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Targeting CD8+ T-cell tolerance for cancer immunotherapy

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Abstract

In the final issue of Science in 2013, the American Association of Science recognized progress in the field of cancer immunotherapy as the 'Breakthrough of the Year.' The achievements were actually twofold, owing to the early success of genetically engineered chimeric antigen receptors (CAR) and to the mounting clinical triumphs achieved with checkpoint blockade antibodies. While fundamentally very different, the common thread of these independent strategies is the ability to prevent or overcome mechanisms of $CD8^+$ T-cell tolerance for improved tumor immunity. Here we discuss how circumventing T-cell tolerance has provided experimental insights that have guided the field of clinical cancer immunotherapy to a place where real breakthroughs can finally be claimed.

Keywords

adoptive cell transfer; cancer immunotherapy; cancer vaccine; $CD8^+$ T-cell checkpoint blockade; chimeric antigen receptor; lymphodepletion; tolerance

Background

The goal of cancer immunotherapy is to enhance antitumor responses by a patient's own immune system. Since the earliest days of immunotherapy, it has been appreciated that immunostimulation is required to elicit endogenous immunity against cancer. This began in the 1890s with Coley's recognition of an apparent relationship between infection and cancer regression [1], which led to decades of effort aimed at developing cancer vaccines with nonspecific immune adjuvants. While this general approach was largely unsuccessful, it was instrumental to the recognition that specific responses would be required for therapy. Evidence for the role of cellular immunity was later demonstrated in 1955 [2], ultimately leading to early efforts at cancer immunotherapy by adoptive transfer of lymphocytes from

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immunized donors and extensive energy directed toward characterizing lymphocyte subpopulations with antitumor reactivity. Despite contributions by other immune cell subsets, $CD8^+$ T cells have emerged as the predominant effector in most cancer immunotherapy settings. Thus, many immunotherapeutic strategies are dedicated to stimulating, enhancing and maintaining responses by tumor-reactive $CD8^+$ T-cells.

A major obstacle to eliciting antitumor $CD8^+$ T-cell immunity is tolerance. Immune tolerance exists to protect healthy tissues from immune-mediated attack. Because tumors commonly express self-antigens characteristic of the healthy tissue from which they are derived, mechanisms of tolerance often prevent appropriate immune responses toward malignant cells. $CD8^+$ T-cell tolerance is broadly categorized into central and peripheral tolerance. In central tolerance, T cells specific for self-antigen are deleted in the thymus before they gain access to peripheral tissues. In contrast, peripheral tolerance serves to attenuate responses by T cells specific for self-antigens not expressed in the thymus, or expressed at such low levels that self-reactive T cells escape negative selection. Since central tolerance effectively creates holes in the tumor-reactive T-cell repertoire by deletion of self-reactive cells, the majority of immunotherapeutic approaches are targeted at preventing or overcoming peripheral tolerance. The mechanisms of peripheral T-cell tolerance are diverse, and can involve the specific deletion of T cells upon engagement of antigen expressed in the periphery by healthy tissues or malignant cells [3]. Alternatively, induction of T-cell dysfunction or anergy can be achieved by an array of stimuli emanating from inhibitory ligands, lack of costimulation, enzymes or the influences of regulatory cell populations [4]. While these processes protect healthy tissues from T-cell-mediated destruction, they represent major obstacles for eliciting immune responses against cancer. Thus, the overarching goal of immunotherapy is to avoid or disable this plethora of tolerizing forces to unleash the power of $CD8^+$ T cells against cancer, while taking care to minimize and manage the inevitable autoimmunity that can arise from such endeavors. Here, we explore the myriad efforts to overcome $CD8^+$ T-cell tolerance to provide durable immunotherapy in patients with cancer, and discuss how past lessons are informing current clinical successes.

Cytokines

Much of the early enthusiasm for cancer immunotherapy arose from the promising results of cytokine-based immunotherapy. The principle of cytokine immunotherapy for cancer is that these small proteins can coordinately regulate the growth and differentiation of lymphocytes, creating a host environment that promotes tumor regression. We have focused on the common gamma chain cytokines, which have well-defined roles in sustaining $CD8^+$ T cells numbers and function. Here we briefly review efforts to harness these cytokines clinically, and highlight the technological advances that exploit their beneficial properties while avoiding the adverse effects that have limited similar application in the past.

IL-2

Initially called T-cell growth factor, IL-2 was first identified as a product of activated T cells that enhanced the proliferation and function of other T cells [5]. These attributes provided the original rationale for exploring IL-2 as an agent for cancer immunotherapy [6–8].

Indeed, high dose IL-2 was found to have clinical efficacy in some patients and was ultimately heralded as the first real success for immunotherapy [9]. However, success with IL-2 as a monotherapy was largely limited to metastatic renal cancer and melanoma, and achieved complete remissions in only a minority (5–10%) of patients, with durable responses in 70% of those patients that experienced complete regression [10].

Since its original characterization, roles for IL-2 in promoting $CD8^+$ T-cell expansion, survival, memory cell development, cytolytic activity and even rescue of $CD8^+$ T-cell tolerance have all been reported [11,12]. Given these qualities, it was not readily obvious why IL-2 ultimately failed to meet high expectations therapeutically, although a deeper appreciation for the pleotropic nature of this cytokine has provided some insight. IL-2 binds with intermediate affinity to the heterodimeric IL-2 receptor composed of the IL-2Rβ chain (CD122) and the common gamma chain (γ_c). In contrast, IL-2 has a much higher affinity for the trimeric receptor that includes IL-2Rβ, γ_c and IL-2Rα (CD25) [13], which is induced on antigen-activated T- cells and constitutively on $FoxP3^+$ Tregs [14]. Consequently, there is potential for IL-2 to boost effector T-cell responses and even circumvent established anergy, but also to mediate tolerance through the expansion of peripheral Tregs [15]. Therefore, the greatest success for IL-2 immunotherapy has come when IL-2 is used to rescue and expand previously unresponsive tumor-reactive $CD8^+$ T cells *in vitro* [16,17], and then as a companion therapy to support adoptive transfer of these T cells into lymphodepleted patients where endogenous Tregs numbers are minimized [18]. Such adoptive T-cell immunotherapy is discussed in more detail later in this review.

The use of high-dose IL-2 monotherapy remains mainstream in the treatment of melanoma and renal carcinoma, with more than 100 current clinical trials aimed at defining optimal dosing regimens, and evaluating IL-2 in combination with other immuno-/chemo-therapeutic approaches. Parallel to these clinical studies, efforts are now being made to improve the efficacy of IL-2 treatment by increasing *in vivo* half-life and enhancing the ability of administered IL-2 to selectively influence desired immune components. For example, association of recombinant IL-2 cytokine with particular anti-IL-2 monoclonal antibodies (mAbs) was shown to form IL-2/mAb complexes that markedly enhance the activity of IL-2 *in vivo*. Depending on the anti-IL-2 mAbs used, these complexes favor binding to either $CD8^+$ effector T-cells or Tregs [19]. Variants of IL-2 known as ‘superkines’ are also being designed with the ability to preferentially target tumor microenvironment (ALT-801, GA504) and/or activate effectors over regulatory T cells [20]. These IL-2 variants are often fused to antibodies or soluble T-cell receptor (TCR) domains specific for tumor proteins/antigens and can be engineered to have enhanced binding to IL-2Rβ in the absence of CD25 [21]. It will be interesting to observe if these antibody-cytokine complexes or supercytokines translate into better clinical outcomes with higher safety profiles in human patients. Representative clinical trials for IL-2 based therapies are provided in Table 1.

IL-15 & IL-21

Advances in our understanding of the cellular and molecular biology of IL-2 and its receptor complex have provided rationale to better exploit IL-2 signaling pathways to expand and

activate T cells in patients with cancer. One such approach is the use of other common-gamma chain cytokines, such as IL-15 and IL-21, which share signaling pathways and immune-modulating activities with IL-2 [22], but may be superior to IL-2 in overcoming mechanisms of tolerance to generate antitumor $CD8^+$ T-cell responses [23,24].

The IL-15 receptor shares the IL-2Rβ and γ_c chain signaling complex with IL-2, but contains a unique IL-15Rα chain that confers selectivity for IL-15. Differences in the distribution and regulation of the unique α chains of the IL-2R and IL-15R, as well as distinct modes of activity largely account for the proposed advantages of using IL-15 over IL-2 in immunotherapy [25]. IL-15 is currently being tested in 14 clinical trials listed on ClinicalTrials.gov as a recombinant cytokine or DNA vaccine to treat a wide range of advanced solid tumors. Its also being examined as a combination therapy to support adoptively transferred T-cells and NK cells in cancer patients. In similar strategies described above for IL-2, boosting the *in vivo* efficacy of IL-15 has been explored by creating a complex consisting of the IL-15 cytokine and a soluble form of the IL-15Rα chain [26]. More recently these efforts have evolved toward the creation of a dimeric IL-15 receptor fusion protein ($\alpha Su/F_c$) complexed with a super agonistic IL-15 mutant cytokine, collectively known as ALT-803 [27]. This IL-15 complex induced robust T-cell and NK cell responses *in vivo*, as well as antitumor activity in a mouse model of multiple myeloma [28]. ALT-803 is now in Phase I/II clinical trials for treatment of metastatic melanoma (NCT01946789) and relapsed hematologic malignancy (NCT01885897).

Another member of the γ_c cytokine family, IL-21 is produced by $CD4^+$ and NK T cells, and binds to a heterodimeric receptor composed of the γ_c chain and a distinct IL-21Rα chain expressed on $CD4^+$ and $CD8^+$ T-cells, as well as other immune cell populations [29]. IL-21 has the potential to impede T-cell tolerance by synergizing with IL-15 to promote activation and proliferation of both memory and naive $CD8^+$ T-cells [30], and by shielding $CD8^+$ T cells from Tregs suppression [31]. Further, IL-21R signaling inhibits Foxp3 expression and attenuates Tregs activity [32,33], representing an important therapeutic advantage over both IL-15 and IL-2. Indeed, IL-21 is being evaluated in clinical trials as a monotherapy for a wide range of cancers, and in combination modalities with more conventional chemotherapies and other immune modulators like anti-PD-1 (NCT01629758), anti-CTLA-4 (NCT01489059). Table 1 provides an overview of clinical trials with γ_c cytokines.

Costimulation & vaccination

The induction of potent and long-lasting $CD8^+$ T-cell responses is a key goal of therapeutic tumor vaccination. Vaccines are designed to overcome mechanisms of tolerance to tumors by providing antigen, costimulation, or both to awaken tumor-specific T cells. Multiple approaches have been developed to augment the immune response to cancer, ranging from general immune stimulants, such as live attenuated vaccines, to molecularly targeted therapeutics designed to trigger specific activating receptors on immune cell subsets. Here we discuss these approaches and the trials that are underway to evaluate their potential in human patients.

Costimulatory agonists

T-cell-mediated rejection of tumors requires signals from the TCR and costimulatory molecules to activate tumor-reactive T cells, promote differentiation, and induce effector functions. Targeting costimulatory molecules for immunotherapy is based on the premise that tumor-specific T cells exist within a cancer-bearing host, but either fail to recognize tumor antigens in an activating context or have been rendered functionally tolerant/anergic by antigen encounter in the absence of appropriate costimulation. By providing these signals therapeutically, it is expected that T-cell numbers and function will be enhanced, leading to better antitumor immunity. Table 2 provides an overview of these clinical efforts.

CD28

The first costimulatory molecule to be discovered and engaged with a mAb to boost T-cell responses against tumor cells was CD28 [34]. Enthusiasm for targeting CD28 therapeutically grew when a CD28 super agonistic antibody (TGN1412) was found to possess strong immunomodulatory properties in pre-clinical models, from the promotion of regulatory T-cell activation to the stimulation of cytotoxic T-cell responses against B-cell leukemia without evoking worrisome proinflammatory mediators. Unfortunately, in a Phase I evaluation in 2006, TGN1412 induced cytokine storm and subsequent multiorgan dysfunction in all healthy volunteers that had received the antibody [35]. Thus, anti-CD28 is not currently being investigated as a therapeutic agent to stimulate T-cell tumor immunity in patients. Indirectly though, treatment with the CTLA-4 antibody, ipilimumab, achieves this objective by blocking ligation of the CD28 homolog and co-inhibitory molecule CTLA-4, which normally outcompetes CD28 for B7 ligand binding, effectively making B7 more available for CD28 costimulation [36]. Additionally, agonistic clinical grade CD28 antibody is used for *ex vivo* expansion of tumor-reactive $CD8^+$ T-cells and TIL for adoptive cellular therapy [37], and the signaling domain of CD28 has been engineered into newer CAR-based cancer therapies [38], all of which are described later in this review.

OX40

OX40 (CD134) represents a transient activation marker on $CD4^+$ and $CD8^+$ T-cells that is upregulated by TCR engagement and provides co-stimulatory signals to the cells upon engagement of its ligand, OX40L [39,40]. OX40L is predominantly found on activated antigen presenting cells (B cells, dendritic cells, macrophages), but is also expressed on smooth muscle, endothelium, and activated T-cells [41]. Ligation of OX40 promotes T-cell proliferation, survival and effector function [42,43]. Importantly, OX40 signaling has also been reported to overcome $CD8^+$ T-cell tolerance in animal models of cancer [44]. This is due not only to T-cell costimulation, but also the ability to impair Tregs suppressor function [45]. The presence of $OX40^+$ T cells in human malignancies prompted evaluation of agonistic OX40 antibodies clinically [46]. In a Phase I human trial, agonistic antibody was well-tolerated, and enhanced T-cell activation and proliferation while leading to regression of at least one metastasis in 40% of patients receiving a single course of the therapeutic [47]. Five additional clinical studies have been initiated to evaluate the efficacy of OX40 agonistic antibodies either alone or in conjunction with radiation and chemotherapy for prostate cancer (NCT01303705) and breast cancer (NCT01862900),or with checkpoint

blockade (ipilimumab: anti-CTLA-4) for metastatic melanoma (NCT01689870). The signaling domain of OX40 is also being incorporated into CAR-expressing T cells for adoptive cell therapy trials to treat neuroblastoma (NCT01822652) and advanced sarcomas (NCT01953900).

4–1BB

4–1BB (CD137) is widely expressed on activated T-cells, NK cells, and other hematopoietic cells, as well as some tumor endothelia [41]. Engagement of 4–1BB with its ligand, 4–1BBL, on activated APC, increases the proliferation of T-cells and their expression of the anti-apoptotic proteins, Bcl-2 and Bcl-xL, promoting their survival [41,48]. In preclinical models, administration of a 4–1BB agonistic antibody reverses $CD8^+$ T-cell tolerance and can promote tumor regression, primarily via its actions on $CD8^+$ T-cells [49–51]. For clinical purposes, the broad distribution of 4–1BB creates concerns regarding toxicity. A Phase I clinical trial using the agonistic 4–1BB antibody BMS-663513 for treatment of metastatic melanoma showed signs of immune stimulation and appeared to be well-tolerated, stabilizing disease in 17% of patients for up to 6 months (NCT00309023) [52]. However, a Phase II trial evaluating the same antibody reported severe hepatitis at the highest doses (NCT00612664). A newer 4–1BB antibody, PF05082566, is now in Phase I trials as a monotherapy or in combination with rituximab for non-Hodgkin's lymphoma (NCT01307267). Like CD28 and OX40, the signaling domain of 4–1BB is being genetically engineered into CAR-expressing T cells for adoptive immunotherapy in multiple cancers, and is discussed later in this review.

CD40

CD40 is broadly expressed, including on professional APC and tumor cells. CD40 ligation stimulates APC maturation and subsequent priming of CD40L-expressing $CD4^+$ T-cells, which then aid in the orchestration of a cytotoxic T-lymphocyte response [53,54]. Thus, targeting CD40 is predominantly an indirect mechanism for promoting immunostimulation of tumor-specific $CD8^+$ T-cells. Agonistic CD40 antibodies have been shown to rescue tolerized cytotoxic lymphocyte responses to poorly immunogenic tumors and have demonstrated potent preclinical antitumor efficacy [54–56]. Several different agonistic antibodies have been studied to treat human hematologic malignancies, including lucatumumab (HCD122) and dacetuzumab (SGN-40), but responses were minimal with some toxicity [57–60]. For advanced solid tumors, Phase I safety and dose escalation trials have been completed using anti-CD40 (CP870–893). In the first trial, CP870–893 was found to be well-tolerated, with an objective response rate of 14%, and 24% of patients achieving stable disease [61]. Two additional trials were recently completed evaluating CP870–893 in combination with checkpoint blockade (tremelimumab: anti-CTLA-4) (NCT01103635) for late-stage melanoma and in a dose-escalation Phase I trial with the chemotherapeutics, paclitaxel and carboplatin, for solid malignancies (NCT00607048). There is also an active study with the chimeric (mouse and human derived) monoclonal CD40 agonistic antibody Chi Lob 7/4 in Europe (NCT 01561911).

DC vaccines

Dendritic cell (DC) vaccination strategies against cancer exploit the ability of DC to capture, process and present antigens to T-cells, and induce tumor-specific $CD8^+$ T cells to reject tumor and provide long-lived memory [62]. The maturation status of the DC dictates whether tolerance or immunity is induced in engaged T cells [63]. Mature, antigen-loaded DC produce greater amounts of immune-modulating cytokines like IL-12 and IL-15 [25,64], and upregulate expression of co-stimulatory molecules required to enhance effector differentiation of $CD8^+$ T cells and overcome tumor-associated tolerance [50,56,65]. To generate mature DC for vaccination, patient-derived DC are cultured with adjuvant, or alternatively, monocytes are differentiated with cytokines (e.g. GM-CSF and IL-4) [66]. Clinical studies have demonstrated that *ex vivo* generated DC vaccines are safe and capable of inducing the expansion of circulating tumor-specific T-cells [67]. Recently, Sipuleucel-T (Provenge) became the first commercial DC vaccine approved by the US FDA for treatment of prostate cancer (Table 3). This personalized vaccine is created through enrichment and activation of patient-derived DC by culturing with GM-CSF and the prostate cancer antigen, prostatic acid phosphatase (PAP). Sipuleucel-T treatment induces T-cell proliferation and PAP-specific immune responses, and provides a 4-month survival benefit to vaccinated patients [68]. Additional DC vaccines (DCVax-L) are now being commercially developed and tested in clinical trials against glioblastoma (NCT00045968), renal carcinoma (NCT01582672), ovarian cancer (NCT00683241), and other solid tumors (NCT01882946).

A related therapeutic approach is *in situ* DC vaccination. Here, tumor antigen is delivered to endogenous DC by coupling antigens to antibodies specific for DC surface receptors such as DEC205 [69]. Receptor binding leads to antigen internalization, processing, and presentation by endogenous DC matured via a co-administered adjuvant. Two Phase I trials have been initiated to investigate vaccination with a DEC-205/NY-ESO-1 fusion protein for vaccination of patients with a broad range of cancers including acute myeloid leukemia or myelodysplastic syndrome [NCT01834248], or with sirolimus in a variety of solid tumors [NCT01522820]. DC vaccines are also being explored in combination with other immune modulating treatments like checkpoint blockade (Table 3). The combination of MART-1 peptide-pulsed DC and the CTLA-4 blocking antibody Tremelimumab increased MART-1 specific $CD8^+$ T cells and achieved objective response in 25% of melanoma patients [70]. A Phase II study using PD-1 blockade in conjunction with the DC/Renal Cell Carcinoma Fusion Cell Vaccine is now recruiting patients (NCT01441765).

Listeria monocytogenes

A variety of live attenuated bacterial and viral vectors are being explored as anticancer agents, with *Listeria monocytogenes* ranking among the most well-characterized for therapy. Its intracellular lifestyle and natural targeting of DC and other professional APC makes *Listeria* particularly adept at eliciting the $CD8^+$ T-cell-mediated immunity required to effectively eliminate tumor [71]. The ability to manipulate the *Listeria* genome to encode desired antigens or adjuvant proteins that are secreted *in situ* in infected cells helps drive tumor-specific responses, including reinvigoration of tolerant self-tumor-reactive $CD8^+$ T-cells [72,73]. Furthermore, the inflammatory responses driven by *Listeria* vaccines

efficiently promote tumor infiltration by activated $CD8^+$ T-cells and reduce suppressive cell populations [74].

Attenuated strains of *L. monocytogenes* being assessed for the clinical treatment of cancer have demonstrated an acceptable safety profile and provided early indications of activity. Lovaxin C (ADXS-11–001, LM-LLO-E7) encodes the Human Papilloma Virus (HPV) E7 antigen fused to Listeriolysin (LLO), an antigenic virulence factor that enhances the immunogenicity of the E7 protein [75]. In a Phase I/II study for advanced cervical cancer, 53% of patients had stable disease and 31% of patients had a reduction in tumor size. [76]. These promising results have lead to Phase II trials for cervical intraepithelial neoplasia (NCT01116245), cervical carcinoma (NCT01266460), and Phase I trials for $HPV16^+$ oropharyngeal cancer (NCT01598792). Similarly, when the mesothelin antigen encoding CRS-207 (*Listeria* ΔactA/ΔinlB-mesothelin) was evaluated in patients with mesothelin-positive malignancies, 35% of patients survived greater than 15 months and 83% of these individuals mounted an LLO-specific T-cell response [77]. Subsequent clinical trials with CRS-207 have been initiated in conjunction with GVAX for metastatic pancreatic cancer (NCT01417000, NCT02004262), or with pemetrexed and cisplatin for pleural mesothelioma (NCT016757650). A Phase I trial examining a newer agent, ADU-623, for glioblastoma multiform (NCT01967758) was recently initiated (Table 3).

Adoptive cell transfer

Adoptive cell transfer (ACT) strives to overcome tolerance by generating large numbers of appropriately activated T cells outside of the immunosuppressive host environment and re-infusing them back into patients where they can mediate tumor regression [37]. Early trials of this approach were limited by their inability to achieve engraftment of large numbers of high affinity tumor-specific T cells, but strategies such as lymphodepletion, elimination of specific suppressor cell subsets, and genetic engineering have enabled adoptive cell-based therapies to achieve dramatic tumor regression in human patients [78].

Genetically engineered T-cell receptors

T-cell receptor (TCR) engineering is a strategy to overcome the obstacles of limited numbers of peripheral tumor-antigen reactive T-cells and the restriction of lower affinity TCR expression. This is accomplished by introducing genes encoding a high affinity TCR to redirect the specificity of patient-derived peripheral T-cells [79]. Such redirected T-cells have specificity for tumor-associated antigens (TAA), and can be re-infused into patients to provide tumor immunity. The first clinical application of genetically engineered TCR for adoptive immunotherapy utilized a MART-1-specific TCR and reported melanoma tumor regression and durable objective responses in two out of 15 patients with rapidly progressive and therapy-resistant metastatic melanoma [80]. These proof of concept studies have led to several subsequent trials, including ongoing Phase II trials for MART-I (NCT00910650) and NY-ESO-1 (NCT01697527, NCT00670748) specific TCR in patients with melanoma or other advanced solid malignancies (Table 4). One limitation of this approach has been the reliance on TCR derived from endogenous TAA-reactive T cells, which are not always high affinity. This is being countered by introducing mutations in the TCR complementarity determining regions, enhancing TCR affinity several orders of magnitude [81]. Affinity-

enhanced TCR directed toward the cancer testis antigen, NY ESO-1, were found to be both safe and effective [82], and are the focus of two current clinical trials (NCT01892293 and NCT01967823). However, there is accumulating evidence of severe off-target toxicity with affinity-enhanced TCR specific for other targets, such as MAGE-A3 [83,84], suggesting the future of this strategy will rely on better toxicity screening. Extensive reviews covering the challenges and advances in TCR gene therapy have been provided elsewhere [79,85].

Chimeric antigen receptors

A promising new approach to prevent tolerance in autologous adoptively transferred tumor-reactive T-cells is expression of genetically engineered chimeric antigen receptors (CAR). Unlike engineered TCR, CAR molecules are completely artificial receptors composed of an antibody subunit capable of binding specific tumor antigens independent of MHC, a trans-membrane domain, and cytoplasmic domains that transduce activation signals to the T-cell upon antigen encounter [38]. Utterly simplistic in concept, the cytoplasmic tails can be tailored to contain signaling domains from costimulatory molecules (e.g., CD28 and 4–1BB) such that antigen recognition is always associated with powerful costimulation [86]. T-cells that express tumor antigen-specific CAR are considerably less vulnerable to mechanisms of tumor evasion and immune suppression (i.e., tolerance) due to this high level of consistent activation. In addition, tumor recognition by CAR is not compromised by alterations in tumor MHC expression or epitope processing, or by TCR complex dissociation on responding T-cells. Furthermore, the antibody-antigen interaction is often of much higher affinity compared with natural TCR binding to MHC/peptide. The fact that CAR do not rely on MHC/peptide-binding carries the added advantage that they are not restricted by a patients' HLA type.

The primary limitation of CAR-mediated immunotherapy is that antigens can only be recognized on the surface of tumors, leaving intracellular tumor-antigens essentially undetectable. However, this is not a serious drawback for those cancers that express well-known and targetable surface antigens, like CD19 on B cell-derived leukemia and lymphoma. T-cells expressing CD19-specific CAR have shown remarkable efficacy at clearing B-cell tumors in animal models [86], and proven clinically promising in human patients [87]. That being said, identification of novel targetable antigens will be key for CAR-based immunotherapy moving forward. This is aided by the antibody–antigen recognition by CAR molecules, which open up a wider range of targets that are not typically involved in TCR-mediated tumor cell recognition, such as carbohydrates and glycolipid tumor antigens [88]. There are approximately 40 studies listed on [89] designed to explore CAR-expressing T cells for immunotherapy in human cancer patients (Table 4).

The next generation of CAR-expressing T cells is being designed in laboratories and tested in animal models. Such T cells are referred to as 'TRUCK's (T-cells redirected for universal cytokine killing), and express CAR along with a separate transgene to allow expression of secreted factors like cytokines [90]. This concept extends the capabilities of CAR-expressing T-cells beyond tumor antigen recognition and direct cytotoxicity, and brings to bear the potential to direct other immune responses within the tumor environment. For example, two reports have demonstrated that IL-12 expression by tumor-specific CAR-T-

cells were more effective at clearing detectable tumors than the same CAR-T-cells that lacked IL-12 expression [91,92]. Moreover, Chmielewski *et al.* showed that IL-12 secretion by these CAR-T-cells even promoted *in vivo* killing of antigen-negative tumors not recognized by the specific CAR being expressed. This is a particularly important enhancement of the CAR concept, as antigen-loss tumor variants represent a real impediment to CAR-based immunotherapy in patients [87]. This compelling result demonstrates the immunomodulatory power of IL-12, a pleiotropic cytokine with the ability to induce expression of the costimulatory ligands CD80 and CD86 on surrounding cells, promote localized Th1 responses (including IFN-γ secretion), recruit natural killer cells and macrophages, and provide resistance to the suppressive effects of Tregs and myeloid derived suppressor cells (MDSCs) [93].

It is tempting to speculate that the bright future of CAR and TRUCK-based immunotherapies for cancer will be limited only by the number of antigens that can successfully be targeted. However managing the risks associated with unleashing such a robust immune response in patients is also key to the future success of these treatment strategies. Engineered T cells can cause autoimmunity, induce cytokine storms, and any genetic modifications can lead to insertional mutagenesis, causing severe toxicity and even death in patients [87,94–95]. Some of these events could potentially be reversed by selective deletion of the engineered T cells themselves, and this is being explored by introducing a ‘suicide gene’ along with other CAR to provide such an option for patients [96].

Lymphodepletion

Host conditioning or ‘lymphodepletion’ prior to adoptive cell transfer provided one of the earliest leaps forward for ACT immunotherapy. A lymphopenic environment promotes T-cell homeostatic proliferation, effector function, memory-like differentiation, and even transient rescue of $CD8^+$ T-cell tolerance [97–99]. In preclinical models, lymphodepletion via total body irradiation (TBI) and/or cytotoxic drugs prior to ACT were found to enhance immune responses to cancer [100,101]. Similarly immunodepleting chemotherapy in human cancer patients prior to T-cell infusion led to the first reported tumor regressions by ACT, with 47–51% objective responses achieved in two separate melanoma trials [18,102]. Several mechanisms by which this disruption of immune homeostasis might overcome tolerance to provide better anticancer immunity have been described, including the creation of space in the lymphoid compartment for low-avidity tumor-reactive clones to expand or for adoptively transferred cells to engraft, the depletion of suppressor cell populations, and the elimination of competition for homeostatic cytokines [103].

The success of patient preconditioning has prompted recent efforts probing the extent of lymphodepletion necessary to improve patient outcomes. Lymphodepletion by chemotherapy alone or in combination with TBI were compared in sequentially treated cohorts of melanoma patients [104]. Here, 40% complete responses and 72% objective responses were observed for highest dose TBI (12 Gy), highlighting the critical role for host manipulation in optimizing ACT. These results are not achieved without consequence, as some autoimmunity toward healthy melanocytes and other toxicities do occur, but are generally well tolerated. Currently, there are 25 clinical trials employing lymphodepletion in

combination with other approaches such as ACT, DC vaccines, systemic IL-2, or molecularly targeted therapeutics. Fifteen of these trials are for metastatic melanoma, while others are aimed at ovarian (NCT01312376), kidney (NCT00091611), and nasopharyngeal (NCT00078546) neoplasms.

Inhibitory cell depletion

One of the presumed reasons for success of lymphodepletion strategies is the reduction of host suppressor cells. Suppressive cell types can dominate the tumor microenvironment, and the most well-defined of these are the Tregs [105]. Tregs are a population of $CD4^+$ $CD25^{hi}$ $Foxp3^+$ T cells whose role in the maintenance of self-tolerance and immune homeostasis is best demonstrated by the lymphoproliferation and inflammatory disease observed in mice and humans unable to generate these cells [14,106]. The accumulation of Tregs in malignancy is generally associated with a poor prognosis due to local immune suppression. But this is somewhat controversial and the current consensus is that the prognostic value of a high Tregs to effector T-cell ratio is likely dependent on the type of cancer [107]. Thus, in select tumors, depletion of Tregs may provide therapeutic benefit. The first strategies aimed at depleting Tregs targeted their high CD25 expression with monoclonal antibodies (daclizumab) or antibody-conjugated toxins (denileukin diftitox), which reduced Tregs numbers in patients and correlated with enhanced T-cell responses post-vaccination [108,109]. However, subsequent studies have failed to consistently observe these promising results, likely attributable to simultaneous depletion of effector T cells that also express CD25 [110]. To further define their clinical activity, both drugs remain under investigation in numerous studies for treatment of diverse human cancers. Additional methods to preferentially eliminate Tregs for cancer therapy have entered clinical trials, including low metronomic doses of cytotoxic drugs such as cyclophosphamide (NCT01581970, NCT01462214), and anti-CCR4 mAb (KW-0761, mogamulizumab) (NCT01929486). Finally, recent studies suggest that the antitumor activity of CTLA-4 targeted antibodies is achieved in part by depletion of $Foxp3^+$ $CD4^+$ Tregs, contributing to the activation of tumor-resident effector T-cells [111,112]. Of note, current therapies targeting Tregs nonspecifically deplete all Tregs, which creates a general risk of autoimmunity in patients. A key advancement would be the ability to specifically target the Treg-cell subpopulations contributing to tumor progression.

Myeloid-derived suppressor cells (MDSC) are a population of myeloid progenitor cells that fail to fully differentiate into DC, macrophages, or granulocytes. They are collectively identified by their suppressive phenotype and expression of the myeloid markers, Gr1 or CD11b in mice, or $CD33^+CD11b^+HLA\text{-}DR^{low/neg}$ in humans [113]. Suppression of $CD8^+$ T cells by MDSC can be achieved by many different mechanisms, including downregulation of the TCRζ, proliferative arrest induced by depletion of amino acids, and altered migration via cleavage of CD62L by the ADAM17 ‘sheddase,’ which have been described in more detail elsewhere [114,115]. Tumor-derived factors promote the accumulation of MDSC in both blood and tumor of cancer-bearing hosts, and frequency inversely correlates with prognosis [116]. A wide range of therapeutic targets have been identified for eliminating MDSC, from preventing their formation and function, to depleting them or inducing their differentiation, which have been reviewed by others [117]. Some of these approaches are in

clinical trials, employing therapeutics already FDA-approved for other indications, such as Tadalafil (a PDE5 inhibitor), which is being evaluated in head and neck cancer (NCT01697800). For depletion of MDSC, cytotoxic therapy with gemcidibine is being evaluated in a Phase I trial for sarcoma (NCT01803152).

Checkpoint blockade

The fate of any responding T-cell is largely determined by a balance between positive (co-stimulatory) and negative (co-inhibitory) signals. Several receptor-based pathways have been identified as negative regulators or 'checkpoints' of T-cell activation, which can induce tolerance or dysfunction in tumor-reactive T-cells. Overcoming tolerance induced by these receptors has recently been transformed from proof-of-principle experiments in laboratories to clinically effective treatments for patients with cancer. The concept of blocking negative regulatory receptors using specific monoclonal antibodies is referred to collectively as checkpoint blockade immunotherapy. Here, we discuss the molecular mechanisms that underlie this potent new therapeutic strategy, and review results from recent clinical trials.

CTLA-4

(CTLA-4; CD152) is expressed on activated $CD4^+$ and $CD8^+$ T cells, and on Tregs. Its functions in T-cell biology, during immune responses to infection, and as a target for cancer immunotherapy have been well described and reviewed elsewhere [118]. CTLA-4 is a homologous counterpart to the co-stimulatory receptor CD28, both of which bind to CD80 and CD86 on APCs. The importance of CTLA-4 for immune tolerance is clear [119], but its role is complex, involving several overlapping mechanisms that serve to blunt T-cell responses in a range of settings. These include out-competing lower affinity CD28 molecules for ligand binding to minimize T-cell co-stimulation, recruitment of inhibitory phosphatases to the TCR complex to disrupt positive signaling cascades, and removing CD80 and CD86 from the surface of APC by trans-endocytosis, thereby diminishing the ability of APC to properly activate otherwise responsive T-cells [120–122].

Based on the criteria above, it is easy to understand why exploitation of the CTLA-4 receptor/pathway is an attractive strategy to modulate T-cell immunity. Indeed, anti-CTLA-4 was the first monoclonal antibody (ipilimumab) to be FDA-approved for checkpoint blockade treatment in cancer patients. There are currently more than 150 ongoing clinical trials investigating the safety and efficacy of CTLA-4 antibodies (ipilimumab and tremelimumab; Table 5, with most focused on boosting immune responses to cancer, predicated on the promising results in melanoma patients [123]. These monoclonal antibodies are thought to work by binding to CTLA-4 on the surface of effector T-cells and blocking natural ligation with B7 molecules. From the perspective of tumor-reactive effector $CD8^+$ T-cells, this prevents negative intracellular signal transduction downstream of CTLA-4, and also results in more unbound B7 available for ligation to CD28 for co-stimulation. On Tregs, there is evidence that CTLA-4 has a direct role in the suppressive phenotype, which may be inhibited with blockade antibody [124,125]. It has also been demonstrated in mice that anti-CTLA-4 binds to CTLA-4^{high} Tregs cells within tumors and promotes antibody-mediated depletion of these Tregs populations while sparing effector T-cells [112]. However, it is important to acknowledge the inevitable flipside of

overcoming these tolerizing mechanisms and reinvigorating a powerful immune response, as blockade of CTLA-4 in patients comes at the cost of potentially severe autoimmunity [123]. Predicting and managing these immune related toxicities is vital for the future success of checkpoint blockade immunotherapy.

PD-1 & PD-L1

Programmed death-1 (PD-1; CD279) is a member of the same family of receptors as CD28 and CTLA-4, and is broadly expressed on lymphoid and myeloid cells [126]. PD-1 binds uniquely to the B7 ligands PD-L1 and PD-L2 on APC and other surrounding tissues, greatly influencing the fate of responding $CD8^+$ T cells in settings of chronic infections and cancer [127–129]. On T-cells, PD-1 is expressed after antigen encounter, but acts almost immediately to impede T-cell activation by recruiting the phosphatases SHP-1 and SHP-2 through signaling motifs in the PD-1 cytoplasmic tail, which reduces Akt phosphorylation, and diminishes T-cell metabolism, proliferation and survival [130]. This is particularly relevant to cancer immunology because many different types of tumor cells can express PD-L1 [131].

Efforts to attenuate the PD-1/PD-L1 pathway have followed a similar blueprint as CTLA-4, focusing on antagonistic antibodies that block receptor ligation. Preclinical work in animal models has demonstrated that *in vivo* administration of such antibodies can restore function in otherwise ineffective T-cells to boost antitumor and antviral responses [127,128]. Three recent clinical trials using different PD-1 or PD-L1 antibodies reported objective responses in 6–38% of patients with different cancer types [132–134]. However, treatment related immune toxicities (> grade 3) were reported in 9–14% of patients treated, a similar frequency reported for the anti-CTLA-4 monoclonal antibody, ipilimumab [123]. The autoimmune events associated with blocking PD-1 are similar to those that occur when blocking CTLA-4 and frequently include skin or gastrointestinal manifestations, such as colitis or dermatitis [135]. Fortunately, as more patients are treated with checkpoint blockade immunotherapy, these autoimmune events are becoming easier to predict and manage by inducing immunosuppression, and altering or discontinuing treatment [136]. There are currently more than 50 trials listed on [89] investigating the safety and efficacy of PD-1 pathway blockade for treatment of a wide range of different human cancers (Table 5).

Combination immunotherapy

The exploitation of negative regulatory receptors to overcome or prevent T-cells tolerance has not stopped at CTLA-4 and PD-1. Several others, including LAG-3 and TIM-3, have been shown to influence T-cell dysfunction [137–139], and are under investigation as potential checkpoints for cancer immunotherapy intervention, which has been discussed elsewhere in more detail [36,140]. While most checkpoint blockade antibodies have been investigated as monotherapies, there is general consensus that combination strategies are likely to be more effective [36,141]. The logic behind this theory lies in the understanding that each regulatory receptor plays a unique role in T-cell tolerance, and simultaneous blockade of multiple pathways represents a means to restore more immune functions. This notion has been demonstrated in animal models of cancer [128,142–144], and recapitulated in melanoma patients treated with CTLA-4 and PD-1 double blockade [145]. Nearly as

remarkable as the 53% objective response rate achieved in patients was the realization that adverse immune-related events associated with this combination blockade was similar in nature to individual antibody regimens, and that these were manageable or even reversible in most cases. There are currently at least nine trials listed on ClinicalTrials.gov testing the combination of anti-PD-1 (8 with Nivolumab and 1 with MK-3475) and anti-CTLA-4 (ipilimumab), and one examining anti-LAG-3 (BMS-986016) and nivolumab. Many more are studying checkpoint blockade antibodies in combination with other more conventional chemotherapies (Table 6).

Conclusion & future perspective

Since the first attempts to harness cellular immunity to eliminate cancer in human patients over 30 years ago, much has been learned about the potential of the immune system to control cancer and how immunotherapy can boost this potential in patients. An appreciation for immune tolerance has aided this process, as we now understand that cancer escapes immune control by failing to elicit sufficient immunity or more actively by inducing peripheral tolerance. Thorough understanding of $CD8^+$ T-cell tolerance has been instrumental to clinical progress, and enabled the design of targeted approaches to achieve antitumor responses by tumor-reactive T cells that otherwise remain ignorant due to low-avidity receptors or anergic by the immuno-suppressive tumor microenvironment. At the same time, advances in biotechnology and molecular techniques have also aided this endeavor, providing a foundation for engineering T cells of desired antigenic specificity. The recent achievements with these approaches for patients with advanced-stage, treatment refractory disease have collectively validated the long-standing idea that immunity is vitally important in the control and elimination of cancer. The paradigm has shifted for treating cancer patients exclusively with tumor-targeted approaches to now include strategies aimed at stimulating immunity. Improving upon these successes will likely require careful incorporation of several treatment modalities in combination approaches, some of which are already being explored in clinical trials. It will also rely on the same ‘bench-to-bedside and back’ translational philosophy that has characterized the development of immunotherapy thus far where observations from animal studies are exploited to provide clinical benefit for human cancer patients, and this success in patients guiding the next steps in preclinical investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

CD8$^+$ T-cell tolerance & cancer immunotherapy

- CD8$^+$ T cells are the predominant effector in most cancer immunotherapy settings.
- Tolerance represents a major obstacle to eliciting antitumor CD8$^+$ T-cell responses; thus, immunotherapeutic approaches aim to overcome tolerance.
- Autoimmunity is a concerning but often manageable risk of cancer immunotherapy.

Cytokines

- IL-2, IL-15, and IL-21 are capable of overcoming tolerance and promoting CD8$^+$ T-cell immunity, with variants and cytokine-antibody immune complexes representing new tools to potentiate the desirable properties of cytokine therapy.
- Current clinical trials focus on cytokine dosing regimens and combination approaches with other therapeutics, to define the safety and efficacy of cytokine based therapeutics.

Costimulation & vaccination

- Costimulatory agonists
  - Ligation of costimulatory receptors (CD28, OX40, 4–1BB, CD40) can prevent the induction of T-cell tolerance and overcome established tolerance.
  - Costimulatory agonistic antibodies are being evaluated as mono/combination therapies for a variety of cancers, and the signaling domains of costimulatory receptors have been incorporated into chimeric antigen receptors (CAR).
- Dendritic cell (DC) vaccines
  - Mature DC presenting tumor antigens promote T-cell immunity over tolerance.
  - Newer approaches include *in situ* DC vaccination, DC-tumor fusion vaccines, and combination therapies.
- *Listeria* vaccines
  - *Listeria* vaccines encoding tumor antigens naturally secrete these products into infected dendritic cells that efficiently prime CD4$^+$ and CD8$^+$ T-cell immune responses.
  - Early trials with these vaccines have found them to be safe, immunogenic and capable of reducing tumor burden; additional trials are underway.

Adoptive cell transfer

- Adoptive cell transfer overcomes tolerance by removing tumor-reactive T cells from the immunosuppressive host, expanding them *ex vivo*, and reinfusing them back into patients to mediate tumor regression.
- T-cell specificity can be redirected by transduction of high affinity T-cell receptors (TCR) or CAR to overcome tolerance-imposed limitations on tumor-specific T-cell number, specificity, and avidity.

Checkpoint blockade

- Inhibitory co-receptors (CTLA-4, PD-1, among others) play a role in the induction and maintenance of T-cell tolerance by transducing negative signals during antigen encounter.
- Antibody blockade of these receptors, individually and in combination, can restore function in tolerant T-cells and boost antitumor responses.

Conclusion & future perspective

- A basic understanding of immune tolerance has elucidated key principles guiding the development of cancer immunotherapeutics.
- Recent success in patients with advanced disease validates the long-standing idea that the immune system can eliminate cancer and is driving new areas of investigation.

Table 1

Cytokines.

Cytokine	Uses	Agent	Developmental stage and clinical trial references†
IL-2	Monotherapy	IL-2, proleukin, aldesleukin	Approved for IV infusion in advanced Renal cell cancer and melanoma Phase II clinical trials (dosing) 1. NCT01702896† – melanoma 2. NCT01702909† – kidney cancer
	Companion therapy to Lymphodepletion (LD)/ACT/DC Vaccination/Ipi	IL-2, proleukin, aldesleukin	Phase II clinical trials for melanoma 1. NCT00338377† – LD + ACT + DC Vax 2. NCT01995344‡–TIL + CTX 3. NCT01701674† – LD + ACT + Ipi 4. NCT02027935† – ACT + CTX + Ipi
	Engineered IL-2 variants	RO6895882 (GA504/CEA-IL2v)	Phase I clinical trial 1. NCT02004106†–solid tumors
		ALT-801 (soluble P53TCR-IL-2 fusion protein)	Phase I/II clinical trial 1. NCT01670994¶– myeloma
		IL-2 superkine (super-2)	Preclinical animal studies
	IL-2/mAb immune complexes	mIL-2/anti-IL-2, rh-IL-2/IL-2Rα	Preclinical animal studies
IL-15	Monotherapy	rhIL-15	Phase 1 clinical trials 1. NCT01572493† –carcinoma, lymphoma 2. NCT01727076† – solid tumors 3. NCT01021059§ – melanoma or RCC
	Companion therapy(with NK cell or TIL transfer)	rhIL-15	Phase I clinical trials 1. NCT01385423† –AML 2. NCT01875601† –solid tumors Phase I/II clinical trial 1.NCT01369888† – melanoma
	IL-15/mAb immune complexes	ALT-803 (IL-15N72D + IL-15RαSu/Fc)	Phase I/II clinical trials 1. NCT01946789‡– melanoma 2. NCT01885897† – hematologic malignancy
IL-21	Monotherapy	rh-IL-21	Phase I clinical trial 1. NCT00095108§ – melanoma or kidney cancer Phase II clinical trial 1. NCT00514085§– melanoma
	Combination therapy (with Ip or nivolumab)	rh-IL-21	Phase I clinical trials 1. NCT01489059¶–melanoma 2. NCT01629758†–solid tumors

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the NIH.

†Recruiting.

‡Not yet recruiting.

§Completed.

¶Active, not recruiting.

ACT: Adoptive cell transfer; AML: Acute myeloid leukemia; CEA: Carcinoembryonic antigen; CTX: Chemotherapy; DC: Dendritic cell; Ipi: Lipilimumab; IV: Intravenous; LD: Lymphodepletion; mAb: Monoclonal antibody; mIL: Mouse interluekin; RCC:Renal cell cancer; TCR: T-cell receptor; TIL: Tumor infiltrating lymphocytes; rbIL: Recombinant human interluekin; Vax: DC vaccination.

Table 2

Costimulation.

Target	Uses	Agent	Developmental stage and clinical trial references[†]
CD28	Agonistic antibody	TGN1412 (CD28-SuperMAB)	Discontinued due to toxicity
	Signaling domain of CAR T-cells	CD19.CAR-CD28Z T-Cells	Phase I clinical trials for CD19$^+$ malignancies 1. NCT02050347[‡] 2. NCT02051257[‡] 3. NCT01815749[†]
OX40	Agonistic antibody (combination therapies)	Anti-OX40 mAb	Phase I clinical trial 1. NCT01644968[¶] – cancers Phase I/II clinical trials 1. NCT01303705[†] – prostate cancer 2. NCT01862900[†] – breast cancer
	Signaling domain of CAR-T cells	iC9-GD2-CD28-OX40(iC9-GD2) T cells	Phase I clinical trials 1.NCT01822652[†] –neuroblastoma 2. NCT01953900[‡] – sarcoma
4-1BB	Agonistic antibody	Urelumab (BMS-663513)	Phase I clinical trial 1. NCT01471210[†] – solid tumors/NHL Phase II clinical trial 1. NCT00612664[§] – melanoma
	Agonistic antibody (combination)	PF-05082566	Phase I clinical trial 1. NCT01307267[†] – NHL
	Signaling domain of CAR-T cells	CD19 scFv TCR:41BB	Phase II clinical trials 1. NCT02030847[†] – B-cell ALL 2. NCT01029366[§] – B-cell leukemia/lymphoma
CD40	Agonistic antibody(combination)	Lucatumumab (HCD122)	Phase lb clinical trial 1. NCT01275209[§] –CD40$^+$follicular lymphoma
	Agonistic antibody	Dacetuzumab (SGN-40)	Phase I clinical trial 1. NCT00079716[§] – myeloma Phase I/II clinical trial 1. NCT00283101[§] –CLL
	Agonistic antibody (combination)	CP870-893	Phase I clinical trials 1. NCT0113635[†] –melanoma 2. NCT00607048[§] – solid tumors
	Chimeric Agonistic Ab	Chi-Lob 7/4	Phase I clinical trial 1. NCT01561911[¶] – neoplasms, lymphoma

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the NIH.

[†]Recruiting.

[‡]Not yet recruiting.

[§]Completed.

[¶]Active, not recruiting.

ALL: Acute lymphoblastic leukemia; CAR: Chimeric antigen receptor: CLL: Chronic lymphocytic leukemia; mAb: Monoclonal antibody NHL: Non-Hodgkin's lymphoma.

Table 3

Vaccination.

Target	Strategy	Agent	Developmental stage and clinical trial references[†]
Dendritic cells	*Ex vivo* generation	Sipuleuel T (ACP8015/ Provenge)	US FDA approved therapeutic cancer vaccine
		DC Vax-L	Phase I, I/II clinical trials 1. NCT00683241[‡]– ovarian cancer 2. NCT01882946[†]– solid tumors Phase III clinical trials 1. NCT00045968[†]– glioma 2. NCT01582672[†] – RCC
	In situ vaccination	CDX-1401 (DEC-205/NYESO-1)	Phase 1 clinical trials 1. NCT01522820[†] – solid tumors 2. NCT01834248[†] – AML/MDS
	DC tumor cell fusion vaccines		Phase I clinical trial 1. NCT00459069[‡] – myeloma Phase I/II, II clinical trials 1. NCT00799110[§] – ovarian cancer 2. NCT00622401[§] – breast cancer
	Combination therapy	Sipuleucel-T + ipilimumab (CTLA-4 blockade)	Phase I clinical trial 1. NCT01832870[§] – prostate cancer Phase II clinical trial 1. NCT01804465[§] – prostate cancer
		DC tumor fusion vaccine + pidilizumab (PD1 blockade)	Phase II clinical trials 1. NCT01441765[†]–RCC 2. NCT01096602[†]–AML 3. NCT01067287[†]– myeloma
Listeria monocytogenes	Live-attenuated *Listeria* encoding LLO-E7 fusion protein	Lovaxin-C (ADXS-11-001, Lm-LLO-E7)	Phase I clinical trial 1. NCT01598792[†] – oropharyngeal cancer Phase II clinical trials 1. NCT01116245–CIN 2. NCT01266460[†]– cervical cancer
	Live-attenuated *Listeria* expressing human mesothelin	CRS-207	Phase I clinical trial 1. NCT01675765[†]–pleural mesothelioma Phase II clinical trial 1. NCT02004262[†] – pancreatic cancer
	Live-attenuated *Listeria* expressing EGFRvlll and NY-ESO-1	ADU-623	Phase I clinical trial 1. NCT01967758[†] – astrocytoma, glioblastoma

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the US NIH.

[†]Recruiting.

[‡]Completed.

[§]Active, not recruiting.

AML: Acute myeloid leukemia; CIN: Cervical intraepithelial neoplasia; DC vax: Dendritic cell vaccination; LLO: Listeriolysin; MDS: Myelodysplastic syndrome; RCC: Renal cell carcinoma.

Table 4

Engineered T-cell receptors/chimeric antigen receptors.

Target antigen	Structure/endodomain	Agent	Developmental stage and clinical trial references[†]
TCR			
MART-1	T-cell receptor	MART-1-specific TCR	Phase II clinical trials in melanoma 1. NCT00706992[#] 2. NCT00509288[§] 3. NCT00910650[†]
NYESO-1		NY ESO-1-specific TCR	Phase I/II clinical trials in myeloma 1. NCT01352286[†] 2. NCT01892293[†] Phase II clinical trials in NY-ESO-1 + cancers 1. NCT00670748[†] 2. NCT01697527[†] 3. NCT01967823[†]
MAGEA3		PG13-MAGE-A3 TCR9W11 (MAGE-A3/12TCR)	Phase I/II clinical trial 1. NCT01273181[#] – MAGEA3/12^{+} cancer
GP100		gp100-specific TCR	Phase II clinical trial 1. NCT00509496[#] – melanoma
CAR			
CD19	CD28-CD3ζ	CD19CAR-28-zetaT cells	Phase I, I/II clinical trials 1. NCT00466531[†]–CD19^{+}CLL/BCL 2. NCT00586391[†] – B-NHL, ALL & CLL
	41BB-CD3ζ	CART-19 TCR zeta:4-1BB (CTL019)	Phase II clinical trials 1. NCT02030834[†] – CD19^{+}lymphomas 2. NCT02030847[†] – B cell ALL 3. NCT01029366[§] – B cell leukemia/lymphoma 4. NCT01747486[†] – CD19^{+}CLL or SLL
CD20	CD28-41BB-CD3ζ	CART-20 TCR zeta:4-1BB	Phase I clinical trials in B- cell leukemia/lymphoma 1. NCT00621452[¶] 2. NCT01735604[†]
CEA	CD28-CD3ζ	Anti-CEA Designer T-cells	Phase II clinical trials 1. NCT01723306[†] – adenocarcinomas
Her2	CD28-CD3ζ	HER.CAR CMV-specific CTLs	Phase I clinical trials 1. NCT01109095[†] – GBM 2. NCT00889954[†] – HER2^{+} cancer
GD2	CD28-OX40-CD3ζ	iC9-GD2-CD28-OX40 (iC9-GD2) T-cells	Phase 1 clinical trials 1. NCT01822652[†] – neuroblastoma 2. NCT01953900[‡] – sarcoma

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the US NIH.

[†]Recruiting.

[‡] Not yet recruiting.

[§] Completed.

[¶] Active, not recruiting.

[#] Terminated.

ALL: Acute lymphocytic leukemia; CAR: Chimeric antigen receptor: CLL: Chronic lymphocytic leukemia; GBM: Glioblastoma multiforme; NHL: Non-Hodgkin's Lymphoma; SLL: Small Lymphocytic Lymphoma; TCR: T-cell receptor.

Table 5

Individual checkpoint blockade.

Target	Agent	Antibody type	Developmental stage and clinical trial references†
CTLA4	Ipilimumab (MDX-010)	Human lgG1 mAb	US FDA approved for melanoma Phase I/II, II clinical trials in melanoma 1. NCT01585194† 2. NCT01689974† Phase III clinical trials in melanoma 1. NCT00094653§ 2. NCT01515189¶
	Tremelimumab (CP-675, 206)	Human lgG2 mAb	Phase II clinical trials 1. NCT00471887¶ – melanoma 2. NCT01649024¶ – mesothelioma
PD1	Nivolumab (BMS-936558 or MDX1106)	Human lgG4 mAb	Phase 1 clinical trials 1. NCT01176461† – melanoma Phase I/II clinical trial 1. NCT01928394† – solid tumors Phase II clinical trial 1. NCT01354431¶ – renal cell cancer Phase III clinical trials 1. NCT01844505† – untreated melanoma
	Lambrolizumab (MK-3475)	Human lgG4 mAb	Phase I clinical trial 1. NCT01295827† – solid tumors Phase II clinical trial 1. NCT02085070† – melanoma and NSCLC Phase III clinical trial 1. NCT01866319¶ – melanoma
	Pidilizumab(CT-011)	Human lgG1 mAb	Phase I/II clinical trial 1. NCT01952769† – glioblastoma multiforme Phase II clinical trials 1. NCT01435369§ – melanoma 2. NCT01313416† – pancreatic cancer
	AMP-224	PDL2 Ig fusion protein	Phase I clinical trial 1. NCT01352884¶ – cancers
PDL1	MDX-1105 (BMS-936559)	Human lgG4 mAb	Phase 1 clinical trial 1. NCT00729664¶ – solid tumors
	MPDL3280A	Engineered lgG1 (no ADCC)	Phase 1 clinical trial 1. NCT01375842† – Solid tumors 2. NCT02013219‡ – NSCLC Phase II clinical trials for NSCLC 1. NCT02031458† 2. NCT01903993†
LAG-3	BMS-986016	Human IgG mAb	Phase 1 clinical trials 1. NCT0206176† – hematologic neoplasms
	IMP321	Soluble LAG-3	Phase I clinical trial 1. NCT00351949§ – renal cell cancer Phase I/II clinical trial 1. NCT01308294† – melanoma

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the NIH.

[†] Recruiting.

[‡] Not yet recruiting.

[§] Completed.

[¶] Active, not recruiting.

ADCC: Antibody-dependent cell-mediated cytotoxicity; mAb: Monoclonal antibody; NSCLC: Non-small-cell lung cancer.

Table 6

Combination checkpoint blockade.

Target	Agent	Antibody type	Developmental stage and clinical trial references[†]
CTLA4 + PD1	Ipilimumab Nivolumab		Phase I clinical trials 1. NCT01024231[†] – melanoma Phase I/II clinical trials 1. NCT01928394[†] – solid tumors 2. NCT02060188[†] – colon cancer 3. NCT01927419[§] – melanoma Phase III clinical trials 1. NCT01844505[†] – untreated melanoma
CTLA4 + PD1	Ipilimumab Lambrolizumab		Phase I/II clinical trials 1. NCT02089685[‡] – melanoma or carcinoma
CTLA4 + PDL1	Tremelimumab+M EDI4736		Phase I clinical trials 1. NCT01975831[†] – solid tumors 2. NCT02000947[†] – NSCLC
PD1 + LAG-3	Nivolumab BMS-986016		Phase I clinical trial 1. NCT01968109[†] – solid tumors

Representative trials, not meant to be exhaustive list of all trials.

Clinical trial status obtained March 2014 from the ClinicalTrials.gov website maintained by the NIH.

[†]Recruiting.

[‡]Not yet recruiting.

[§]Active, not recruiting.

mAb: Monoclonal antibody; NSCLC: Non-small-cell lung cancer.