the near future, alone and in combination with immune checkpoint blocking agents.

8. Summary and conclusions

Cancer immunotherapy has evolved significantly, from the time of Coley's observations of a spontaneous sarcoma regression to the recent clinical testing of immune checkpoint blockade and genetically modified T cells. Our increased understanding of lymphocyte biology and immunosuppressive pathways has facilitated translation of animal work into groundbreaking clinical trials. For patients with solid tumors resistant to conventional therapy, immunotherapy alone or in combination with traditional interventions is an emerging therapeutic option. Highly specific immunotherapeutic agents, such as genetically modified T cells, will be increasingly used in combination with immune checkpoint blocking agents. Usage of patient and tumor surrogates of response to immunotherapeutic agents will likely refine our selection of patients for these novel treatments. As our knowledge of basic and clinical immunology continues to evolve, we can expect that an increasing number of our cancer patients will benefit from the innovative immunotherapies described in this review.

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Fig.

Overview of antitumor immunity and tumor-induced immunosuppression. Left: A dendritic cell fulfills its role as a professional antigen-presenting cell. Dendritic cell process and present tumor antigen from the carcinoma cell to effector CD4 or CD8 T cells (Teff). Presentation of antigen in the context of MHC molecules, along with co-stimulatory second signals, induces Teff activation. Activated Teff produce IL-2 and interferon-gamma, which help drive a tumoricidal response. Right: Unfortunately, tumors actively promote an immunosuppressive microenvironment through several mechanisms. Tumors promote the expansion and influx of suppressor cells, including Treg and MDSCs. Treg and MDSC suppress Teff via suppressive cytokines (IL-10 and TGF β) and PD-1 activation in Teff. Tumor cells themselves can express PD-L1, which binds to PD-1 on Teff to induce exhaustion and anergy.

Table 1

Immune evasion and escape mechanisms.

Mechanism	
Secreted factors	
TGFβ	Inhibits T cell and NK function, promotes expansion of Treg and MDSC [21–25].
IL-10	Inhibition of NK, TNF, suppression of T and B cells. [26–29].
PGs	Promotion of anti-apoptotic Bcl-2 in tumor cells [30]. Secreted by suppressive immune cells to inhibit T cell activity. Promotes IL-10 production [31].
Suppressive cells	
Treg	Suppress antigen-specific T cells by activation of CTLA-4, secretion of IL-10 and TGFβ, induction of IDO, and promotion of effector T cell apoptosis [32–37].
MDSC	Suppression of T cell responses through multiple mechanisms, including nitric oxide production, IL-10 secretion, and PD-L1 engagement of PD-1 on activated T cells [22,23,38].
Suppressive pathways	
Fas-FasL	FasL-to-Fas interaction induces T cell apoptosis and suppression [39].
CTLA-4	Immunoinhibitory receptor. Increases the threshold for T cell activation. Limits T cell division and cytokine production.[40,41].
PD-1/PD-L1	Immunoinhibitory receptor. Limits T cell division and cytokine production. [42,43]

Bcl-2 = B cell lymphoma gene; IDO = indoleamine 2,3-dioxygenase; TNF = tumor necrosis factor.

Table 2

Summary of immunotherapy trials for advanced solid tumors.

Author	Year	Disease	Agent	n	Outcomes
Atkins <i>et al.</i> [109]	1999	Melanoma	HD IL-2	270	RR 16% PR 10% CR 6% MDR 8.9 mo MS 11.4 mo
Butts <i>et al.</i> [68]	2005	NSCLC	MUC1 antigen (BLP-25 liposome vaccine) <i>versus</i> best supportive care	171	MS ↑ 4.4 mo ($P = 0.07$)
Kantoff <i>et al.</i> [71]	2010	Prostate cancer	Prostatic acid phosphatase/GM-CSF vaccine (Sipuleucel-T) <i>versus</i> placebo	512	MS ↑ 4.1 mo 22% ↓ risk of death ↑ 3 y OS 8.7%
Hodi <i>et al.</i> [41]	2010	Melanoma	Ipilimumab (anti-CTLA-4) +/- gp100 vaccine <i>versus</i> gp100	676	MOS ↑ 3.7 mo
Robert <i>et al.</i> [92]	2011	Melanoma	Ipilimumab + dacarbazine <i>versus</i> dacarbazine	502	MOS ↑ 2.1 mo 3 y OS ↑ 8.6% MDR ↑ 11.2 mo
Rosenberg <i>et al.</i> [77]	2011	Melanoma	TIL + IL-2 + lymphodepletion	93	RR/CR (chemo) 49%/12% RR/CR (chemo + 2 Gy) 52%/20% RR/CR (chemo + 12 Gy) 72%/40%
Louis <i>et al.</i> [103]	2011	Neuroblastoma	Anti-GD2 CAR ⁺ dTc	19	CR 27% (three of 11 patients with active disease)
Brahmer <i>et al.</i> [42]	2012	NSCLC, melanoma, RCC, ovarian, colorectal, pancreatic	Anti-PD-L1	207	Melanoma RR 17% NSCLC RR 10% Ovarian RR 6% RCC RR 12% 12%–41% stable at 24 wk
Topalian <i>et al.</i> [48]	2012	NSCLC, melanoma, prostate cancer	Nivolumab (anti-PD-1)	296	Melanoma RR 28% NSCLC RR 18% RCC RR 27% 20/31 responses >12 mo
Hamid <i>et al.</i> [49]	2013	Melanoma	Lambrolizumab (anti-PD-1)	135	RR 38% MPFS >7 mo
Wolchok <i>et al.</i> [50]	2013	Melanoma	Ipilimumab + nivolumab; concurrent or sequenced	53	RRc 40% CA: 65% RRs 20% CA: 43%

CA = clinical activity defined as objective, immune related or unconfirmed response for at least 24 wk; CR = complete response; GM-CSF = granulocyte-macrophage colony-stimulating factor; HD IL-2 = high dose IL-2; MDFS = median disease-free survival; MDR = median duration response; MOS = median overall survival; MPFS = median progression free survival; MS = median survival; OS = overall survival; PR = partial response; RFR = recurrence free rate; RR = response rate; RRc = response rate concurrent therapy; RRs = response rate sequenced therapy.